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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Oral cancer, also known as **mouth cancer**, is a type of head and neck cancer and is any cancerous tissue growth located in the oral cavity.

It may arise as a primary lesion originating in any of the tissues in the mouth, by metastasis from a distant site of origin, or by extension from a neighboring anatomic structure, such as the nasal cavity. Alternatively, the oral cancers may originate in any of the tissues of the mouth, and may be of varied histologic types: teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment-producing cells of the oral mucosa. There are several types of oral cancers, but around 90% are squamous cell carcinomas, originating in the tissues that line the mouth and lips. Oral or mouth cancer most commonly involves the tongue. It may also occur on the floor of the mouth, cheek lining, gingiva (gums), lips, or palate (roof of the mouth). Most oral cancers look very similar under the microscope and are called squamous cell carcinoma, but less commonly other types of oral cancer occur such as melanoma and Kaposi's sarcoma. The **"DISEASE DIAGNOSIS"** segment discusses in-depth all clinic-diagnostic aspects of ORAL CARCINOMA. Also discussed is the Therapeutic approach to the disease.

As the diagnosis of the lesions mentioned above are based on histopathology/ biopsy, the **"INTEPRETATION"** section of this communiqué highlights the various Histological stains alongwith what the stain and in what colour. Also given are Fun Facts as related to them.

"TROUBLESHOOTING" portion lays threadbare the problems associated with H & E staining and how to overcome them.

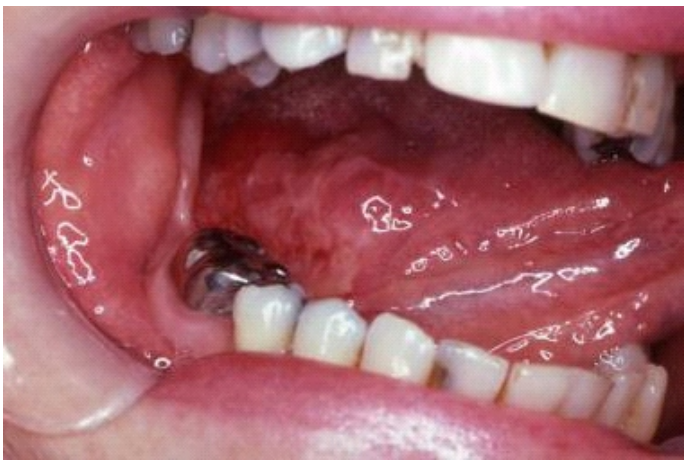
Among all the serious talk, **"BOUQUET"** has not been forgotten, a few jokes, a few words of advice and four simple but tricky questions cap it all.

DISEASE DIAGNOSIS

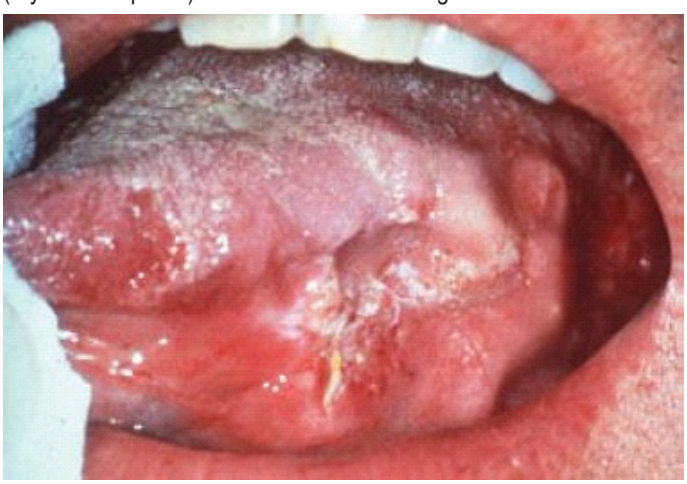
ORAL MUCOUSA SQUAMOUS CELL CARCINOMA

Background

The image below shows early oral squamous cell carcinoma (OSCC) on the lateral border of the tongue.



Different clinical presentations of oral squamous cell carcinoma (OSCC) on the anterior and posterior lateral border of the tongue illustrate the importance of differential diagnosis. This first case was thought to be an allergic reaction to amalgam. Therefore, the importance of a good differential diagnosis. Mouth (oral) cancer is a major neoplasm worldwide and accounts for most head and neck cancers. It theoretically should be largely preventable or detectable at an early stage. Approximately 90% of oral cancers are SCC, which is seen typically on the lateral border of the tongue, oropharynx, and floor of the mouth, as a red lesion (erythroplakia), white lesion (leukoplakia), or a mix of the two (erythroleukoplakia) with an ulcer. See the image below.



Oral squamous cell carcinoma in the most common intraoral site manifesting as a chronic, indurated ulcer. Early oral cancer is asymptomatic, which contributes to delayed diagnosis. Any single ulcerated lesion persisting for more than 3 weeks should be regarded with suspicion, and a biopsy should be performed. The mnemonic RULE (red, ulcerated, lump, extending for 3 or more weeks) is an aid to diagnosis. OSCC is particularly common in the developing world, mostly in older males. There is concern about an ongoing increase in younger patients and in women in particular, as well as in the oropharynx, possible due to human papillomavirus (HPV) infection. The etiology of OSCC appears to be multifactorial and strongly related to lifestyle, mostly habits and diet (particularly tobacco alone or in combination with betel, and alcohol use). Other factors such as infective agents may also be implicated, particularly in oropharyngeal cancer (HPV). Immune defects or immunosuppression, defects of carcinogen metabolism, or defects in DNA-repair enzymes underlie some cases of SCC. Sunlight exposure predisposes to lip cancer. Findings from the history and clinical examination by a trained dentist are the primary indicators of OSCC, but the diagnosis must always be confirmed histologically with tissue biopsies, even if the clinical picture is consistent with OSCC.

Pathophysiology

In oral squamous cell carcinoma (OSCC), modern DNA technology, especially allelic imbalance (loss of heterozygosity) studies, have identified chromosomal changes suggestive of the involvement of tumor suppressor genes (TSGs), particularly in chromosomes 3, 9, 11, and 17. Functional TSGs seem to assist growth control, while their mutation can unbridle these control mechanisms. The regions most commonly identified thus far have included some on the short arm of chromosome 3, a TSG termed *P16* on chromosome 9, and the TSG termed *TP53* on chromosome 17, but multiple other genes are being discovered. As well as damage to TSGs, cancer may also involve damage to other genes involved in growth control, mainly those involved in cell signaling (oncogenes), especially some on chromosome 11 (*PRAD1* in particular) and chromosome 17 (Harvey ras [H-*ras*]). Changes in these and other oncogenes can disrupt cell growth control, ultimately leading to the uncontrolled growth of cancer. H-*ras* was one of the oncogenes that first caught the attention of molecular biologists interested in cell signaling, cell growth control, and cancer. It and the gene for epidermal growth factor receptor (*EGFR*) are involved in cell signaling. The genetic aberrations involve, in order of decreasing frequency, chromosomes 9, 3, 17, 13, and 11 in particular, and probably other chromosomes, and involve inactivated TSGs, especially *P16,* and *TP53* and overexpressed oncogenes, especially *PRAD1.* The molecular changes found in OSCC from Western countries (eg, United Kingdom, United States, Australia), particularly *TP53* mutations, are infrequent in Eastern countries (eg, India, Southeast Asia), where the involvement of *ras* oncogenes is more common, suggesting genetic differences that might be involved in explaining the susceptibility of certain groups to OSCC. Carcinogen-metabolizing enzymes are implicated in some patients. Alcohol dehydrogenase oxidizes ethanol to acetaldehyde, which is cytotoxic and results in the production of free radicals and DNA hydroxylated bases; alcohol dehydrogenase type 3 genotypes appear predisposed to OSCC. Cytochrome P450 can activate many environmental procarcinogens. Ethanol is also metabolized to some extent by cytochrome P450 IIEI (CYP2E1) to acetaldehyde. Mutations in some TSGs may be related to cytochrome P450 genotypes and predispose to OSCC. Glutathione S transferase (GST) genotypes may have impaired activity; for example, the null genotype of GSTM1 has a decreased capacity to detoxify tobacco carcinogens. Some GSTM1 and GSTP1 polymorphic genotypes and GSTM1 and GSTT1 null genotypes have been shown to predispose to OSCC. *N*-acetyltransferases NAT1 and NAT2 acetylate procarcinogens. *N*-acetyl transferase NAT1*10 genotypes may be a genetic determinant of OSCC, at least in some populations. Tobacco is a potent risk factor for oral cancer. An interaction occurs between redox-active metals in saliva and the low reactive free radicals in cigarette smoke. The result may be that saliva loses its antioxidant capacity and

instead becomes a potent pro-oxidant milieu. DNA repair genes are clearly involved in the pathogenesis of some rare cancers, such as those that occur in association with xeroderma pigmentosum, but, more recently, evidence of defective DNA repair has also been found to underlie some OSCCs. An immune deficiency state may predispose one to a higher risk of developing OSCC, especially lip cancer.

