

EDITORIAL

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OLGA PREUSS^{A-F}, TOMASZ SMEKTAŁA^{A-F}, KATARZYNA SPORNIAK-TUTAK^{A-F}

Early Diagnosis Methods of Cancer Lesions in Oral Cavity – Systematic Review

Metody wczesnej diagnostyki stanów nowotworowych jamy ustnej – przegląd systematyczny

Department of Dental Surgery, Pomeranian Medical University in Szczecin, Szczecin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Oral cancers comprise between 1% and 2% of all malignant neoplasms. A delay in recognition decreases the chances of successful treatment. Recently, numerous procedures have been introduced to help detect oral cancer lesions at the earliest stage. The objective of this article is to review early diagnostic methods in medical and scientific literature. A systematic literature search was carried out in MEDLINE (PubMed), MEDLINE (Ovid) and the ISI Web of Knowledge from 2000 to January 10, 2013. Only the articles whose main subject was the early detection of pre-cancerous and cancerous lesions were included. Staining and other methods that use light, besides detecting dysplasia and metaplasia, also increase the visibility of lesions. Thanks to chemiluminescence, pathologically changed tissue becomes clearer and more visible during sample collection. Incision biopsy has some disadvantages, which may lead to false negative results. These disadvantages can be reduced through careful adherence to the procedure and use of additional visualization methods, but invasiveness make this the last of choice technique. Nowadays, incision biopsy is considered the gold standard of diagnosis. However, many less invasive methods that are highly sensitive and specific also seem to be effective (**Dent. Med. Probl. 2014, 51, 4, 421–429**).

Key words: mouth neoplasms, staining and labeling, biopsy techniques.

Streszczenie

Nowotwory regionu jamy ustnej stanowią 1–2% wszystkich nowotworów złośliwych. Opóźnienie w postawieniu prawidłowego rozpoznania zmniejsza szanse na skuteczne leczenie. W ostatnim czasie zostały wprowadzone liczne metody wykrywania zmian nowotworowych jamy ustnej na najwcześniejszym etapie. Celem pracy jest omówienie, na podstawie piśmiennictwa, metod wczesnej diagnostyki zmian nowotworowych. Przegląd dostępnej literatury został przeprowadzony w bazach MEDLINE (PubMed), MEDLINE (Ovid) oraz ISI Web of Knowledge z zastosowaniem ramy czasowej 01.01.2000–10.01.2013. Do analizy zostały włączone wyłącznie artykuły, których główną tematyką było wykrywanie zmian nowotworowych na wczesnym etapie. Metody bazujące na barwieniu oraz wykorzystaniu źródeł światła umożliwiają wykrycie zarówno dysplazji i metaplazji, jak i wzmagają widoczność granic zmiany. Dzięki chemiluminescencji patologicznie zmienione tkanki stają się jaśniejsze oraz bardziej widoczne podczas pobierania materiału do badania histopatologicznego. Wady biopsji nacięciowej mogą prowadzić do uzyskania wyników fałszywie negatywnych. Część tych wad może zostać zmniejszona dzięki starannemu przestrzeganiu zasad procedury oraz zastosowaniu dodatkowych metod wizualizacji, lecz inwazyjność czyni tę technikę ostatnią z wyboru. Obecnie biopsja nacięciowa jest uważana za złoty standard, istnieje jednak wiele mniej inwazyjnych technik mających podobną czułość oraz i swoistość (**Dent. Med. Probl. 2014, 51, 4, 421–429**).

Słowa kluczowe: nowotwory jamy ustnej, metody barwienia tkanek, biopsja.

Cancer is a global health problem that concerns an increasing number of individuals, and oral cancers comprise between 1% and 2% of all

cancers. The most common oral cavity cancer is squamous cell carcinoma (SCC), which comprises 90–94% of such cases. Despite the accessibil-

ity of the oral cavity and the ease of its examination, SCC is often only diagnosed when additional symptoms occur, such as ulceration, loss of sensation, surrounding tissue oedema or an enlargement of the lymph nodes [1]. The 5-year survival rate is currently approx. 50% [2–4]. However, if treatment is administered at stage I-T1N0M0 or II-T2N0M0, the survival rate increases to 80% [2, 3, 5–8].

