REVIEW

Illuminating Tumor Diagnosis: A Comprehensive Exploration of Immunohistochemistry in Head and Neck Pathology

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Received : 1-01-2024
Revised : 19-01-2024
Accepted : 26-01-2024

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Abstract:

Introduction: Immunohistochemistry (IHC) is a valuable tool for diagnosis and characterization of oral tumors for their behavior, origin, and prognosis. This article takes an in-depth look at how immunohistochemistry (IHC) is used to diagnose oral tumors, starting with a simple explanation of how antibodies and antigens interact, and the different types of antibodies involved. It also covers the various labeling methods and techniques used in IHC step-by-step. In addition to that, it gives a brief history of IHC, showing how it has evolved from its early days to becoming an essential tool in modern biomedical research and diagnostics.

Discussion: The article explores the diagnostic utility of different markers within the head and neck region. It highlights the importance of classification and treatment planning facilitated by IHC. The article also addresses the limitations of IHC, including subjective interpretation and the need for standardization and expertise.

Conclusion: The article underscores the pivotal role of IHC in enhancing diagnostic accuracy, understanding tumor behavior, and guiding treatment decisions, especially in the realm of head and neck tumors. By elucidating the principles, methodologies, and clinical applications of IHC, this article serves as a comprehensive resource for researchers, pathologists, and clinicians involved in the diagnosis and management of oral tumors.

Keywords: Tumour, diagnosis, immunohistochemistry, head and neck tumour.

INTRODUCTION

Immunohistochemistry (IHC) is vital in diagnosing oral tumors, utilizing antibodies to detect antigens in tissues. It complements basic staining techniques and aids in understanding tumor behavior and origins. IHC protocols use chromogen-tagged antibodies analyzed under an optical microscope. Specific markers help identify neoplasm types and origins, enhancing diagnostic accuracy. It's applicable to formalin-fixed, paraffin-embedded, and frozen tissues, significantly improving microscopic diagnosis. While Hematoxylin and Eosin staining remain fundamental, IHC has become indispensable in diagnosing challenging tumors, providing insights into histopathogenesis and prognosis. Tumor classification by IHC relies on predictable antigen expression, aiding in distinguishing undifferentiated neoplasms. In summary, IHC is crucial for diagnosing, investigating, and understanding head and neck tumors.

Antigens provoke an immune response and are typically foreign proteins. They contain epitopes, which are the parts recognized by antibodies. Antibodies, produced by B-lymphocytes, can be soluble or membrane-bound. They bind specifically to antigens, with each antibody recognizing a distinct epitope. Antigenic sites (epitopes) can vary, allowing multiple antibodies to bind[1].

Antibodies, also known as immunoglobulins, are a type of gamma globulin protein. They have a Y-shaped structure composed of two light chains and two heavy chains linked by disulfide bonds[2].

Antibodies consist of identical heavy and light chains, each with constant and variable regions. The variable regions contain paratopes, which bind antigens. The constant region of the heavy chain has domains, including a hinge region found in IgD, IgG, and IgA, providing flexibility[3]. Antibodies can be divided into antigen-binding (Fab) and fragment crystallizable (Fc) regions. The Fab region binds antigens, while the Fc region interacts with Fc receptors on immune cells, activating the immune response[4].

Immunoglobulins are categorized into five classes (IgG, IgA, IgM, IgD, and IgE) based on the structure of their heavy chains[1]. Clonality in immunohistochemistry refers to the genetic identity of antibodies, which can be polyclonal or monoclonal[5]. Polyclonal antibodies are suitable for detecting low antigen levels but may increase background staining in IHC.[6,7] However, they are more robust with routinely processed tissues[8]. Monoclonal antibodies offer higher specificity but may lack robustness[7,8]. Cross-reactivity can occur between antigens and antibodies, particularly during antigen retrieval or with cross-species reactions6. Choosing primary and secondary antibodies from different species minimizes cross-reactivity[6]. Dilution of antibodies, specified in data sheets, ensures optimal experimental conditions[9].

Types of Antibodies:

1. **Polyclonal Antibodies:**

   Polyclonal antibodies react with various epitopes on the antigen against which they are raised. They are generated by different B-cell clones, each directed against various epitopes on the same antigen[7]. The antibodies are collected from several different B-cell clones activated by the immune system of the host animal, such as rabbits, sheep, goats, or rats. When the sera of the host animals are collected, it contains a heterogeneous mixture of high-affinity polyclonal antibodies.