Epidemiology

The oral cavity is one of the 10 most frequent sites of cancer internationally, with three quarters of cases affecting people in the developing world, where, overall, oral cancer is the third most common cancer after stomach and cervical cancer. An estimated 378,500 new cases of intraoral cancer are diagnosed annually worldwide. Unfortunately, the parts of the world where oral cancer is most common are also those where descriptive information (ie, incidence, mortality, prevalence) is least available. In certain countries, such as Sri Lanka, India, Pakistan, and Bangladesh, oral cancer is the most common cancer. In parts of India, oral cancer can represent more than 50% of all cancers. The worldwide incidence of oral cancer is estimated to be around 260,000 cases annually, although there is great variation in the incidences across the world. Countries like Taiwan, Hungary, Brazil, France, and parts of South Africa have higher incidences compared with some other countries, such as Japan. In developed countries, oral cancer is less common but is the eighth most common form of cancer overall. For example, in areas of northern France, oral cancer is the most common form of cancer in men. Estimates show that in 1980, more than 32,000 new cases of oral cancer were diagnosed throughout the European community. The prevalence of lip cancer appears to be decreasing, but the prevalence of intraoral cancer appears to be rising in many countries, especially in younger people. This is especially true in Central and Eastern Europe, especially Hungary and Northern France. Within the United States, oral cancer represents the eleventh most common cancer in males and the sixteenth most common in females. Approximately 27,000 new cases of oral cancer are diagnosed each year, with about 5,500 patients succumbing to the disease annually.

Race

The prevalence of tongue cancer is consistently found to be higher (by approximately 50%) in blacks compared with whites within the same regions of the United States. The prevalence of oral cancer is also generally higher in ethnic minorities in other developed countries.

Sex

Oral cancer affects males more frequently than females, although the ratio is equalizing.

Age

Oral cancer is predominantly found in middle-aged and older persons. However, in recent years, an increase in younger patients has been observed.

Prognosis

In general, the prognosis for oral cancer depends on tumor staging and the location of the tumor. At most times, the staging of the tumor is associated with the timing of the diagnosis. The earlier the diagnosis, the lower the tumor stage, and hence, a better survival rate (83.7%) is noted compared with a lower survival rate with a late diagnosis, leading to a higher stage III-IV (38.5%). However, other factors also have to be taken into account, such as the location of the tumor, the patient's general health, age, tobacco usage, and the presence of human papillomavirus (HPV) infection. Based on 2017 data from the United States, the estimated 5-year survival rate for oral and pharyngeal cancers is approximately 66%. Lip carcinomas generally has the best 5-year survival rate (88%) and the floor of mouth has the worst (54%). Tumor staging is the best prognostic factor for intraoral cancers and lip carcinomas, while the status of transcriptionally active HPV is considered the most important prognostic factor for oropharyngeal cancers. Those with HPV-positive tumors tend to respond better to chemotherapy and/or radiation therapy compared with those with HPV-negative tumors.

Patient Education

Unfortunately, little has been done in regard to patient education as it concerns oral cancer. Like melanoma, oral cancer can be easily seen, except those in the posterior regions of the tongue, by the patient and the primary care physician. However, this is true only if they know how to identify it. Of interest, the National Cancer Institute (NCI) estimates 87,110 new cases of melanoma for 2017, with 9,730 estimated deaths, while for oral cancer, they estimate 49,670 new cases, with a mortality of 9,700. This difference can only be explained by the aggressive campaign sponsored by the America Academy of Dermatology (AAD) against melanoma, which produced and distributed visual teaching material for patients about the risk of melanoma and the typical clinical presentation. Increased awareness instituted by relevant dental societies to educate the public on the risks and typical clinical presentation of oral squamous cell carcinoma (OSCC) is recommended. Educate patients regarding lifestyle changes, including a diet richer in vegetables and fruits, discontinued smoking, and moderation of alcohol consumption. Furthermore, patients should be encouraged to learn about their oral condition, prevention, treatment options, and complications from therapy. For patient education resources, see the following:

CLINICAL PRESENTATION

History

Some oral squamous cell carcinomas (OSCCs) arise in apparently normal mucosa, but many are preceded by clinically obvious potentially malignant disorders, especially erythroplakia (red patch), leukoplakia (white patch), erythroleukoplakia (red and white patch), or verrucous leukoplakia. Many others are associated with such lesions (especially in Southeast Asia). The challenges in predicting which oral mucosal potentially malignant disorder will progress to neoplasia are discussed more fully elsewhere. Erythroplastic lesions are velvety red plaques, with a prevalence ranging from 0.01-0.21%, which, in at least 90% of cases, show severe dysplasia or frank malignancy. In contrast, most white lesions are not malignant or premalignant. Speckled or verrucous leukoplakias are more likely to be premalignant. The prevalence of leukoplakias as compared with erythroplakia is higher, and severe dysplasia or carcinomatous change is more common in erythroplakia. Homogeneous leukoplakias are only very occasionally premalignant, but speckled or verrucous leukoplakias are more likely to be premalignant. In a study of 257 patients with oral leukoplakia, Silverman et al followed these patients over a mean period of 7.2 years. Of these patients, 17.5% developed carcinoma. The time from initial diagnosis of either epithelial dysplasia or hyperkeratosis to carcinoma ranged from 6 months to 39 years. In most cases, a biopsy with histologic examination is required because dysplasia may precede malignant changes. The rate of malignant changes can be as high as 36% when moderate or severe dysplasia is present. Be aware that single ulcers, lumps, red patches, or white patches (particularly if they persist >3 wk) may be manifestations of malignancy.

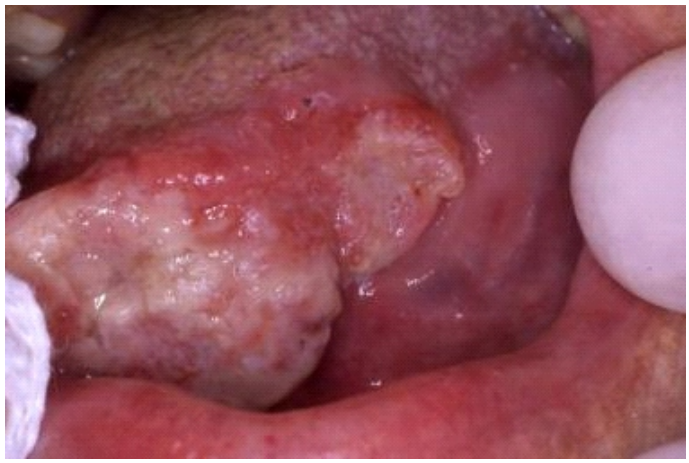
OSCC may manifest as the following:

- A red lesion (erythroplakia)
- A granular ulcer with fissuring or raised exophytic margins
- A white or mixed white and red lesion
- An indurated lump/ulcer (ie, a firm infiltration beneath the mucosa)
- A nonhealing extraction socket
- A lesion fixed to deeper tissues or to overlying skin or mucosa
- Cervical lymph node enlargement, especially if hardness is present in a lymph node or fixation. Enlarged nodes in a patient with oral carcinoma may be caused by infection, reactive hyperplasia secondary to the tumor, or metastatic disease. Occasionally, a lymph node is detected in the absence of any obvious primary tumor. Nodal enlargement is a feature particularly in oropharyngeal cancers.

These potentially malignant disorders and OSCC should be detected at an early stage; however, many OSCCs still are seen only when advanced. Diagnosis is often delayed by up to 6 months, even in developed countries, despite exhortations over the past 25 years to increase the index of suspicion. Early detection and treatment is the short-term goal because this results in considerably better survival rates. Early carcinomas may not be painful; however, later, they may cause pain and difficulty with speech and swallowing. Dental practitioners and dental care professionals should remain vigilant for signs of potentially malignant disorders and oral cancer while performing routine oral examinations.

Physical Examination

A systematic and thorough examination of the mouth, fauces, and cervical lymph nodes should be performed by a clinician trained in the diagnosis of oral diseases, and a general physical examination is indicated. Dental practitioners and dental care professionals are trained in the examination of the mouth. Examine the teeth, periodontium, and entire mucosa in good lighting. In those with oral squamous cell carcinoma (OSCC), advanced caries, periodontal disease, or periapical lesions may need early attention, especially if radiotherapy is to be used in the management of OSCC. The most common sites of OSCC include the tongue, mainly the lateral and ventrolateral aspects, and the floor of the mouth; however, all areas should be scrutinized. A common site for OSCC is the posterior portion of the tongue, which may be missed on cursory inspection; hence, special care is needed to ensure close examination. The clinical appearance of oral cancer is highly variable and includes ulcers, red or white areas, lumps, or fissures. Lesions always must be palpated after inspection to detect induration and fixation to deeper tissues. Erythroplakia is a red and often velvety lesion, which, unlike leukoplakias, may not form a plaque but is level with or depressed below the surrounding mucosa. Of these lesions, 75-90% may show severe epithelial dysplasia, carcinoma in situ, or invasive changes. Erythroplakia can affect patients of either sex in their sixth and seventh decades and typically involves the floor of the mouth, the ventral surface of the tongue, or the soft palate. Red oral lesions usually are more dangerous than white oral lesions. Oral mucosal white patches usually result from increased keratinization or candidosis. Leukoplakia is restricted to white patches for which a cause cannot be established; therefore, the term implies a diagnosis by exclusion (eg, lichen planus, candidiasis). The term leukoplakia is also used irrespective of the presence or absence of epithelial dysplasia. Leukoplakia is a clinical term for a persistent adherent white patch with no histologic connotation and no implied premalignant potential. Some OSCC can also appear as a white patch. Late OSCC may manifest as an exophytic lesion or an area of ulceration with induration. A typical malignant ulcer is hard with heaped-up and often everted or rolled edges and a granular floor, as shown in the image below.