Many new procedures have been introduced to help detect cancer lesions at their earliest stages. The objective of this article is to review the early diagnostic methods that have been described in the medical and scientific literature.

Methods

Database searches were made at MEDLINE (PubMed), MEDLINE (Ovid) and the ISI Web of Knowledge from 2000 to Jan. 10, 2013 using one search equation: (diagnosis[TI] OR detection[TI] OR diagnostic[TI] OR detect[TI]) AND oral[TI] AND (“Mouth Neoplasms”[Majr] OR “Precancerous Conditions”[Majr]) AND (cancer[TI] OR carcinoma[TI] OR lesions[TI]) AND ((systematic[sb] OR Meta-Analysis[ptyp] OR Review[ptyp] OR Clini-

cal Trial[ptyp] OR Comparative Study[ptyp] OR Controlled Clinical Trial[ptyp] OR Randomized Controlled Trial[ptyp] OR Evaluation Studies[ptyp] OR Multicenter Study[ptyp] OR Validation Studies[ptyp]) AND “2000/01/01”[PDat] : “2013/01/10”[PDat] AND “humans”[MeSH Terms] AND English[lang]). The total number of chosen identified articles was 192. Following the automatic rejection of duplicates using Endonote X5, 160 articles remained. Inspection of the abstract was performed by two independent readers. Only articles written in English were included in this review whose main subject was the early detection of pre-cancerous and cancerous lesions. Articles describing biopsies, lymph node examinations or metastases were excluded. Possible conflicts were resolved by the inclusion of a third reader. After selecting articles from the database, the references of 36 included papers were reviewed to search for other possibly important publications. This effort resulted in an additional 12 works. The screening process is presented in Fig. 1.

In the next part of the article we are going to show the early diagnosis methods for the recognition of oral cancer lesions in the oral cavity that have been described in reviewed articles:

1) toluidine blue, methylene blue, Lugol’s iodine,

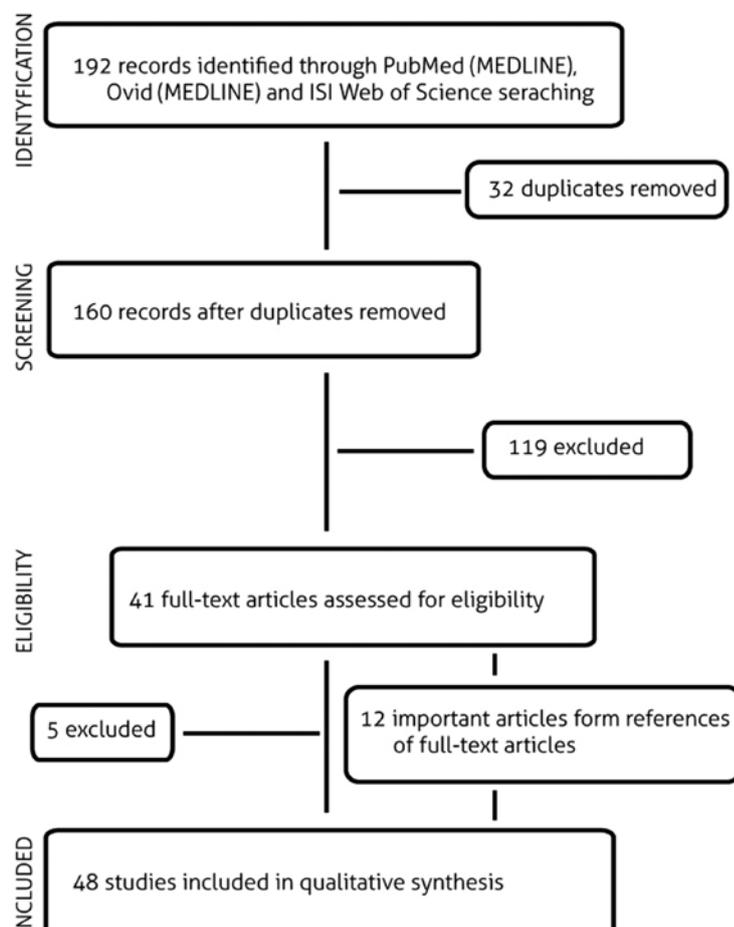


Fig. 1. Diagram presenting screening process

Ryc. 1. Diagram przedstawiający proces selekcji artykułów

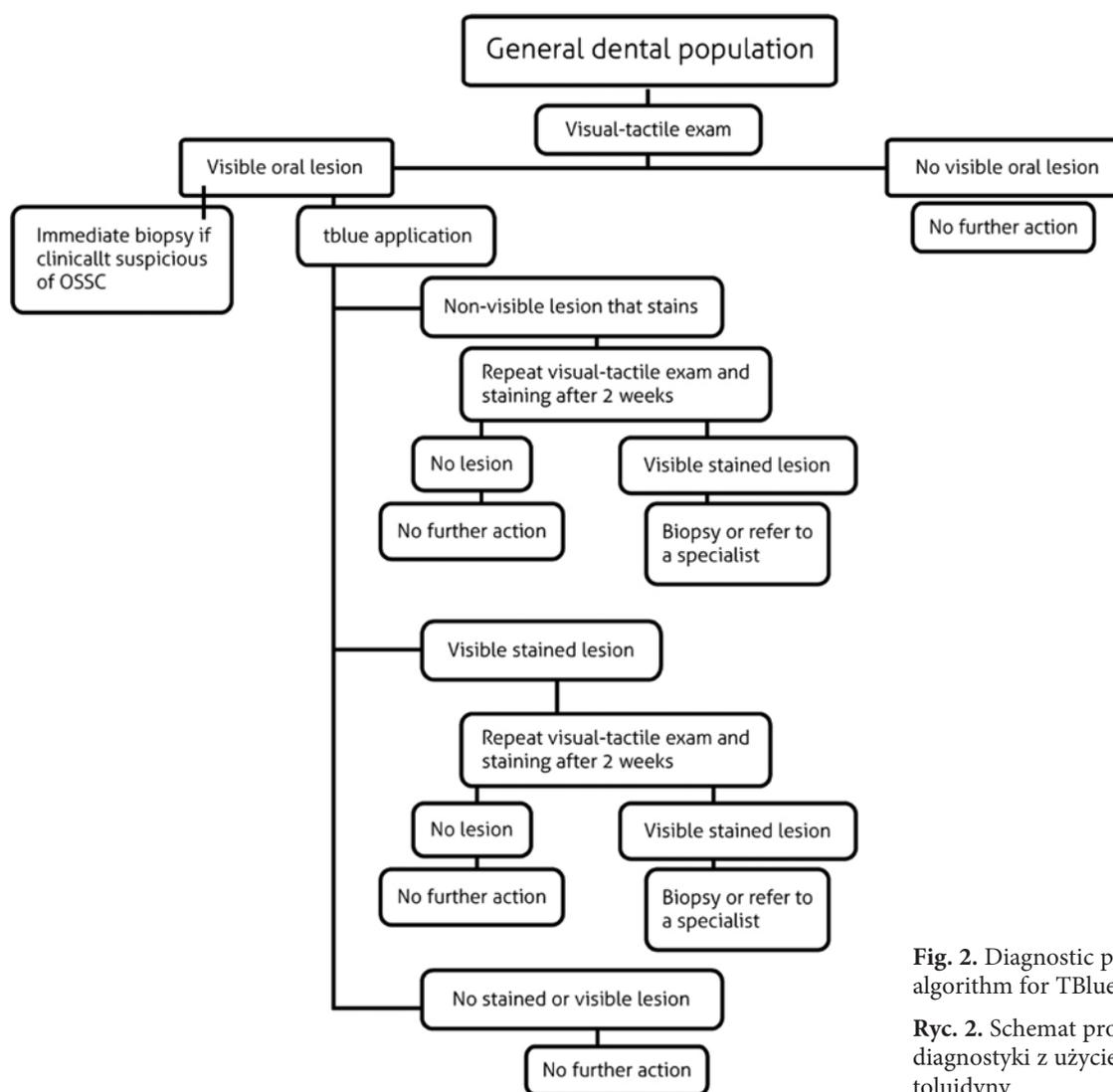


Fig. 2. Diagnostic procedure algorithm for TBlue staining

Ryc. 2. Schemat prowadzenia diagnostyki z użyciem błękitu toluidyny

- 2) chemiluminescence,
- 3) fluorescence,
- 4) colposcopy,
- 5) biopsy: brush, needle and incision,
- 6) radiological methods,
- 7) biomarkers saliva, blood.

Tissue Staining Methods

Toluidine Blue

Pathologically altered tissue staining with Toluidine blue (TBlue) 0.5–1%, also known as toluidine chloride (C₁₅H₁₆ClN₃S), is a commonly used non-invasive method. TBlue is a positively charged and metachromatic thiazine dye [1, 9–12] that acts similarly to methylene blue. TBlue dyes cellular acid components, both RNA and DNA, and can enter the intercellular spaces of dysplastic epithelium. The color that is held by the tissue can be connected to the loss of heterozygosity, which can indicate a transformation between pre-

-cancerous and cancerous lesions [13]. This staining is a highly effective method for the detection of cancer *in situ* as well as invasive cancer [14]. The method's sensitivity is 56.5–73.9%, and its specificity ranges from 25% to 74.1% [12–14]. Epstein et al. [1] proposed a diagnostic procedure algorithm that accounts for TBlue staining (Fig. 2). There are few disadvantages of this method. TBlue can stain nucleic acids in mucosal ulcerations, granulation tissue and inflammatory lesions [1, 9]. To confirm the results, a biopsy should be conducted. The color of the lesion stained with TBlue could be misleading because of difficulties in differentiating between shades of blue [10]. Portions of stained tissues can also be invisible without the use of special light sources [10]. Finally, toluidine blue has shown a toxic effect to fibroblasts [10].

Methylene Blue

Methylene blue is an organic compound that began to be used relatively recently in the diagnosis of cancerous lesions in the oral cavity. This dye