2. **Monoclonal Antibodies:**

   Monoclonal antibodies, first described by Georges Kohler and Cesar Milstein (1975), are immunochemically identical and react with a specific epitope on the antigen against which they are raised. They are generated by a single B-cell clone, resulting in a homogenous population[7]. If the antibody-producing B-cell is fused with a myeloma cell, the unit is immortalized, providing a constant supply of antibodies. Monoclonal antibodies offer high specificity and are recommended for detecting specific antigens, single proteins, or staining cells with less background staining, making them ideal for use in IHC. Polyclonal antibodies, on the other hand, tend to have more non-specific reactivity and high variability from batch to batch.

Table 1: Differences between monoclonal and polyclonal antibodies

The antibody-antigen reaction relies on non-covalent forces, facilitated by a close proximity between interacting sites. A precise 3D fitting between antigen and antibody binding sites is crucial. Additionally, these non-covalent forces allow for reversible binding, enabling the dissociation of the antibody-antigen complex[10].

The equilibrium between epitopes and paratopes involves continuous association and dissociation.

Various factors, including temperature, pH, ionic strength, enzyme treatment, antibody and antigen concentration, antigen site density, and incubation duration, influence the reaction[11]. The strength of binding and affinity depends on the direction of the equilibrium, which can favor immune complex formation or dissociation into single components11. Temperature plays a significant role in affecting the equilibrium constant, with lower temperatures and longer incubation periods often resolving issues with antibody binding[11].

Principles of Immunohistochemistry:

The term "Immunohistochemistry" combines "Immuno" (Antibodies), "Histo" (Tissue), and "Chemistry" (Staining process). Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) through antigen-antibody interactions, with the site of antibody binding identified either by direct labeling of the antibody or by a secondary antibody labeling method[1].

Localization Principle:

The localization of antigens in tissue sections is achieved using labeled antibodies as specific reagents through antigen-antibody interactions, visualized by markers such as fluorescent dyes, enzymes, radioactive elements, or colloidal gold[1].

Labeling Methods:

• Enzyme Labels:

- Enzymes like Horse-Radish Peroxidase (HRP), Alkaline Phosphatase, Glucose oxidase, and β-D-

Table 1: Differential diagnosis of Anaplastic tumors

Origin	Cytokeratins (AE1/3, MNF116, Cam 5.2)	Melan A, S-100, HMB 45	Vimentin (low specificity)	Lymphoid markers (CD 45)
Epithelial origin/carcinoma	+	-	Some are positive	-
Sarcoma	**+/-**	**+/-**	+	-
Hematopoietic/lymphoid	-	-	-	+
Melanocytic	-	+	-	-

Galactosidase are commonly used. The selection depends on factors such as availability, stability, and interference with staining[3].

- HRP, commonly used due to its stability and small size, generates colored products upon oxidation[3].
- Examples of chromogens include 3,3' DiAminoBenzidine Tetrahydrochloride (DAB), 3 Amino-9-EthylCarbazole (AEC), 4 Chloro 11 Naphthol (CN), and p phenylenediamine dihydrochloride/pyrocatechol[3].
- Alkaline Phosphatase hydrolyzes naphthol phosphate esters to produce insoluble, colored azo dyes[3].

• Fluorescent Labels:

- Fluorescein, Rhodamine, Texas red, and Phycoerythrin (PE) are commonly used fluorochromes with various absorption and emission properties[3].

• Radiolabels:

- Radioisotopes serve as tracers and require autoradiographic facilities, primarily used in quantification studies[3].

• Colloidal Metal Labels:

- Colloidal gold and silver can be used, with colloidal gold widely used in electron microscopy[3].

Historical Context:

The principle of Immunohistochemistry has been known since the 1930s, with the first study reported by Coons et al. in 1942, where they identified pneumococcal agents in infected tissue[12]. Today, Immunohistochemistry is widely utilized for localizing antigens in biological cells or tissues, aiding in disease diagnosis, drug development, and biological research[13]. Antibodies, with their high selectivity in recognizing antigens, are essential tools for Immunohistochemistry. Synthetic antigens resembling naturally occurring proteins are injected into host animals (e.g., rats, sheep, goats), prompting the production of antibodies against them[14].