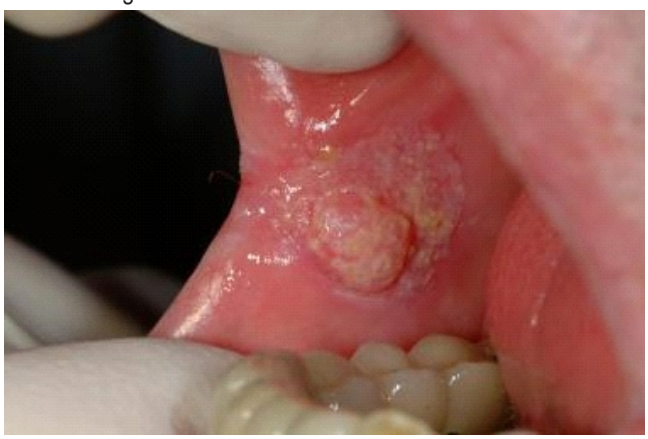
Oral squamous cell carcinoma (OSCC) presenting as a large, ulcerated lump on the left anterior lateral border of the tongue. The floor of the mouth is the second most common intraoral site for cancer and more commonly is associated with leukoplakia. Most cancer arises in the anterior floor of the mouth as an indurated mass that soon ulcerates, resulting in slurring of speech. Carcinomas of the alveolus or gingiva can present as an exophytic mass or a persistent ulcer. The underlying alveolar bone is invaded in 50% of cases, even in the absence of radiographic changes, and adjacent teeth may be loose. Carcinomas of the buccal mucosa are mostly seen at the commissure or in the retromolar area. Most are ulcerated lumps, and some arise in candidal leukoplakias. Any single lesion that persists more than 3 weeks, especially if red, ulcerated, or a lump, especially with induration (ie, the RULE mnemonic) should be regarded with suspicion and a histopathological diagnosis established. Second primary tumors are additional primary carcinomas (synchronous tumors) present in as many as 10-15% of persons with oral carcinoma and are most commonly seen in the mouth in patients with gingival, floor of mouth, lingual, or buccal carcinoma. Second primary tumors may also be present elsewhere in the upper aerodigestive tract. Lymph node examination is of paramount importance, and general examination and, possibly, endoscopy, may be indicated to detect metastases or second primary tumors. From 30-80% of patients with oral cancer have metastases in the cervical lymph nodes at presentation. Oral cancer predominantly metastasizes locally and to regional lymph nodes, primarily in the anterior neck. Later, dissemination to the lungs, liver, or bones may occur. Any chronic oral lesion should be regarded with suspicion, especially when found in an older patient, when lesions appear (see History), with induration, with fixation to underlying tissues, with any recent changes in appearance, with associated lymphadenopathy, or with no obvious explanation for the lesion. Examine the entire mucosa because widespread dysplastic mucosa (field change) or a second neoplasm (see Staging) may be present. Carefully record the location of suspicious lesions, preferably on a standard topographic diagram.

Causes

Tobacco and alcohol use are independent risk factors for mouth cancer and tongue cancer. Heavy tobacco smokers have a 20-fold greater risk; heavy alcohol drinkers a 5-fold greater risk; those who do both have a 50-fold greater risk. Betel-quid chewing and oral snuff are important risk factors in people from specific geographic areas (eg, betel chewing in Southeast Asia). Finally, a diet low in fresh vegetables and fruits has also

been implicated in causing oral squamous cell carcinoma (OSCC), and human papillomaviruses (HPVs) have been implicated in oropharyngeal cancers.

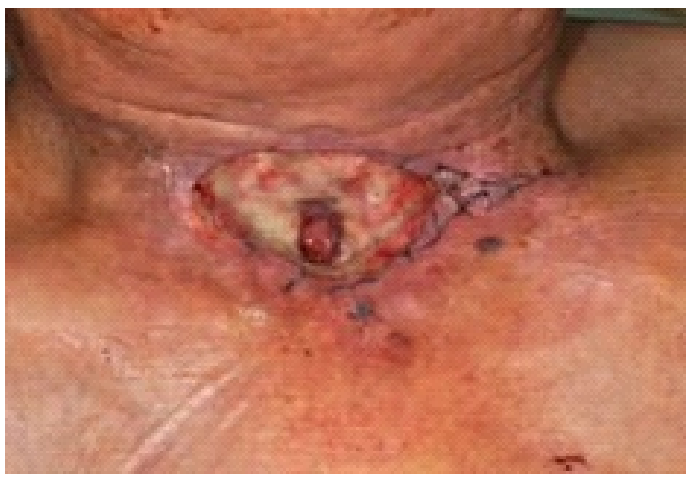
- Cigarette smoking: Compared with persons who do not smoke, the risk of oral cancer in persons who smoke low/medium-tar cigarettes and high-tar cigarettes was 8.5- and 16.4-fold greater, respectively. (Note that cigarettes are classified as low/medium if the tar yield is less than 22 mg and high tar if the tar yield is greater than 22 mg). Note the image below.



Early oral squamous cell carcinoma in the buccal mucosa arising from a chronic candidal leukoplakia in a person who smokes heavily. The lesion was a painless, chronic indurated lump.

- Alcohol: Growing evidence is associating increased alcohol consumption with the risk of developing OSCC. Alcoholic beverages may contain carcinogens or procarcinogens, including nitrosamine and urethane contaminants and ethanol. Ethanol is metabolized by alcohol dehydrogenase and, to some extent, by cytochrome P450 to acetaldehyde, which may be carcinogenic. The combined effects of tobacco use and alcohol consumption are found to be multiplicative. Compared with persons who do not drink and do not smoke, the risk of developing OSCC is increased 80-fold in persons with the highest levels of smoking and alcohol consumption.
- Betel and similar habits: The betel quid contains a variety of ingredients, including betel vine leaf, betel (areca) nut, catechu, and, often, slaked lime together with tobacco. Some persons chew the nut only, and others prefer paan, which includes tobacco and sometimes lime and catechu. In 1986, the International Agency for Research on Cancer deemed betel-quid chewing an important risk factor, and the areca (betel) nut habit with or without tobacco use can cause cytogenetic changes in oral epithelium. Various other chewing habits, usually combinations that contain tobacco, are used in different cultures (eg, Qat, Shammah, Toombak). Tobacco chewing in people from parts of Asia appears to predispose to OSCC, particularly when it is started early in life and is used frequently and for prolonged periods. Studies from India have confirmed the association between paan tobacco chewing and OSCC, particularly cancer of the buccal and labial mucosa.
- Diet: Dietary habits may play a role in the development of oral cancer. A diet rich in fresh fruits and vegetables with limited consumption of meats is recommended for prevention of cancer. Health supplements (vitamins, minerals, and other bioactive compounds) have not been shown to confer the same level of effectiveness in replacing these nutrients. Hence, these health supplements should not be used as substitute for vegetables and fruits in meals.
- Oral health: A case-control study (ie, every oral cancer case prior to surgery and every control at the time of interview had a structured oral examination) from China found that wearing dentures, per se, is not a risk factor, although the risk was increased in men who wore dentures made from metal. Poor dentition, as reflected by missing teeth, emerged as a strong risk factor independent of other established risk factors.
- Mouthwash use: The effect of the alcohol in mouthwash appears to be similar to that of alcohol used for drinking, although the contribution of mouthwash use to oral cancer must be small in terms of attributable risk. This controversy continues.
- Socioeconomic status: Behaviors that lead to social instability or social instability itself have been linked to an increased risk of oral cancer, but many other explanations may exist (eg, habits, oral health, diet, nutrition).
- Infective agents: *Candida albicans* and viruses, such as herpes viruses and papillomaviruses, may be implicated in some cases. HPVs are particularly implicated in oropharyngeal cancers. HPV-related tumors tend to be seen in younger patients, in the fauces, and have usually a better prognosis.
- Others: Associations also are apparent between oral cancer and other various oral conditions (eg, oral submucous fibrosis, oral lichen planus, lupus erythematosus, dyskeratosis congenita, Fanconi anemia).

DIFFERENTIAL DIAGNOSES



- Actinic Keratosis
  If patient presents with a lump on vermillion border of the lip.
- Erythroplasia
  Brightly colored, smooth, red patch usually found on floor of the mouth, oropharynx area, and lateral border of the tongue.
- Lichen Planus
  Bilateral or symmetric reticular changes with or without ulcers and erythema occurring anywhere in the oral cavity.
- Lichenoid lesions
  A single white reticular patch with erythema and/or ulceration either due to a drug reaction or adjacent to dental material such as amalgam.
- Mucosal Candidiasis
  A group of yeast like fungal infections involving the skin and mucous membrane including the mouth.

- Traumatic lesion
  Appearing adjacent to a sharp or broken tooth.

WORKUP

Laboratory Studies

To confirm the diagnosis of oral squamous cell carcinoma (OSCC), a tissue biopsy must be performed to allow histopathologic examination of the lesional tissue. In addition, to determine whether malignant disease is present elsewhere after the initial diagnosis of OSCC is rendered, further investigations can be performed to look for the following:

- Bone, muscle, or primary tumors: Other primary tumors are typically located in the upper aerodigestive tract (eg, mouth, nares, pharynx, larynx, esophagus). Whether endoscopy is warranted to detect such tumors in all cases remains controversial.
- Metastases: This initially occurs to regional lymph nodes and later to the liver, bones, and brain. Imaging studies may help detect abnormalities missed during the clinical examination.