has been widely used in the detection of metaplasia of the epithelium, the prostate, bladder, lower parts of the digestive system, and in combination with endoscopic examinations for Barrett's oesophagus [10, 15, 16]. Recent research has shown that methylene blue is less toxic than toluidine blue and that its physicochemical properties and chemical structure are similar. The influence of methylene blue on cells is not fully known, although it has been accepted that the dye is absorbed by areas of metaplasia. The dye penetrates cells with abnormally increased levels of nucleic acids, resulting in tell-tale navy blue staining of the tissue that is affected by cancer. This dye also shows bactericidal effects. For staining mucosa, it is used in a 1% solution with the addition of the following: 1% malachite, 0.5% eosin, glycerol and dimethyl sulfoxide [15]. Research conducted by Ya-Wei Chen et al. [16] showed a 90% sensitivity and 69% specificity in screening for oral malignant or precancerous lesions. One disadvantage was the high percentage of false positive results, which was connected with dye retention in inflammatory, post-traumatic tissues. The reason underlying these false positive results is the mechanical retention of methylene blue in any irregularities on the lesion surface. The high number of false positive results leads to the requirement of additional, unnecessary biopsies [16]. *In vivo* staining must take place in conditions without saliva and plaque retention, which can influence any results. False results can also be attributed to inflammation, which very often accompanies cancerous lesions [15].

Lugol's Iodine

In Lugol's iodine (I₂KI) staining, also known as Schiller's test, iodine reacts with glycogen in the epithelium, resulting in the ability to differentiate inflammatory or cancerous epithelium (high glycogen content) from healthy tissue (low glycogen contents) [17]. The glycogen content in tissue is inversely proportional to the keratinisation levels. Because pathological lesions have a similar color to keratotic epithelium, the use of Schiller's test in the oral cavity is limited solely to the non-keratotic area of the mucosa. Lugol's iodine is applied at concentrations of 2–10% onto the lesion, with the hue of the healthy tissue ranging from brown to mahogany and dysplastic tissue exhibiting no change in color. According to research conducted by Petruzzi et al. [13], the sensitivity of this method is 87.5%, and its specificity is 84.2%. Lugol's iodine can also be used in the detection of regular border lesions, enlarging them by approximately 5 mm.