IMMUNOHISTOCHEMICAL METHODS

I. Traditional direct technique

II. Two step indirect technique

Polymer chain two step indirect technique

Unlabeled antibody-enzyme complex techniques

III. Immunogold silver staining technique (IGSS)

IV. (Strept) avidin-biotin techniques

V. Hapten labeling technique

Biotinylated tyramide signal amplification

Biotin free catalysed signal amplification (CSA II)

I. Traditional Direct Technique: In this method, the primary antibody is directly conjugated to a label, which could be a fluorochrome or an enzyme. The labeled antibody then directly reacts with the antigen. While this technique is quick and easy to use, it lacks the sensitivity achieved by other techniques[15].

II. Two-Step Indirect Technique: This technique involves using a labeled secondary antibody directed against the immunoglobulin of the animal species in which the primary antibody was raised. Commonly, horseradish peroxidase (HRP) labeling is used. This method is more sensitive as multiple secondary antibodies may react with different antigenic sites on the primary antibody, thus increasing signal amplification.

Polymer Chain Two-Step Indirect Technique: In this method, an unconjugated primary antibody is used first, followed by a secondary antibody conjugated to an enzyme-labeled polymer dextran chain. This technique is biotin-free and does not react with endogenous biotin. It is proving useful for multi-color staining on single slide preparations.

Unlabeled Antibody-Enzyme Complex Techniques:

Peroxidase Antiperoxidase (PAP) Complexes: In this technique, the secondary antibody has two binding sites: one binds to the primary antibody, and the other binds to a rabbit Peroxidase-anti-Peroxidase complex, as described by Sternberger in 1979.

Alkaline Phosphatase Antibodies: Antibodies raised in mouse against alkaline phosphatase can be utilized to form Alkaline Phosphatase-Anti-Alkaline Phosphatase complexes (APAAP)[16,17,18,19].

III. Immunogold Silver Staining Technique (IGSS): The use of colloidal gold as a label was introduced by Faulk and Taylor in 1971. This technique, applicable in both direct and indirect methods, is primarily utilized in ultrastructural immunolocalization but is not widely employed in light microscope immunohistochemistry[15]. Gold particles are enhanced

with metallic silver layers, producing a metallic silver precipitate overlaying the colloidal gold marker. While more sensitive than the PAP technique, IGSS may suffer from the formation of fine silver deposits in the background, which can be confusing when identifying small amounts of antigen[15].

IV. Avidin-Biotin Technique: Developed by Hsu et al. in 1981, this method enhances immunoenzyme techniques using high-affinity molecules avidin and biotin[20]. The binding of avidin to biotin is nearly irreversible, rapid, and strong, remaining unaffected by extremes of pH, temperature, or other denaturing agents[21]. Biotin, also known as vitamin B7 or vitamin H, is readily conjugated to antibodies and enzyme markers. Avidin, easily purified from chicken egg whites, contains about 10% carbohydrate, contributing to its high solubility but also potential nonspecific binding. Streptavidin, isolated from Streptomyces avidini, shares similar binding properties with avidin but exhibits lower nonspecific binding due to lack of glycosylation and a lower isoelectric point. Streptavidin-biotin techniques, the most widely used, involve adding biotinylated label and streptavidin together before use to form a fully complex. This technique increases sensitivity and allows for higher dilution of the primary antibody. Tissues rich in endogenous biotin may require avidin/biotin blocking before applying the primary antibody[22].

Immunohistochemistry (IHC) is a vital technique for identifying cellular or tissue constituents by means of antigen-antibody interactions, utilizing either direct labeling of antibodies or secondary antibody labeling methods. It involves localizing antigens in tissue sections using labeled antibodies, with markers such as fluorescent dyes, enzymes, radioactive elements, or colloidal gold. Enzyme labels, such as horse-radish peroxidase (HRP), are commonly used due to their stability and ease of purification. HRP, derived from the horseradish plant, is widely used in antibody labeling due to its small size and stability. Another commonly used enzyme label is alkaline phosphatase, which hydrolyzes substrates to produce insoluble, colored azo dyes.

Fluorescent labels like fluorescein and rhodamine are also widely used in IHC, offering distinctive emission spectra for visualization. Radiolabels, though less common due to their requirement for autoradiographic facilities, are used for quantification studies in IHC. Two-step indirect techniques involving labeled secondary antibodies are more sensitive, allowing for signal amplification by reacting with multiple antigenic sites on the primary antibody. Unlabeled antibody-enzyme complex techniques, such as peroxidase-antiperoxidase (PAP) complexes, offer additional options for IHC staining.

Immunogold silver staining technique (IGSS) employs colloidal gold as a label, enhanced with metallic silver layers, offering higher sensitivity for ultrastructural immunolocalization. However, it may suffer from background staining issues. The avidin-biotin technique enhances immunoenzyme methods using high-affinity avidin-biotin interactions, allowing for irreversible binding and signal amplification.