Blood tests include the following:

- Liver function tests: Results may reveal metastases in persons with advanced disease.
- Complete blood cell count and hemoglobin value
- Urea and electrolyte measurements
- Blood group testing and cross-matching
- Calcium level: As many as 4% of patients with cancer in the head and neck may have elevated serum calcium levels. This is a poor prognostic indicator primarily found in persons with advanced disease.
- Serum ferritin, alpha-antitrypsin, and alpha-antiglycoprotein levels: Persons with high-stage cancer of the head and neck also have increased levels of serum ferritin, alpha-antitrypsin, and alpha-antiglycoprotein, while those at any stage of disease have increased haptoglobin levels (although not known if this is true specifically for oral cancer). Additionally, prealbumin levels are decreased slightly in persons at any stage. Results from assays of these serum constituents cannot be regarded as sufficiently specific or sensitive to be of reliable clinical value, and this, unfortunately, is also true of the many tissue markers thus far described.

Imaging Studies

Photography to create a photographic record is especially useful for monitoring the clinical state and site of premalignant lesions. Chest radiography and endoscopy are valuable procedures for excluding synchronous second primary tumors. Chest radiography may be indicated because the lungs are the most common site for metastases and a site for second primary carcinomas. Radiography, sometimes including axial CT scanning or, possibly, other imaging techniques, may be needed to determine the degree of spread of some tumors, particularly to exclude bone invasion and lymph node involvement. Chest radiography is important as a preanesthetic check, especially in patients with known pulmonary or airway disease and to demonstrate metastasis to the lungs or hilar lymph nodes, ribs, or vertebrae. Jaw radiography (often rotating pantomography) may show invasion, although it is inadequate to exclude bone invasion. Other imaging investigations include MRI or CT scanning of the primary site, of the head and neck, and of suspected sites of lymph node or distant metastases. Sentinel node biopsy, MRI, 18-fluorodeoxyglucose positron emission tomography (FDG-PET) scanning, or ultrasonography of the neck (or combinations) can be used to delineate the extent of cervical node metastasis. Radionuclide scanning occasionally is useful. Bone scanning is of little value in screening because findings are positive only where bone involvement is symptomatic. Bone scanning is primarily used to determine the extent of tumor spread. Liver radionuclide scanning shows abnormal findings in as many as 6% of patients with cancer in the head and neck, but two thirds are false-positive findings; therefore, liver scanning normally is not indicated. Routine panendoscopy helps identify simultaneous second primary carcinomas in the esophagus, larynx, or lungs in as many as 14% of patients. Endoscopy is widely recommended, although it is not performed in all centers. More than one third of second primary tumors are detectable by endoscopy at or within 1 year of diagnosis of the index tumor.

Other Tests

Electrocardiography may be useful.

Procedures

Incisional biopsy, guided when appropriate by vital staining, is essential to confirm the diagnosis. A biopsy must be performed on any oral mucosal lesion suggestive of possible malignant changes, such as an ulcer that does not heal within 2-3 weeks. Lesional tissue must be obtained to allow histopathologic examination of the tissue. It is a good rule to obtain the tissue specimen from the worst-looking area. Always take a biopsy specimen of the red lesions if both red and white lesions are present because red, rather than white, areas are more likely to show dysplasia, or from the ulcer if the lesion is ulcerated. Vital staining maybe helpful if difficultly arises when deciding which area is most appropriate for the biopsy, particularly if widespread lesions are present. Staining with toluidine blue followed by a rinse with 1% acetic acid and then saline may stain the areas most suggestive of findings and indicate which need a biopsy. Oral carcinoma in situ and early invasive carcinoma have an affinity for toluidine blue dye, and although several false-positive results may be encountered, these can be minimized by restaining after 14 days. Toluidine blue clearly is more effective in experienced hands and when used with appropriate clinical judgment. Various light sources are becoming available to help delineate areas for biopsy, but it is most crucial to perform a direct oral examination under good light. When taking a biopsy specimen, ensure that sufficient tissue is obtained to avoid the need for a rebiopsy. Fix tissue biopsies in 10% formalin as soon as possible after the procedure. Avoid excisional biopsies unless the lesion is small because the procedure is unlikely to have achieved excision of an adequately wide margin of tissue if the lesion is malignant but will have destroyed clinical evidence of the site and the character of the lesion for the surgeon or radiotherapist. This can be avoided by tattooing the site, and/or taking a good quality photo prior to biopsy to be presented at your local tumor board with the final diagnosis. Lymph node biopsy is generally performed by an interventional radiologist should there be suspicion for involvement of regional lymph nodes. This is done using a fine-bore needle to aspirate cells for cytologic examination. Ultrasound-guided fine-needle aspiration cytology is now favored. False-negative results are possible, but the primary danger of a fine-needle aspiration biopsy is that it may seed malignant cells. In practical terms, ipsilateral, firm or hard, enlarged regional lymph nodes in a patient with an obvious oral carcinoma are likely to include metastases.

Histologic Findings

Microscopic examination of oral squamous cell carcinoma (OSCC) reveals nests and islands of squamous cells invading the underlying connective tissue. In some of these tumor islands, there is aberrant keratinization, forming whorls of keratin within. The presence of aberrant keratinization is a feature of well-differentiated carcinoma. Occasionally, an endogenous foreign body giant cell reaction to the keratin from ruptured pearls occurs. Poorly differentiated OSCC consists of sheets of cells showing extreme pleomorphism, giant nuclei, and multiple and

bizarre mitoses and often is difficult to distinguish from other malignancies, particularly poorly differentiated lymphoma or melanoma. In this instance, immunocytochemical markers such as keratins, common leukocyte antigen, and melanoma-specific antibodies are indicated.

Staging

The 1993 American Joint Committee on Cancer tumor, node, metastasis (TNM) classification and staging of oral cancer is as follows:

Classification

Primary tumor, as follows:

- T0 - No primary tumor
- Tis - Carcinoma in situ
- T1 - Tumor 2 cm or smaller
- T2 - Tumor 4 cm or smaller
- T3 - Tumor larger than 4 cm
- T4 - Tumor larger than 4 cm and deep invasion to muscle, bone, or deep structures (eg, antrum)

Lymphatic node involvement, as follows:

- N0 - No nodes
- N1 - Single homolateral node smaller than 3 cm
- N2 - Nodes(s) homolateral smaller than 6 cm
- N3 - Nodes(s) larger than 6 cm and/or bilateral

Tumor metastasis, as follows:

- M0 - No metastasis
- M1 - Metastasis noted

Staging

Stage I is T1, N0, M0.
Stage II is T2, N0, M0.
Stage III is as follows:

- T3, N0, M0
- T1, T2, T3, N1, M0

Stage IV is as follows:

- T4, N0, M0
- Any T, N2 or N3, M0
- Any T, any N, any M

Treatment & Management

Medical Care

Combined clinics that include surgeons, oncologists, and support staff usually have an agreed treatment policy and offer the best outcomes. Oral squamous cell carcinoma (OSCC) currently is treated largely by surgery and/or irradiation, although few unequivocal controlled trials of treatment modalities have been conducted. Photodynamic therapy and chemotherapy have occasional applications, and there is an increased use of chemotherapy, including targeted therapy (discussed below). An immunochemistry study of nuclear accumulation of (phosphorylated signal transducer and activator of transcription (pSTAT3) in OSCC concluded that increased nuclear pSTAT3, found in 49 of 90 leukoplakias and 63 of 94 OSCCs, correlated with tumor state, nodal metastasis, and poor prognosis.

Radiotherapy

Advantages of radiotherapy include the facts that (1) normal anatomy and function are maintained, and (2) general anesthesia is not needed. Disadvantages mainly include the facts that (1) adverse effects are common; (2) cure is uncommon, especially for large tumors; and (3) subsequent surgery is more difficult and hazardous and survival is reduced further. Radiotherapy can be performed by external beam radiation (teletherapy), which is commonly accompanied by adverse effects, or interstitial therapy (eg, brachytherapy, plesiotherapy). Implants of iridium Ir 192 for a few days are often used, supplying a radiation dose equivalent to teletherapy but one that is confined to the lesion and immediate area. Plesiotherapy causes fewer complications but is suitable only for tumors that are smaller than 2 cm and located in selected sites.

Of short-term complications, the oral mucositis that invariably follows external beam radiotherapy involving the oral tissues or cancer chemotherapy can be the most distressing and may have a significant effect on quality of life. Occasionally, oral mucositis is so severe that cancer therapy needs to be curtailed. As many as 40% of patients can be affected. Longer-term complications of radiotherapy, such as dry mouth (xerostomia), loss of taste, osteoradionecrosis (ORN) (less commonly), and other problems also may be distressing. Radiotherapy also complicates further surgery, because in particular, the endarteritis impoverishes healing. Prevention and treatment of oral complications whenever possible are important and should be performed by an oncologic team including a dental practitioner and an oral hygienist.

Prevention and treatment planning before cancer therapy

Prevention of oral disease and careful treatment planning are essential to minimize oral disease and the need for, and possible adverse consequences of, operative intervention. Adults with malignant head and neck disease unfortunately often have poor oral hygiene and care and are poorly compliant with oral health care. Most (97%) need oral health care before radiotherapy or chemotherapy for cancer. Almost one third of patients need oral care before bone marrow transplantation. Extremely important, but often overlooked, is the need for psychosocial counseling; patients must be counseled carefully to ensure they can adjust, at least partially, to the complications of cancer therapy. Many patients undergoing head and neck cancer surgery, particularly of the neck, can have life-threatening postoperative complications. These can often be predicted by preoperative assessment using a specific activity scale questionnaire, an assessment of alcohol abuse, and a platelet count, because thrombocytosis identifies patients at risk for wound infection. Fruits and vegetables appear to offer some protective effect. The potential of topical gel formulations for local delivery of chemopreventive plant anthocyanins is being investigated.