Chemiluminescence

Chemiluminescence has been used for many years in gynaecology to diagnose cervical cancer. Currently, this chemical light wave emission method is also used in the early diagnosis of oral cavity lesions. The systems used for this purpose are as follows: ViziLite® (Zila Pharmaceuticals, Phoenix, AZ, USA) and ViziLite Plus [11, 18–22]. Before a test, it is necessary to rinse the oral cavity with 1% acetic acid for 60 s, which cleans the testing surface of saliva glycoproteins and any debris. The acetic acid also causes a minor dehydration of the mucosa [7, 19]. Then, a diffuse chemiluminescent light with a 490–510 nm wavelength is applied to the mucosa [7]. The healthy cells absorb light and take on a blue hue. Pathologically altered tissue can be clearly seen as white in the chemiluminescent light [19]. With the ViziLite Plus system, toluidine blue is used to stain the lesions [10]. Most research has used the ViziLite system [19, 21]. The visibility of the lesions increases markedly after the use of chemiluminescent light. The lesions are seen as smaller and brighter, and their borders are more visible than those examined under a dental lamplight. Additionally, the lesion surface structure can be revealed [21]. Chemiluminescence is also used in white and white-red lesion diagnosis, although red lesions do not react to chemiluminescent light. This examination cannot distinguish between benign lesions and early stages cancer, but it can be used to better visualise the range of the lesion. The inability to differentiate between high- and low-risk lesions can lead to unnecessary biopsies for confirmation [23]. Epstein et al. [13] noted that 55% of pre-cancer lesions that were suspected of dysplasia or SCC were more visible after using light, with the lesions' brightness increasing and their borders becoming clearer. However, Laskin et al. [22] did not notice an increase in brightness after using chemiluminescence. Awan et al. [23] examined 164 patients, reporting the sensitivity of this method to be 75.4%. This method offered a lower specificity (26%), staining lesions that had previously been diagnosed as benign. Other authors estimated the sensitivity of this method to be as high as 100% [3, 20, 21], with a specificity not exceeding 14.5% [3, 20, 22].

Fluorescence

Attempts to expose tissues at specific wavelengths of light have resulted in the discovery of differences between the autofluorescence properties of different tissues [10, 24]. Fluorophores are

the parts of molecules that are responsible for fluorescence; therefore, the loss of fluorescence is characteristic of tissues in which the internal fluorophore environment has changed. Tissue absorbs fluorescent light and can take on a colour ranging from brown to black. This is unlike healthy tissue, which emits pale green autofluorescence light. The most commonly used device that can detect the loss of fluorescence in tissues is the VELscope® (Visually Enhanced Lesion Scope; LED Dental Inc., White Rock, BC, Canada), which emits waves at a length of 400–460 nm [25]. Research has shown that the most effective light color is from red to green, with a wavelength of approximately 405 nm [26, 27]. The sensitivity of this method is 97–98%, and its specificity is 94–100% [3, 26]. Lane et al. [27] achieved 100% specificity when discriminating normal mucosa from severe dysplasia/carcinoma *in situ* or invasive carcinoma in 50 biopsy sites from 44 patients. Measurements conducted by Poh [24] proved that autofluorescence can be used to define a tissue margin, which should be excised from the cancer lesion. No recurrence was reported among patients who qualified for lesion excision using autofluorescence as a guide. In contrast, of those subjects who were only tested with a visual testing method, recurrence was noted in 32% of the cases [19]. The examination of cancer lesions with fluorescence revealed cancer tissue an average of 25 mm beyond the lesion, with this later being confirmed by biopsy in 89% of the cases [10].

