Protocol for the Examination of Specimens from Patients with Hematopoietic Neoplasms of the Ocular Adnexa

Protocol applies to primary hematopoietic neoplasms of the conjunctiva, orbital soft tissue, lacrimal gland, lacrimal drainage apparatus and eyelid. This protocol does not apply to intraocular lymphomas and secondary hematopoietic neoplasms.

Based on:

AJCC/UICC TNM, 8th edition
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None

Summary of Changes:

This protocol is revised to the 8th edition of the AJCC Cancer Staging Manual. This protocol was retired by CAP, September 2017.

Procedures Covered in this Protocol:

- Biopsy
- Resection

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The purpose of the Protocols is to support Laboratory Personnel engaged in the macroscopic examination of cancer resection specimens. The Protocols are based on specified relevant source documents, drafted by pathologists' assistant experts, and supported by information provided by the College of American Pathologists (CAP) and the American Joint Committee on Cancer (AJCC). These Protocols are intended to serve patients by ensuring that the macroscopic examination of cancer resection specimens is compliant with CAP Cancer Protocols, the AJCC Cancer Staging Manual, and provide optimization of the pre-analytic steps necessary to promote appropriate molecular studies.

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Molecular Considerations:

The detection of several specific genetic alterations gives both diagnostic and prognostic information and can also be used to aid in the detection of minimal residual disease.
The most common molecular techniques available include:

- Next-generation sequencing techniques
- Polymerase chain reaction (PCR)
- FISH

These examination elements will allow for a diagnosis, but if not processed cautiously, the tissue harvesting steps could adversely affect the pathologist's ability to work up the case.

- In the setting of bulky disease, submit fresh tumor in RPMI1640 or other suitable transport medium for flow cytometric analysis.

- In the setting of non-bulky disease where an inadequate amount of tissue is available for flow cytometric analysis, the specimen should be formalin fixed. If bone is present, separate and decalcify separately from remaining tissue (use caution when separating the bone as bone involvement is an element of staging).

Cytogenetic Analysis:

Fluorescence in situ hybridization (FISH) is important in the work-up and classification of hematologic malignancies but plays a limited role in the evaluation of lymphomas of the ocular adnexa. Namely, FISH may be performed primarily to assess for *IGH/BCL2* or *MALT1* rearrangements in the diagnostic workup for possible follicular lymphoma or extranodal marginal zone lymphoma, respectively.

Immunohistochemistry:

Immunophenotyping by immunohistochemistry is a critical tool in the evaluation of ocular adnexal lymphoma. In addition to its diagnostic utility, immunohistochemistry for Ki-67 is used to determine the proliferation index.

These tests can be performed on formalin fixed paraffin embedded tissue sections. The macroscopic description should provide the fixative used. 10% neutral buffered formalin is the preferred fixative. It is recommended that the duration of fixation be provided as well.

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol:**

  - Biopsy
  - Resection

- **Specimen Size and Extent of Resection:** *(Figure 1)*

  - Provide three dimensions of the specimen.
  - Document and measure organs/structures included:
    - Conjunctiva
    - Orbital soft tissue
    - Lacrimal gland
    - Lacrimal sac or nasolacrimal duct (lacrimal drainage apparatus)
    - Eyelid
    - Other (specify)

- **Specimen Integrity, Adequacy, and Macroscopic Features:**

  - Provide an assessment of the specimen integrity.
  - State the presence or absence of ulceration.
  - State whether the specimen has an exophytic, verrucous, fleshy, or infiltrative appearance, or if the specimen is pigmented.

  Statements should include, with as much clarity as can be provided, the anatomic location of the defects or disruptions, and should also incorporate statements that assess the relationship of any defects or disruptions to the tumor. Consultation with the surgeon for clarification should be considered, as well as differentially inking the areas affected by defects or disruptions as this may assist in creating a post-surgery treatment plan.