Oral health and disease in cancer therapy

Complications of cancer therapy depend on the type of malignancy and location, the treatment modality used (ie, agents, sequencing, rate of delivery, dosage), and host factors. For example, the severity of oral mucositis following radiation therapy depends on the ionizing radiation used, the rate at which it is delivered, and the total dose given. Manifestations of cancer therapy may include mucositis and oral ulceration, infections, bleeding, pain, xerostomia, ORN, taste loss, trismus, and caries. These require prevention and management.

Mucositis

Mucositis can be induced either by chemotherapy or by radiotherapy. Mucositis appears from 3-15 days after cancer treatment, earlier with chemotherapy than with radiotherapy. Pain can be so intense that it interferes with eating and quality of life. Occasionally, therapy must be stopped for several days to allow healing. In addition to causing local pain and ulceration, mucositis can provide a portal for microbial entry and thus, can result in local and, sometimes, systemic infection. The acute mucosal reaction to radiotherapy results from mitotic death of cells in the epithelium. The cell cycle time of basal epithelial cells is approximately 4 days, and because this epithelium is 3-4 cells thick, radiation changes begin to appear at approximately 12 days after the start of irradiation, independently of the dose, fractionation, or radiation technique. Initial mucosal erythema is followed after a few days by the

appearance of a patchy fibrinous exudate. If a high dose of radiation is given over a short time, ulceration may supervene, with a thick fibrinous membrane covering the denuded surface. Surviving epithelial cells respond to radiation damage by dividing more rapidly; therefore, complete healing is the rule. The duration mucositis takes to heal depends on the dose intensity of the radiotherapy, but usually, healing is complete within 3 weeks after the end of treatment. Tobacco smoking delays resolution. Cytotoxic drugs, which have a selective action on cells in the mitotic cycle, kill regenerating epithelial cells; therefore, the simultaneous use of chemotherapy and radiotherapy results in more severe and prolonged mucosal toxicity. Frank oral ulceration may be a portal for infection and septicemia. Preventing or ameliorating mucositis may be possible by minimizing exposure to radiation and by taking active measures. For example, radiotherapy radically increases oral gram-negative enterobacteria and pseudomonads. The presence of gram-negative bacilli may contribute to the mucositis. In addition, these microorganisms release powerful endotoxins that themselves cause both systemic and local effects on the host. If gram-negative bacilli have a role in the etiology of irradiation mucositis, preventing, treating, or ameliorating mucositis may be possible by abolishing the gram-negative florae. Promising results have been reported in two clinical trials using polymyxin E and tobramycin applied locally 4 times daily. This regimen has not been evaluated fully for the treatment of existing irradiation mucositis. Oral hygiene should be maintained with brushing the teeth. Advise the patient to eat a soft, bland diet and avoid irritants such as smoking, spirits, or spicy foods. Topical analgesics (eg, aspirin, benzydamine, lignocaine, dyclonine, diphenhydramine) may provide symptomatic relief. Topical chlorhexidine gluconate and sucralfate may reduce the frequency and severity of mucositis.

Oral infections

Levels of *Streptococcus mutans, Lactobacillus* species, and candidal species significantly increase after radiotherapy. These changes are maximal from 3-6 months after radiotherapy, after which no further change or a partial return towards the baseline florae occurs. The frequency and severity of oral infections with virus, bacteria, and fungi significantly increase after cytotoxic chemotherapy and radiochemotherapy. The primary symptomatic viral infections affecting the mouth in patients with cancer include herpes simplex virus (HSV) and herpes varicella-zoster virus infections. Acyclovir remains the primary treatment, but new agents, such as famciclovir, penciclovir, sorivudine, foscarnet, and other agents, may be needed in cases of acyclovir resistance. Homeostatic microbial communities are protective in health by preventing or interfering with the colonization of exogenous pathogens (colonization resistance). When oral tissues are irradiated, colonization resistance is practically abolished, and alteration of the oral microflora occurs, with increases in yeasts and some gram-negative organisms. The possible role of yeasts in irradiation mucositis has garnered considerable interest because the number of candidal subspecies, in particular, appears to increase. Candidosis is the most common oral fungal infection in patients with cancer and may cause soreness and, occasionally, may be responsible for dissemination of infection. Xerostomia, dental prostheses, alcohol use, and tobacco smoking predispose patients to oral candidosis. A meta-analysis of numerous studies has shown the prophylactic value of clotrimazole or fluconazole.

Hyposalivation

Salivary tissue, particularly serous acini, is highly vulnerable to radiation damage, and the parotid glands are damaged most readily. A radiation dose as small as 20 Gy can cause permanent cessation of salivary flow if given as a single dose, and with the conventional treatments for oral carcinoma (60-70 Gy), a rapid decrease in flow occurs during the first week of radiotherapy, with an eventual approximate 95% reduction. Salivary flow begins to diminish. After 5 weeks of radiotherapy, the flow virtually ceases and rarely completely recovers. Both resting and stimulated salivary flow are inhibited. Nevertheless, the sensation of dryness of the mouth tends to diminish after a few months to a year, partly as a result of compensatory hypertrophy of unirradiated salivary glandular tissue. After 1 year, little further improvement occurs. The degree of hyposalivation depends on the degree of exposure of the salivary tissue. Hyposalivation occurs when the upper border of the radiation field is above the submental area, particularly when the parotid glands are involved. Partially irradiated glands have resultant higher flow rates than fully irradiated glands. Mantle, unilateral, and bilateral fields of radiation can be associated with a reduction in salivary flow of 30-40%, 50-60%, and approximately 80%, respectively. A high initial salivary flow rate is associated with higher flow rates after radiotherapy. Radiotherapy to the nasopharynx damages both of the parotid glands and causes severe and permanent hyposalivation. Radiotherapy to a salivary tumor may avoid the contralateral gland and not cause severe hyposalivation. Radiotherapy fields used in the treatment of oral cancer normally avoid at least part of the parotid glands; therefore, hyposalivation tends not to be as severe as it would be if both glands were irradiated in their entirety. Hyposalivation leads to discomfort and loss of taste and appetite. In addition to minimizing unnecessary glandular irradiation, stimulating the salivary glands prior to radiotherapy has been suggested as valuable for reducing glandular damage. The use of pilocarpine during radiotherapy has shown encouraging results. In hyposalivation, residual salivary tissue may be stimulated by gustatory or pharmacologic stimuli. Sugar-free chewing gum may be a useful stimulus, is inexpensive, and has no adverse effects. Drugs that may be effective particularly include various cholinergic agents such as pilocarpine, given as ophthalmic drops placed intraorally or as tablets, is effective in relieving symptoms and in improving salivation when used in doses of up to 5 mg administered 3 times daily. Individuals with dry mouth frequently sip water, particularly during eating, and they often need to keep water by their bedsides. Several saliva substitutes or mouth-wetting agents are currently marketed. Most contain carboxymethylcellulose, although some that contain animal mucins and some also contain constituents that may facilitate enamel remineralization. Some patients find these products useful, but clinical experience suggests that they are not always well accepted. Some studies have suggested that mucin-containing preparations are accepted better by patients and may promote the establishment of normal oral florae; however, when cost and convenience are taken into consideration, many patients prefer to simply sip water frequently or to use an aerosol pump of water. Advise xerostomic patients to avoid agents such as medications, tobacco, and alcohol that may further impair salivation.

Dental problems

Although periodontal disease is not usually a problem, patients who undergo cancer therapy may be predisposed to caries because of hyposalivation, foods with a high sucrose content, and, possibly, a shift to more cariogenic oral microflora. Several types of carious lesions have been identified, most involving the incisal edges and cervical areas. The direct effect of radiation on tooth structure is probably less than the indirect effect (eg, xerostomia). Patients must achieve a good level of oral hygiene before radiotherapy or chemotherapy commences. Dietary control and topical fluoride therapy are essential and must be continued for life. Fluoride is applied best to the entire surface of all teeth to have

the maximal protective effect. This is achieved best by providing custom-built carriers for each patient. A gel containing 1% sodium fluoride is put into the carrier and applied to the teeth for 5 min/d. Fluoride mouth rinses are also useful. Sodium fluoride mouth rinses with chlorhexidine diacetate may be particularly effective. Amorphous calcium phosphate preparations are also protective.

Loss of taste sensation

Patients receiving radiotherapy to the mouth invariably experience some disturbance or loss of taste sensation. The taste receptor cells are relatively radioresistant, and the mechanism of this loss of taste has not been elucidated. Xerostomia probably contributes because disturbance of taste is common after irradiation of the parotid glands. Taste loss can be a distressing symptom and contributes to poor nutrition in patients receiving radiotherapy. Fortunately, taste perception usually recovers slowly within a few months after the end of radiotherapy, although sometimes loss is permanent. Zinc sulphate may help improve taste sensation in some patients.