A new technique based on tissue fluorescence is known as Multispectral Fluorescence and Reflectance. This method uses 3 types of light: white, purple (405 nm), and amber (560 nm). The first two types result in tissue fluorescence, whereas the amber light improves the visibility of blood vessels in the tissue. Tissue that has changed shows a diffuse, blurred area of vascularisation [9]. Diffuse reflectance spectroscopy (DRS) is a method that uses light to measure the amount of oxygenated haemoglobin in tissue [28]. The length of the absorption wave is dependent on changes in blood content and oxygenation, with research indicating 100% sensitivity and 86% specificity.

The narrow band imaging method is an endoscopic technique that uses optical filters employing a narrow spectrum band for the enhanced visualisation of microcirculation in tissue. It was discovered that for this method, the depth of light penetration depends on the wavelength of the light. The filters used in this system can select blue and green light (415 and 540 nm), which are consistent with the absorption of haemoglobin. The light reveals the superficial layer of mucosa; the sensitivity of this method was assessed at 96%, with a specificity of 100% [1, 18].

Colposcopy

Colposcopy (direct microscopy) is diagnostic procedure used to examine the vagina, vulva and cervix. The magnifications obtained while using the colposcope allow the practitioner to observe and identify characteristic features of pre-cancerous and cancerous lesions and to designate the most appropriate area for biopsy [29]. This procedure is painless and easy to perform. Before examination, a 3% solution of acetic acid is applied to the mucosa for coagulation and to facilitate mucus removal. Lugol's iodine should then be used if no lesions are visible after using the acetic acid. Healthy oral cavity mucosa is pink and smooth with small, regular blood vessels. This normal vascularisation can be altered through inflammation or cancer. The colposcopic signs that suggest invasion are as follows: 1. abnormal vascular pattern, 2. irregular contour with a loss of surface epithelium and 3. color tone change. Compared with toluidine blue staining, the main advantage of direct microscopy is that catamnesis is available earlier, and the expected result of the treatment can be controlled. The accuracy of colposcopy is approximately 80–90% [30].

Biopsy

Biopsy is a diagnostic procedure that utilises an invasive method of collecting biological material from potentially altered tissue.

Brush Biopsy

A non-invasive biopsy method is the (small) oral brush biopsy that is used in the early diagnosis of oral cavity cancer and has found a growing number of supporters. The advantages of this method are as follows: a small amount of discomfort during the procedure, a lack of necessity for anaesthesia, a small amount of bleeding, and ease of performance [31]. The technique of collecting an oral brush biopsy must be very accurate due to the necessity that the sample contains an epithelial layer. Initially, the oral cavity should first be cleaned with water to remove any food remains or debris [32]. Currently, a brush biopsy is used with computational analysis of the tested material. This method, known as cytormorphometry, has found wide application in the USA as Oral Cdx® (OralScan Laboratorie Inc, Suffern, NY, USA) [9, 33]. The smears to be analysed are enlarged and show pleomorphism, nuclear borders, nuclear numbers, keratinisation, hyperchromasia, and the distribution of chromatin. Remmerbach

et al. [34] combined cytometry with DNA analysis and obtained a 98% sensitivity and 100% specificity in the detection malignant lesions. However, oral brush biopsy alone should only be used for lesions that are not suspicious [35]. In the research conducted by Mehron et al. [36] that included 79 patients, all of the oral brush biopsies were re-examined with incision biopsy examination. In 5 cases, the lesions were found to be benign when the oral brush biopsy defined them as atypical. However, Bhoopathi et al. [37] found a high number of false positive results due to the low predictive value of 7.9% [37]. These authors stated that this can cause unnecessary anxiety among patients, in addition to the necessity for additional confirmation examinations.