Biotinylated tyramide signal amplification, first described by Bobrow et al., offers highly sensitive detection systems in IHC. This technique utilizes biotinylated tyramide to enhance signal amplification, enabling the use of primary antibodies at greater dilutions. Biotin-free Catalyzed Signal Amplification (CSA II) systems, such as those offered by Dako, provide alternatives to reduce background staining associated with endogenous biotin.

Procedures for immunohistochemistry involve antigen retrieval, blocking steps, incubation with primary and secondary antibodies, introduction of substrate, nuclear counterstaining, and mounting. Antigen retrieval methods include proteolytic enzyme digestion and heat-mediated techniques, which help recover antigenicity masked by formalin fixation and paraffin embedding[1].

Various wash buffers are used to remove excess reagents during the staining process, including Tris Buffered Saline (TBS) and Phosphate Buffered Saline (PBS). Background staining issues in IHC are addressed through pre-staining steps and protein blocks to minimize nonspecific reactions[1].

Controls are essential in IHC to validate results, including negative and positive tissue controls, and internal tissue controls. Quantitative analysis in IHC involves counting labeled and unlabeled cells to determine labeling index. Immunohistochemistry finds wide applications in disease diagnosis, drug development, and biological research, offering valuable insights into cellular and tissue constituents[1].

However, IHC has limitations, including subjective interpretation, inability to detect small quantities of proteins, and the need for standardization and interpre-

tation expertise. Additionally, the absence of staining in poorly preserved tissues may lead to inconclusive results. Despite its limitations, IHC remains a powerful tool in biomedical research and diagnostics, providing valuable information about cellular components and disease pathology[1].

Table 2: Classification of diagnostic IHC markers

DIAGNOSTIC MARKER FOR EPITHELIAL TUMORS

Cytokeratins (CK) constitute a significant subgroup of epithelial-specific intermediate filament proteins and are the most abundant proteins in epithelial cells[23]. They exhibit site-specific and differentiation-dependent expression, with over 60 CK genes identified in the human genome, out of which fifty-four are functional[24]. CKs are categorized into Type I (acidic) and Type II (basic) cytokeratins, with varying immunoreactivities and charge characteristics[25]. The pan-cytokeratin AE1/AE3 antibody cocktail offers broad reactivity, targeting both low and high molecular weight cytokeratins across various epithelia[26,27].

Table 2: Differential tumors of Round cell tumors

Differential Diagnosis	CD 99	CD45	Cytokeratin	Desmin	Neural markers (CD 56)	Muscle markers (MyoD1)
Ewing sarcoma/ PNET	+	+	**+/-**	**+/-**	+	**-**
Neuroblastoma	-	+	**-**	**-**	+	**-**
Rhabdomyosarcoma	+	+	**-**	+	**-**	+
Lymphoma	+	-	**-**	**-**	**-**	**-**
Synovial sarcoma	+	+	+	**-**	**-**	**-**

DIAGNOSTIC MARKER FOR MESENCHYMAL TUMORS

Vimentin, traditionally associated with mesenchymal cells and tumors, is expressed in a variety of cells during early embryonic development and is a marker for mesenchymal tissues. It is commonly found in fibroblasts, smooth muscle cells, melanocytes, and endothelial cells[28]. Immunohistochemical staining for vimentin is observed in several sarcomas and some epithelial neoplasms, highlighting its diagnostic significance[29].

DIAGNOSTIC MARKERS FOR TUMORS WITH NEURAL DIFFERENTIATION

S100 protein, widely distributed in the nervous system, is utilized as a marker for Schwann cells, glial cells, and various histiocytes. Its expression pattern aids in the diagnosis of tumors with neural differentiation, including neurofibromas and neuroendocrine tumors[30, 31,32]. Glial fibrillary acidic protein (GFAP), an intermediate filament protein of glial cells, distinguishes glial hamartomas from non-glial lesions[33]. Additionally, neuron-specific enolase (NSE), a glycolytic enzyme, is identified in neuroendocrine cells and tumors, including neuroblastomas and malignant melanomas[34,35].

DIAGNOSTIC MARKERS FOR TUMORS WITH MUSCLE CELL DIFFERENTIATION

Desmin, an intermediate filament protein present in muscle tissues, serves as a marker for tumors with muscle cell differentiation, such as rhabdomyosarcomas and leiomyosarcomas[4]. Actin, a family of contractile proteins, is expressed in various tissues including skeletal muscle, smooth muscle, and myofibroblasts[36, 37, 38].