Osteoradionecrosis

ORN, although uncommon, is potentially the most serious oral complication of radiation therapy. Radiation results in thrombosis of small blood vessels; fibrosis of the periosteum and mucosa; and damage to osteocytes, osteoblasts, and fibroblasts. The damaged osteoclasts and osteoblasts survive until they attempt to divide, at which time mitotic death occurs. An individual bone cell may not divide for months or years after irradiation, or it may not divide unless stimulated by trauma. Therefore, a slow protracted loss of bone cells occurs after radiotherapy, with a consequent slowing of remodeling, which eventually may result in thinning and reduced bone strength. The mandible consists of more compact bone with a higher density than the maxilla; therefore, it absorbs more radiation than the maxilla. The predisposition to ORN occurs because the blood supply of the mandible in the age group that develops cancer is poor and is almost entirely via the periosteum (which also becomes less vascular). The maxilla, with its lower density and rich vasculature, is rarely the site of ORN. Various factors predispose patients to ORN, but generally the risk is greatest in the mandible, in higher radiation doses, fraction size, number of fractions, and when teeth are extracted after radiotherapy. Nevertheless, ORN also may occur unrelated to trauma. Depending on educational level, socioeconomic strata, and cultural habits, patients with oral cancer abuse alcohol and tobacco and may be in poor general condition, which together with poor nutritional status and oral hygiene, make them particularly prone to oral ulceration and ORN. In the United States, ORN is seen less frequently in medical centers where special prophylactic protocols are in place. In a study done by Morrish et al, the incidence of ORN appears to be directly related to the radiation dose. ORN is most unlikely with radiation doses below 65 Gy; in doses up to 70 Gy, the rate is 1.8%, and in doses higher than 70 Gy, the rate is approximately 9%. In modern series, 5-15% of patients who undergo radiotherapy to the head and neck region develop ORN. Radiation shields decrease the radiation dose received by the bone and minimize the risk of ORN. Because infection or trauma (including surgical intervention) may result in local infection, delayed healing, and ORN, these should be kept to a minimum. Dentate patients are at higher risk than edentulous patients for developing ORN; this may be because of possible infection from periodontal disease and trauma from tooth extraction. Tooth loss after high-dose irradiation is by no means inevitable, and the prevalence of bone necrosis is lowest if extraction can be avoided altogether. The only teeth that need to be extracted before radiotherapy include those that are not vital, need root filling or elaborate restorative techniques, or are associated with active periodontal disease. Extractions of these teeth should be performed atraumatically, the tissues sutured to promote rapid healing, and antimicrobial therapy instituted. All other teeth should be cleaned and restored before radiotherapy begins, and patients should be recalled on a regular basis for oral hygiene treatments by trained oral hygienists. If dental extraction is performed shortly after radiotherapy, when devascularization occurs in addition to damage to the osteoblasts, the risk of ORN is particularly high. The risk of ORN is less if dental extraction is performed well before radiotherapy, but, regardless, the risk remains as a consequence of the enhanced remodeling of bone that continues for some months after the extraction. Dental extractions typically are best performed judiciously and a minimum of 2-3 weeks before commencement of irradiation therapy. If surgery later becomes necessary in the management of malignant disease, irradiated tissue should be handled as gently as possible. The highest rate of mandibular ORN occurs in patients who have dental extractions immediately prior to radiotherapy or immediately after radiotherapy. Many authors agree that postradiation extractions should be avoided if possible. A conservative approach to the treatment of ORN is indicated because up to approximately 60% of cases of ORN resolve with conservative therapy. ORN is treated best in a progressive manner, depending on results and the healing of the lesion. Therapeutic approaches include local wound care, topical or systemic antibiotics, ultrasound, hyperbaric oxygen (HBO) therapy, and minor-to-extended surgery with reconstruction procedures. Meticulous oral hygiene is essential, including the use of 0.2% aqueous chlorhexidine mouthwashes after meals. Irrigate away debris and allow sequestra to separate spontaneously because any surgical interference only encourages extension of the necrotic process. Any sequestrum that becomes loose should be removed gently along with any sharp edges of spicules of bone. Antimicrobials are not especially effective because the tissues are avascular; therefore, prolonged treatment is necessary. Tetracyclines are useful because of their selective bone uptake, and a regimen of 250 mg of tetracycline 4 times a day for 10 days, followed by 250 mg twice daily continued for several months, is recommended. Add metronidazole at 200 mg 3 times a day in cases of severe infection or when anaerobes are implicated. HBO therapy also has been shown to promote healing. HBO therapy at 2-2.5 atmospheres of pressure for 1.5-2 h/d for up to 84 sessions is recommended. Adverse effects with HBO therapy are uncommon but include transient myopia, seizures, and otic or pulmonary barotrauma; the latter potentially results in air embolism. Concern has been expressed that HBO therapy may exacerbate a variety of autoimmune and immunosuppressive disorders and viremia, although little evidence supports this concern. Relative contraindications to HBO therapy include upper respiratory tract infection, chronic sinusitis, epilepsy, chronic obstructive airways disease, high fever, a history of spontaneous pneumothorax or thoracic or ear surgery, viral infections, congenital spherocytosis, and a history of optic neuritis. Untreated pneumothorax is the only absolute contraindication. Risks of HBO therapy may be minimized by a careful pretreatment assessment including chest radiography and electrocardiography. Some advise otolaryngologic and ophthalmologic assessment. Therapeutic ultrasound at a frequency of 3 MHz pulsed 1 in 4 at an intensity of 1 W/cm applied to the mandible for 10 minutes daily for 50 days also may effectively improve ORN. Surgical management also has played a role in the treatment of ORN and may include sequestrectomy, alveolectomy with primary closure, closure of orocutaneous fistulae, or hemimandibulectomy.

Surgical Care

The goal of surgery for oral squamous cell carcinoma (OSCC) is to

remove the primary tumor together with a margin of clinically normal tissue to ensure complete excision of malignant tissue. Surgery thus provides a one-stage definitive procedure, from which the patient normally recovers within 10-14 days. Although modern reconstructive techniques can produce good orofacial aesthetics and function, neither can be totally ensured. Cancer centers receive many patients with advanced disease, and many operations fail to remove the tumor completely, resulting in a poor outcome and recurrence of the tumor. Ensuring that the patient is as prepared as possible for the major surgery required, particularly in terms of general anesthesia, potential blood loss, and ability to metabolize drugs, is important. In addition, address any potential dental or oral problems preoperatively in order to avoid later complications such as osteoradionecrosis (ORN). Surgery provides complete tumor and lymph node excision. A full histologic examination can then be performed for staging purposes and to help predict prognosis and the need for adjuvant radiotherapy. Surgery also provides another option of treatment for radiotherapy-resistant tumors. Disadvantages primarily are perioperative mortality and morbidity, but modern techniques have significantly decreased these risks, as well as the aesthetic and functional defects. When OSCC is fatal, it almost always is either because of failure to control the primary tumor or because of nodal metastases. Death resulting from distant metastasis is unusual. Ablative surgery ideally excises the cancer with at least a 2-cm margin of clinically normal tissue. If at least one node has clinical signs of invasion, a reasonable presumption is that others may be involved and must be removed by traditional radical neck dissection. Functional neck dissections (modified to preserve the jugular, sternomastoid, or accessory nerve, while ensuring complete removal of involved nodes) have gained popularity. Moderate-dose radiotherapy occasionally is used to "sterilize" such necks. Reconstruction is tailored to the patient's ability to cope with a long operation and the risk of significant morbidity. For soft-tissue reconstruction, tissue often must be brought into the region to close the defect using split skin grafts or flaps. Local flaps (eg, nasolabial flaps) provide thin, reliable flaps suitable for repairing small defects. Distant flaps required to repair larger defects include the following:

- Free flaps: Microvascular surgery facilitates excellent reconstruction in a single operation using, for example, forearm flaps based on radial vessels, which are particularly useful to replace soft tissue, or those based on the fibula when bone is required.
- Pedicle flaps: Myocutaneous or osteomyocutaneous flaps based on a feeding vessel to muscle and perforators to the skin paddle (eg, flaps based on the pectoralis major, latissimus dorsi, or trapezius) may be used in a one-stage operation to replace skin, and because they also contain muscle, they have adequate bulk to repair defects and may be used to import bone (usually rib). Forehead or deltopectoral pedicle flaps, once the mainstay, required a 2-stage operation, replaced only skin, and relied on a tenuous blood supply.
- Hard tissue: Hard-tissue reconstruction ideally is performed at the time of tumor resection. Dental implants can be inserted at that point to carry a prosthesis. Bone is traditionally taken as free nonvascularized bone grafts from the iliac crest or rib but may survive poorly if contaminated or if the vascularity is impaired after irradiation. In such cases, or when a large defect is present, an osteomyocutaneous flap greatly improves the graft vascular bed. True free vascularized bone grafts (eg, fibula grafts) have great benefits but are time consuming and require considerable expertise. The benefits of bone grafting for maxillary defects are less certain, and maxillary reconstruction is usually with an obturator (bung), which has the advantage that the cavity can be readily inspected.

Specific complications from the surgery of OSCC may include infection and rupture of the carotid artery, salivary fistulae, and thoracic duct leakage (chylorrhea).

Complications

Local complications from the treatment of oral squamous cell carcinoma (OSCC) can be transient or chronic depending on disease stage and treatment. They include, among others, limitation of and/or restriction of tongue movements or limited mouth aperture due to surgery, which can be improved by regular exercise; mucositis; altered taste during the course of radiation, which usually resolves a few weeks after radiation treatment is over, and xerostomia during and after treatment, which can be helped by topical and/or systemic treatments as described under Medical Care. However, it is important to monitor patients during the course of treatment, preferable weekly, for yeast and/or bacterial infections, onset of osteoradionecrosis (ORN), and/or any other complications. Specific complications from the surgery of OSCC may include infection and rupture of the carotid artery, salivary fistulae, and thoracic duct leakage (chylorrhea).