Needle Biopsy

There are two different types of needle biopsy: oligobiopsy and fine-needle biopsy. Fine-needle biopsies are less invasive but are less sensitive than incision or oligobiopsies because of the small amount of material that is tested. In fine-needle biopsies, the structure of a cell evaluated, whereas in oligobiopsies, the architecture of the actual tissue is examined. Needle biopsies are applied to assess lesions in deeply localised tissues, which is difficult to evaluate using other methods. Needle biopsies have one important advantage, namely, low infection risk, but the disadvantages are as follows: difficulty in reaching the lesion, a small movement area, the possibility of damage to other anatomical structures, bleeding risk, and the spread of cancer cells through the route travelled by the needle [38]. This technique can also lead to infection, nerve injury, oedema, haematoma, swelling and bruising. The procedure is performed without anaesthesia and can be painful. Occasionally, needle biopsies in children should be performed under general anaesthesia. The sensitivity and specificity of this method vary from 80% to 100% [38].

Incision Biopsy

The incision biopsy is currently considered the gold standard for oral cancer lesion diagnosis. There are two different types of incision biopsy: a direct biopsy, in which the lesion is located in the oral mucosa and can be easily assessed with a scalpel; and indirect biopsy, in which the lesion is covered by apparently normal oral mucosa. In addition to its use in incision biopsy, it can be utilised in excision biopsy in cases where it is necessary to remove the whole lesion. It is theoretically a better method because of its complete lesion examination; however, this may lead to incomplete malig-

nant lesion treatment or to the radical treatment of a benign lesion [22]. The examined material can then be sent for a histopathological analysis. However, this examination has some disadvantages that should be considered. Specifically, it is an invasive procedure, and some patients may not agree to proceed with this treatment. Additionally, in the case of a large number or wide lesions, choosing the right incision point may be difficult. Due to the risk of destroying the tissue architectonic layer, the procedure should take place under block anaesthesia to minimize trauma [39]. Preliminary research for the removal of tissue samples using laser light has shown that it can prevent the spread of suspected dysplasia cells [25]. However, the use of this method comes with negative effects, such as the creation of thermal artifacts at the sample borders that may influence the outcome of the examination. The laser can cause cytological atypia, which can result in artifacts. A similar phenomenon also occurs in electrocoagulation. Excision biopsy carries the risk of incomplete treatment of malignant tissue and the overtreatment of benign lesions [22, 39]. Following an incision biopsy, pain and bleeding from the wound can occur. If necessary, bleeding can be controlled by sutures [33]. The result of samples taken from lesions that are not homogeneous may depend on the location from which the sample is taken [12, 22]. Biopsies require the use of local anaesthesia.

Molecular Techniques

The knowledge of cancer cell structures, their metabolism and predisposing factors for the occurrence of cancer has led to the development of modern diagnostic methods. The material for examination that is used in molecular techniques can be taken from oral cavity fluids, such as saliva or blood (blood is a reliable source of DNA). The advantages of saliva are as follows: availability, repeatability, and ease of performance [28, 40, 41]. Markers in the blood include (VEGF)-A, VEGF-C, cyclooxygenase (COX)-2, and phosphodiesterases (PDE) [42, 43]. Markers in the saliva include RNA, IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT. Other saliva markers include squamous cell carcinoma associated antigen (SCC), carcinogenic embryonic antigen (CEA), CA19-9, carcinoma-antigen CA128, CA125, serum tumour marker, cyfra 21-1, intermediate filament protein, tissue polypeptide specific antigen (TPS), reactive nitrogen species (RNS), 8-OHdG (a DNA damage marker), lactate dehydrogenase (LDH) (a marker of tissue breakdown), IgG immunoglobulin, Sec

IgA mucosal immunoglobulin, IGF growth factor, MMP-2 metalloproteinase, MMP-11 metalloproteinase, loss of heterozygosity (LOH), loss of specific chromosomal regions, DNA hypermethylation, and gene inactivation [44–49].

For a biomarker examination such as that of interleukin or albumin levels, immunoblots are most often used. Brinkmann et al. [28] examined biomarkers included in the saliva. These authors stated that IL8, IL1B, M2BP proteins and mRNA markers were similar in patients with oral cancer. M2BP was determined to be an important marker for early stage of cancer (T1/T2), and IL1B and mRNA were found to mark stage T3/T4. Increase levels of the markers IL1B and IL8 was also observed in periodontal disease, which may cause false positive results [28]. The specificity and sensitivity of mRNA biomarkers are both 91%.