MyoD1, a regulator of muscle differentiation, is strongly expressed in alveolar rhabdomyosarcomas, aiding in their diagnosis[39]. Factor VIII-associated antigen (von Willebrand factor), CD-34, and CD-31 are markers for tumors with vascular cell differentiation, with varying expression patterns across different vascular tumors40. Histiocytic markers such as CD-68 are utilized in the diagnosis of histiocytic and presumed histiocytic lesions, providing valuable diagnostic information. These immunohistochemical markers play a crucial role in tumor diagnosis, aiding pathologists in accurate classification and treatment planning[41].

Myoglobulin, present in cardiac and skeletal muscles, is a protein that emerges during early muscle differentiation but is not sufficiently expressed in tumors. Studies have shown that myoglobulin is detected in only 50% of rhabdomyosarcomas[42,43,44].

DIAGNOSTIC MARKERS FOR TUMORS WITH VASCULAR CELL DIFFERENTIATION

Diagnostic markers for tumors with vascular cell differentiation include Factor-VIII-Associated Antigen (Von Willebrand Factor), CD-34, and CD-31 (Platelet-Endothelial Cell Adhesion Molecule). Factor VIII, a complex protein synthesized by endothelial cells, platelets, and megakaryocytes, serves as a reliable marker for endothelial differentiation. It is prominently expressed in normal endothelium, benign vascular tumors such as hemangiomas and pyogenic granulomas, as well as low-grade vascular tumors like epithelioid hemangioendothelioma and spindle cell hemangioendothelioma. However, its expression is notably low in angiosarcoma and absent in Kaposi sarcoma, although variable in lymphangioma and other types of hemangiomas. CD-34, expressed on hematopoietic progenitor cells, vascular endothelial cells, and dendritic cells, is another significant marker found in most benign vascular tumors and up to 80-90% of malignant tumors, including Kaposi's sarcoma. Its expression is also observed in various other malignancies such as fibrohistiocytoma, dermatofibrosarcoma, solitary fibrous tumor, malignant peripheral nerve sheath tumor, and epithelioid sarcoma. CD-31, a transmembrane glycoprotein, is expressed by endothelial cells and certain hematopoietic cells. It is universally present in benign vascular tumors and highly prevalent (80-100%) in angiosarcomas, with occasional expression in malignant mesothelioma, leiomyosarcoma, and carcinoma. Lymphangioma also exhibits variable expression of CD-31. Regarding histiocytic markers, CD-68, a lysosomal constituent, is expressed in various histiocytic, myeloid, and myelomonocytic malignant tumors, as well as in lymphomas of B-cell lineage. It is also detected in certain carcinomas and granular cell tumors, aiding in the identification of malignant fibrous histiocytoma and angiomatoid fibrous histiocytoma[33].

IMMUNOHISTOCHEMISTRY FOR DIAGNOSIS OF HEAD AND NECK TUMORS

Various immunohistochemical markers are used to identify tissue differentiation. For epithelial tumors, cytokeratin, integrin, filaggrin, involucrin, and desmosomal proteins are the primary markers. In

mesenchymal tumors, vimentin is commonly expressed. For melanocytic differentiation, S100, HMB-45, Melan A, and microphthalmia factor are used. Muscle-specific actin, desmin, myogenin, myoglobin, and MyoD1 are used for muscle tumors. Neural markers include S100, CD57, neurofilament, GFAP, and neuron-specific enolase. Vascular cell markers like Von Willebrand factor, CD-34, and CD-31 are crucial for identifying vascular differentiation, and histiocytes are identified by CD-68. Bone markers include alkaline phosphatase, type 1 collagen, type 5 collagen, BMP-2, BMP-7, and other factors related to bone differentiation.

ODONTOGENIC TUMORS[45,46]

For odontogenic tumors, CK14 differentiates odontogenic epithelial tumors from others, while amelogenin and ameloblastin are expressed in odontogenic tumors with odontogenic epithelial components. Nestin is a marker for odontogenic ectomesenchyme, and calretinin helps distinguish ameloblastoma from other tumors, including unicystic ameloblastoma from odontogenic cysts. Bone morphogenetic proteins and tenascin are expressed in tumors forming calcified masses.