Prevention

Instruct patients to minimize risk factors such as tobacco and alcohol use and to maintain a well-balanced diet, consuming fresh fruits and vegetables whenever possible. Furthermore, the risk of recurrence, and how to prevent it, should be clearly addressed.

Long-Term Monitoring

Schedule routine follow-up visits for oral squamous cell carcinoma (OSCC) patients. Depending on treatment provided, the OSCC patient is followed initially by otolaryngology oncology surgeon, radiation oncologist, and the oral medicine specialist, who monitors for recurrence and treats any adverse effects resulting from radiation or chemotherapy.

INTERPRETATION

THE A TO Z OF HISTOLOGICAL STAINS

With the use of stains and dyes, histology allows researchers to visualize particular tissue structures, chemical elements within cells, tissues and even microorganisms. The advent and evolution of histology follows that of microscopy as outlined in 'A (very) Short History of Histology'. Histology, which means 'tissue science' became an academic discipline in its own right in the 19th century, after the French anatomist, Bichat, introduced the concept of tissue in 1801. Karl Meyer, a German anatomist, however, was the first to coin the term "histology" in 1819. Historically, histologists relied on readily available chemicals. Although some older staining methods have since been abandoned because the chemicals proved to be toxic (YIKES!), many, which are still in use today, have stood the test of time. They have proven to be efficient, accurate, and less complex. BitesizeBio has already covered some of these staining procedures in depth. The aim of this article is to provide you with a brief overview of many of the available histological stains. Although by no means exhaustive, the table below gives a rundown of the top dyes and stains dominating today's world of medical/diagnostic histology.
Happy Staining!

20 Stains Table

	Name of the Stain	Specifically Stains	'Fun Facts'
1	4', 6-diamidino-2-phenylindole (DAPI)	Nuclei: blue	First synthesized in 1971 by Otto Dann's lab as a drug to treat trypanosomiasis. Selectively binds to double-stranded DNA, with no (or very little) cytoplasmic staining. Can be easily used as a counterstain for green or red fluorescent labels. Can be used to stain DNA in mammalian, bacterial, and metazoan cells.
2	Acid Fast	Rod-shaped bacteria: red-pink	The Ziehl-Neelsen approach for Acid Fast Red staining, which was first described in 1800s, is the most widely used. A differential stain used to identify acid-fast bacterial organisms, such as the members of the generaus Mycobacterium and Nocardia. Particularly important for in the diagnosis of tuberculosis.
3	Alkaline phosphatase	Pluripotent cells: red/purple	Universal stem cell membrane marker. Commonly used to screen colonies during early stages of the reprogramming workflow (the stain maintains stem cell viability). Can also be used as a negative selection tool at later stages to identify undifferentiated cells.
4	Bielschowsky Stain	Axons, plaque neurites, and tangles: black Plaque and vascular amyloid: generally brown to dark brown Background: yellow to brown	A silver staining method introduced by Max Bielschowsky, who improved the approach developed by Ramon y Cajal. It can be used to visualize nerve fibers. Routinely used to study Alzheimer's disease.
5	Congo Red	Amyloid fibrils: pale orange-red (apple green birefringence under polarized light)	Although it had been around for a while, German physician, Hermann Bennhold, was the first to discover that Congo red binds to amyloid, in 1923. Remains the 'gold standard' test by amongst diagnosticians to identify amyloid in in the tissues (most often in patients with Alzheimer's disease).
6	Gram Stain	Gram-positive bacteria: blue-black Gram-negative bacteria: red-pink	Developed by Hans Christian Gram in 1884. This stain classifies bacteria as either, gram-positive cells (e.g. Staphylococcus spp.), which usually has have a thicker peptidoglycan mesh, or gram-negative cells (e.g. Escherichia coli, Salmonella spp.), which usually haves a lipid-polysaccharide layer external to the peptidoglycans.
7	Grocott-Gomori's (or Gömöri) Methenamine Silver	Fungal elements: black (with sharp margins and cleared center) Background: light green	A 'broad spectrum' fungal stain, which is better than the PAS stain (see below) at detecting even degenerated and dead fungi. Particularly useful for staining carbohydrates. Used for general screening for fungal infections.

	Name of the Stain	Specifically Stains	'Fun Facts'
8, 9	**Hematoxylin and Eosin**	Nuclei: blue (hematoxylin) Endoplasmic reticulum: blue (hematoxylin) Elastic fibers: pink (eosin) Collagen fibers: pink (eosin) Reticular fibers: pink (eosin) Red blood cells: orange/red	Most popular 'general purpose stain' used for routine tissue preparation. Hematoxylin is extracted from the Haematoxylum campechianum tree; it was first used by CG Reichel in 1758, but only produced good results as a histological stain in 1865, when it was used in combination with alum by Böhmer. It binds and stains acidic structures. Eosin, a yellow-red dye, was synthesizsed by a Polish chemist, Heinrich Caro (who named it after the nickname of a girl he liked!). Emil Fischer, a German chemist, then worked on the novel compound first publishing a paper on Eosin Y (for 'yellowish') in 1875. Eosin binds and stains basic structures. Hematoxylin and eosin were used in combination by a chemist, Wissowzky, in 1876.
10	**Hoechst stain**	Nuclei: blue	Part of a family of blue fluorescent dyes developed by a German life sciences company, Hoechst AG. Hoechst dyes specifically binds to A/T-rich regions of double stranded DNA, with no (or very little) cytoplasmic staining. It is these dyes are less toxic and more cell-permeable than DAPI (see above).
11	**Luxol Fast Blue**	Myelin fibres: blue to blue/green Neurons: violet (when counterstained) Red blood cells: blue	Created by Heinrich Klüver and Elizabeth Barrera in 1953. AIt is a copper phthalocyanine dye that is soluble in alcohol and attracted to the bases found in the lipoproteins of the myelin sheath. Commonly used to detect (de)myelination in the central nervous system. Often accompanied by a counterstain (e.g. hematoxylin and eosin).
12	**Methylene Blue**	Nucleus and cytoplasm: blue	Cationic dye that binds to anions in the tissue, such as carboxylic acid, sulfuric acid, and phosphoric acid groups.
13	**Oil Red O**	Neutral triglycerides and lipids (frozen sections), lipoproteins (paraffin sections), and lipofuscin: Bright red	Introduced in 1926 by RW French Closely related to the Sudan dyes Used to demonstrate the presence of fats or lipids in fresh, frozen tissue sections. Also used in forensic pathology to enhance latent prints produced by oily fingers.
14	**Periodic-acid Schiff**	Glycogen and other carbohydrates: magenta Nuclei: blue Collagen fibers: pink	Useful for researching/diagnosing glycogen storage diseases or diseases of the basement membrane. Hematoxylin is typically used as a counter stain to visualizse other tissue elements. A light green counter stain is preferred when PAS is used to demonstrate fungal organisms.
15	**Perl's Prussian Blue**	Iron deposits: Blue or purple Other tissue components: red (when counterstained with neutral red)	Prussian blue was accidently developed in 1704 by a chemist, but it was first introduced as a histological stain by the German pathologist, Max Perls, in 1867. Important stain to identify patients with hemosiderin (type of iron-storage complex found inside cells) deposits, for instance in some liver diseases or hemolytic anemia.
16	**Sudan Black B**	Neutral triglycerides and lipids (frozen sections); some lipoproteins (paraffin-embedded sections): blue-black Nuclei: red	Similar to other synthetic Sudan stains (including Oil Red O). Usually tissue sections are counterstained (with hematoxylin/ nuclear fast red). Can also be used to stain myeloblasts, but not lymphoblasts.

	Name of the Stain	Specifically Stains	'Fun Facts'
17	**Toluidine Blue**	Mast cell granules and polysaccharides: violet Nuclei: blue Cytoplasm: blue Red blood cells: blue Collagen fibres: blue	Developed by William Henry Perkin in 1856. Basic dye that selectively stains acidic tissue components. It is also useful for staining thin sections of resin-embedded tissues for electron microscopy, in order to help with the orientation and visualization of samples.
18	**Trichrome**	Collagen, bone: green-blue Muscle, fibrin, cytoplasm: red Red blood cells: yellow or red Nuclei: dark red-black	Original trichrome recipe was formulated by pathologist, Claude L. Pierre Masson, in the early 1900s. Technique using three (acidic) dyes to produce different colouration of (basic) tissue elements. This stain is routinely used in diagnostic labs to evaluate liver diseases, such as cirrhosis. Various staining approaches exist, of which Masson's Trichrome and Gömöri's Trichrome are the most commonly used today.
19	**Verhoeff-van Gieson Stain**	Elastic fibers and cell nuclei: black (Verhoeff component) Collagen and muscle: Red (van Gieson component) Cell cytoplasm and other components: yellow	Ira van Gieson first described this staining procedure in 1889, which was modified by Frederick Herman Verhoeff in 1908. The Verhoeff component is an iron-hematoxylin stain, while the van Gieson component is a collagen-specific counterstain, which is comprised of picric acid and acid fuchsin. Used to validate the presence or absence of elastic fibers in tissues.
20	**Warthin-Starry**	Microorganism: dark brown to black Background: golden brown (due to lower concentrations of silver deposits)	First developed by American pathologists, Aldred Scott Warthin and Allen Chronister Starry, in 1920. Silver nitrate-based staining method Considered the best approach to detect Gram-negative organisms, such as small bacilli and spirochaetes.