Radiological Methods

In the early diagnosis of cancer, radiological methods such as the following are also used: F-FDG PET, computer tomography (CT), optical coherence tomography (OCT), and optical Doppler tomography (ODT). OCT can provide non-invasive imaging of the epithelial and subepithelial structures and can detect and diagnose oral premalignant lesions and inflammation [22, 39]. OCT also allows the identification of architectural changes in malignant cells; however, it cannot differentiate between lesions [39]. CT is used to evaluate the primary tumour invasion to lymph nodes and bone. F-FDG PET- 2-fluoro-[18F]-deoxy-2-D-glucose positron emission tomography is a visualisation technique that provides information about tissue metabolism. It is often used to detect metastases to the lymph nodes. The sensitivity of this method has reached 90%. The research of Shu-Hang et al. [50] proved that this method can also detect primary tumors. Specifically, this method confirmed 122 cases of 124 cancer lesions [50]. This method is very sensitive for detecting primary tumors that are not observed in morphologic imaging modalities but does not provide essential information for surgical planning, such as the depth of cancer penetration and changes in neighboring tissues. It is, however, helpful in identifying primary oral cavity tumors that cannot be seen using different methods [50]. Recently, a hybrid PET/CT has achieved better diagnostic accuracy.

Summary

Most oral cavity lesions are currently detected by physical and visual examination with incandescent light. However, the use of additional diagnostic methods can improve the effectiveness of such examinations. This article describes less invasive diagnostic methods that have a relatively high sensitivity and specificity (Table 1). The described staining methods can be widely used due to their availability, low invasiveness and cost. TBlue staining is an effective, inexpensive and rapid method [1]. This technique might be useful in determining which clinically suspicious lesion could progress to cancer [10]. However, it should be remembered that this technique could only be performed on superficial tissues. Also portions of stained tissues can be invisible without the use of special light sources and toluidine blue has toxic effect to fibroblasts [10]. Staining and other methods that use light, in addition to detecting dysplasia and metaplasia, increase the visibility of lesions but requires additional equipment. Through the use of chemiluminescence, pathologically changed tissue becomes clearer and more visible, which is helpful when taking samples [3]. Needle biopsies should be applied to assess lesions in deeply localised tissues, which is difficult to evaluate using other methods. Difficulty in reaching the lesion necessitates the use of ultrasound imaging and sometimes requires a few attempts to obtain a proper sample. This technique also carries the risk of damage to adjacent anatomical structures and infection. Needle as well as incisional biopsy needs to be performed under local or sometimes general anaesthesia. Incisional biopsy could lead to incomplete treatment of malignant tissue while excisional to overtreatment of benign lesions. These disadvantages can be reduced through careful adherence to the pro-

Table 1. Comparison of sensitivity and specificity of presented diagnostic techniques

Tabela 1. Porównanie czułości oraz swoistości metod diagnostycznych prezentowanych w pracy

Method	Sensitivity – %	Specificity – %
Toluidine blue	56.5–73.9	25–74.1
Methylene blue	90	69
Lugol's iodine	87.5	84.2
Chemiluminescence	75.4–100	14.5–26.8
Fluorescence	97–98	94–100
Direct microscopy	80–90	–
Brush biopsy	77–98	100
Needle biopsy	80–100	80–100
Molecular techniques	91	91

cedure and use of additional visualization methods but, invasiveness make this the last of choice technique. In the future, it seems likely that biomarkers will replace traditional methods. However, at this time, there is no molecular marker that

can determine the range and behavior of a tumor or lesion [18]. Using additional diagnostic methods, such as incision biopsy, allows for the removal of materials in a much more accurate manner, thereby lowering the risk of false negative results.

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Address for correspondence:

Tomasz Smektała
Department of Dental Surgery
Pomeranian Medical University in Szczecin
Powstańców Wlkp. 72
70-111 Szczecin
Poland
E-mail: smektała.t@gmail.com

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