Salivary gland tumors can be differentiated using several markers. Pan-cytokeratin (AE1/AE3) is expressed in both luminal and abluminal cells of the salivary glands. Epithelial membrane antigen (EMA) marks luminal cells, and carcinoembryonic antigen (CEA) also targets ductal luminal cells. Myoepithelial markers include α-smooth muscle actin, calponin, muscle-specific actin, p63, and CK14. Markers like S-100 protein, vimentin, and glial fibrillary acidic protein (GFAP) are used to identify myoepithelial cells, while cell proliferation and tumor prognosis are assessed using markers like Ki-67 (MIB-1) and p[53].

SALIVARY GLAND TUMORS[47,48,49]

In benign salivary gland tumors, markers like S-100, actins, and calponin are expressed. Warthin's tumor is positive for epithelial markers like AE1/AE3 and lymphoid markers (B cells: CD20; T cells: CD3). Myoepitheliomas show positivity for pan-cytokeratin, S-100 protein, vimentin, and p63, while basal cell adenomas express CK, EMA, and CEA. Other benign tumors like sebaceous adenomas and ductal papillomas also express epithelial markers like CK and EMA.

Malignant salivary gland tumors, such as mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, and polymorphous adenocarcinoma, exhibit specific marker patterns that aid in diagnosis. For example, acinic cell carcinoma is positive for CK, CEA, and vasoactive intestinal polypeptide, while adenoid cystic carcinoma expresses low molecular weight CK, CEA, and EMA, with Myb as a reliable marker.

MESENCHYMAL NEOPLASMS[52,53]

Markers are also used in diagnosing mesenchymal neoplasms. For example, gingival fibromatosis shows fibroblastic cells positive for vimentin, while angiofibromas are positive for vimentin and VEGF. Myofibromas are positive for vimentin and SMA but show limited expression of desmin. Hemangiopericytomas and solitary fibrous tumors are positive for CD34 and vimentin but negative for other common markers like CD31, actin, and keratins.

VASCULAR & PERIVASCULAR LESIONS[54, 55]

In vascular and perivascular lesions, markers like CD31, CD34, and anti-factor VIII are used for hemangiomas and lymphangiomas, while GLUT-1 and VEGFR-3 are sensitive markers for lymphatic-derived lesions. Malignant vascular tumors like Kaposi's sarcoma show positivity for HHV-8, aiding in diagnosis.

MUSCLE TUMORS[56]

For muscle tumors, rhabdomyomas express muscle-specific actin, desmin, myoglobin, myogenin, and MyoD1, while leiomyomas are positive for desmin, α-smooth muscle actin, and h-caldesmon. Rhabdomyosarcomas and leiomyosarcomas also express these markers, along with vimentin and other muscle-related proteins.

NEURAL ORIGIN NEOPLASMS[57]

Neural origin neoplasms express specific markers such as neurofilaments, S-100 protein, and synaptophysin for neurosarcoma and olfactory neuroblastoma. Malignant fibrosarcoma and lymphomas also exhibit characteristic marker patterns, such as CD45 for lymphomas and CD20 or CD19 for B-cell lymphomas.

DIFFERENTIAL DIAGNOSIS OF HEAD AND NECK TUMORS[58]

Table 1: Differential diagnosis of Anaplastic tumors

Table 2: Differential tumors of Round cell tumors

Table 3: Differential tumors of Spindle cell neoplasms

Conclusion

Immunohistochemistry (IHC) enhances diagnostic accuracy by assessing cell nature, differentiation status, proliferation, and protein expression, particularly in metastatic disease. It aids in identifying primary tumor sites, especially in sarcomas and lymphomas, improving classification and guiding therapy. Clear indication criteria optimize its use, particularly in head and neck tumors, aiding in accurate diagnosis and treatment decisions.

Conflict of interest: None
Source of support: Nil

Table 3: Differential tumors of Spindle cell neoplasms

Diagnosis	CD34	α- SMA	Desmin	S100	CK	CD99
Synovial sarcoma	-	-	-	-	+	+
MPNST	-	-	-	+	-	-
Leiomyosarcoma	-	+	+	-	**Sometimes positive**	-
Myofibrosarcoma	-	+	-	-	-	-
Fibrosarcoma	-	-	-	-	-	-
Spindle cell carcinoma	-	-	-	-	+	-
Malignant melanoma	-	-	-	+	-	-

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How to cite this article: Balla H, Uppala D, Kotina S, Yinti R. Illuminating tumor diagnosis: A comprehensive exploration of immunohistochemistry in head and neck pathology. J Oral Biomed Sci 2024; 3:24-35.