TROUBLESHOOTING

H and E: Method, Tips and Troubleshooting

The complete method for H&E staining is contained in the tables below, but we'll take a look at each of the stages in turn and explain the process which should help you should you need to troubleshoot.

Dewax and rehydrate

The first stage in any histological staining or immunohistochemistry method is to dewax your sections on the slides. The paraffin needs to be removed to enable the aqueous solutions to penetrate the fixed tissue. This is usually done using either xylene or 'Histoclear'. After the wax is removed (typically in three separate 5 minute immersions in dewaxing solution), the tissue needs to be rehydrated which is done through a decreasing series of alcohol solutions. In these first stages of an H&E protocol (or any immunohistochemical method) there's not much to troubleshoot- you should ensure that most of the xylene is drained from the rack and slides before placing them in the 100% alcohol and also ensure that the slides are exposed to the air for the minimum amount of time between solutions. For the tap water stages of this protocol, I would recommend using a container within the sink with constantly running water- this helps to contain the excess stains from the slides which would otherwise stain the whole sink blue and pink!

Got the blues

As mentioned in the previous article, haematoxylin is used to dye the nucleus and binds to the histones with the aid of the metal salt, otherwise known as the 'mordant'. A five minute submersion in haematoxylin is standard- this relatively long time ensures that all of the available nuclear binding sites are saturated. This method of staining is known as 'regressive' staining. The tissue is incubated for a long period to allow for deliberate over-staining and then some of the stain is removed in a step called 'differentiation'. The other method is 'progressive' staining where the solution of haematoxylin may not be as concentrated and the tissue is checked at regular intervals until the desired intensity is reached. Personally, I have always used the former method as it's probably the easiest and in the end is less time-consuming as you don't need to keep taking the slides from the haematoxylin, submersing them in tap water and checking the intensity using a microscope. When you take your slides out of the haematoxylin, all will be blue! Initially, the nuclei will be deep purple/blue, but the differentiation stage will render the stained nuclei dark blue. If you over-differentiate, fear not! Most of the stages of the H&E method can be repeated and the final colour can be adjusted.

Differentiation and Bluing

To achieve the blue coloured nuclei, there are two stages. The first is the differentiation stage. After the tap water rinse, the slides are submerged for a short period of time in an acid-alcohol solution which removes the excess staining. At this stage, the nuclei will be very dark and deep purple in colour. To achieve the classic H&E blue colour, the slides are rinsed then submerged in a solution called 'Scotts Tap Water'. This second stage is known as 'bluing'. The use of tap water (as opposed to distilled water) is important in these stages and bluing can be achieved with tap water alone (depending on the intensity of the staining following the haematoxylin stage). Tap water is slightly acidic (pH of around 6.0 to 6.8), but this is more alkaline than the pH of the haematoxylin which tends to be around pH 2.7. Two to five minutes in running tap water will remove most of the excess mordant giving sharp blue nuclear staining.

Eosin

The aim of H&E staining is to achieve a contrast between the deep blue nuclei and red/pink of the other cellular components. Eosin is used to stain cytoplasm, muscle fibres and so on. This pink counterstain also helps to differentiate between nuclei and non-nuclear components in cells. Don't be tempted to over-stain at this stage- always err on the side of caution. Over-staining can result in cells which are not immediately easy to differentiate. You can remove a certain amount of over-stain in running tap water. Some methods suggest that Eosin is diluted in ethanol, but I would recommend a solution in water as the ethanol has the potential to remove the haematoxylin staining from the previous stages.

Method

This is a basic method for H&E staining in which I have split into two sections: the dehydration/rehydration section (for rehydration following the eosin/tap water rinse, simply follow Step 9 to Step 1 before mounting the slides).

Dehydration/Rehydration

Step	Solution	Time	Notes
1	Xylene	5 mins	These steps should be carried out in a fume hood or downflow bench
2	Xylene	5 mins	
3	Xylene	5 mins	Drain off excess xylene before (4)
4	Absolute ethanol	20 secs	
5	Absolute ethanol	20 secs	
6	Absolute ethanol	20 secs	
7	95% ethanol	20 secs	
8	80% ethanol	20 secs	
9	70% ethanol	20 secs	
10	Tap water	2 mins	Running tap water in a sink

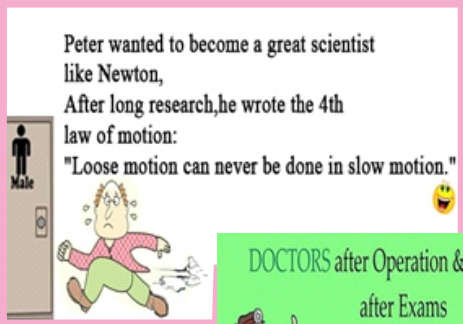
H&E Staining

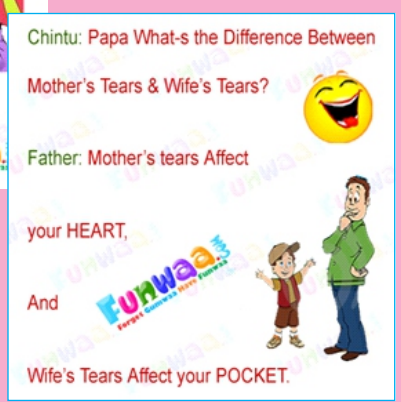
Step	Solution	Time	Notes
1	Harris' Haematoxylin	5 mins	Run control slides first to check times and staining.
2	Tap water	20 secs	Running tap water in a sink
3	1% Acid alcohol	5 secs	5 secs is maximum time in this solution
4	Scotts Tap Water	2 mins	Until tissue sections turn blue
5	Eosin	2 mins	
6	Tap water	20 secs	Running tap water in a sink, then check slides.

The nuclei in the cells should be dark blue whereas the cytoplasm will be pink. Fibrin and muscle will be deep pink and collagen will stain pale pink. Any erythrocytes will be red. Remember to allow any excess fluid to drain back into the staining dish before proceeding to the next step. Slides are mounted directly from the last xylene step.

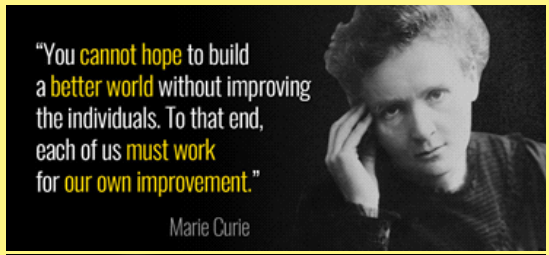
BOUQUET

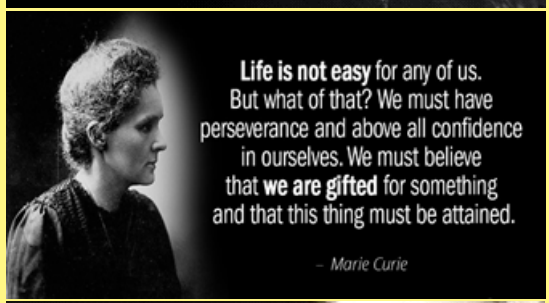
In Lighter Vein

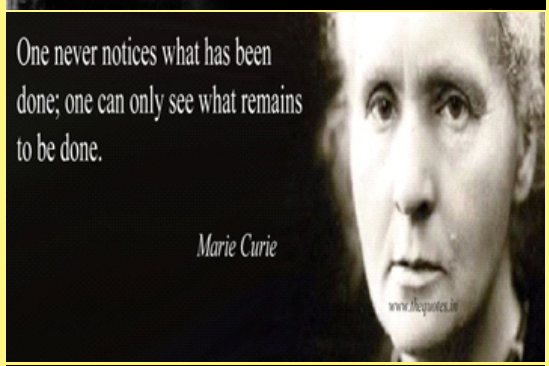


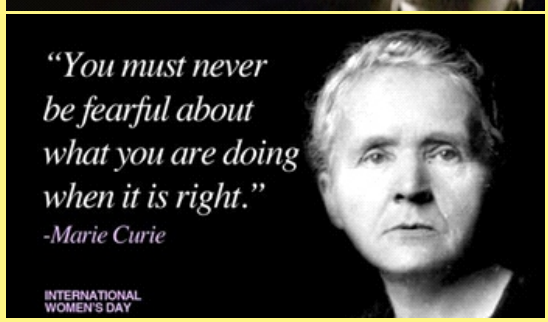


Wisdom Whispers









Brain Teasers

1. **Which test would diagnose typhoid at the earliest?**
   - A. Blood culture
   - B. Detecting IgM antibodies to S typhi "O" antigen
   - C. Widal test
   - D. Reduced Widal antigen test.
2. **Which of the following malarial enzymes is detected while diagnosing various malarial infection?**
   - A. pLDH
   - B. Aldolase
   - C. HRP II
   - D. All of the above.
3. **Which of the following malarial species is not responsible for causing malaria in humans?**
   - A. *P. vivax*
   - B. *P. falciparum*
   - C. *P. knowlesii*
   - D. *P. ovale.*
4. **What is not true about using pLDH as a diagnostic tool to detect malarial infections?**
   - A. Can be used as a screening tool for all malarial species
   - B. It detects only viable parasites
   - C. It detects dead parasites
   - D. Can be used as a screening tool in the blood banks.

ANSWER: 1. B, 2. D, 3. C, 4. C

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