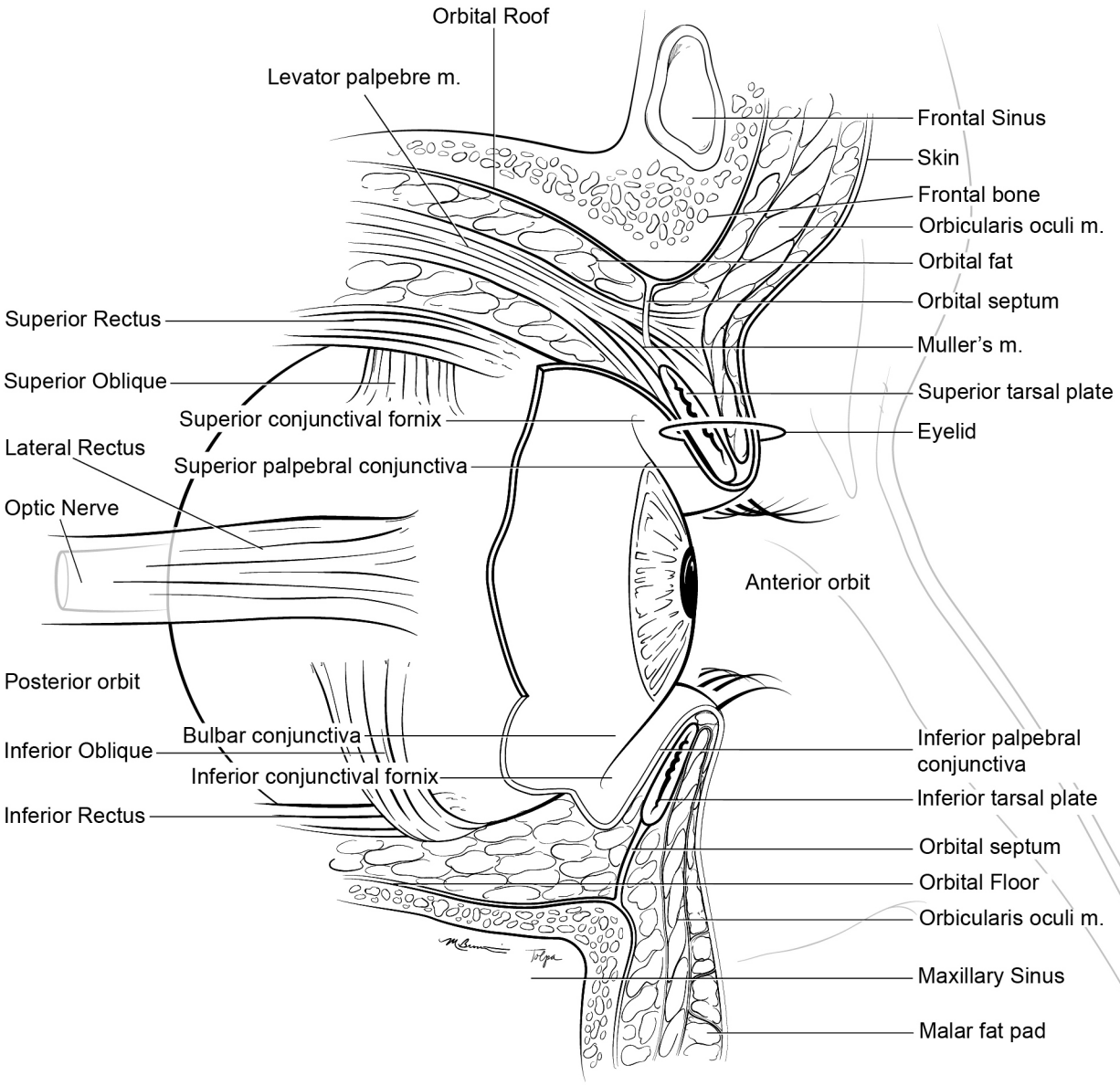

Figure 1: Ocular Adnexal Anatomy Sagittal Right Orbit

- **Ocular Adnexal Anatomy:**

The ocular adnexa include those structures surrounding the eyeball, which protect it from injury and facilitate its functioning. These structures include the conjunctiva (palpebral and bulbar), orbital cavity soft tissues, main and accessory lacrimal glands, nasolacrimal drainage system (including upper and lower canaliculi, lacrimal sac and nasolacrimal duct), and the

Conjunctiva

- Palpebral conjunctiva – Inner surface membrane of the eyelid
- Bulbar conjunctiva - Anterior surface of eye adherent to periphery of cornea
- Conjunctival sac – space between palpebral and bulbar conjunctiva
- Superior and inferior conjunctival fornices – recesses formed by reflection of the palpebral conjunctiva onto the eyeball
- Caruncle - located at the inner canthus and marks a transition zone between the conjunctiva and skin

Orbit

- The soft tissues of the orbital cavity posterior to the orbital septum in the eyelid including the extraocular muscles
- Orbital lymphoma is defined according to its location in relation to the equator of the globe
  - Anterior orbit - area between the orbital septum and equator of the globe
  - Posterior orbit - area posterior to the equator of the globe extending to the orbital apex
  - All lymphomas posterior to the orbital septum are considered to involve the orbit

Lacrimal drainage system

- Lacrimal glands secrete lacrimal fluid, which is drained by the lacrimal canaliculi into the lacrimal sac then into the nasal cavity by the nasolacrimal duct
- Main lacrimal gland - located in the superolateral part of the orbit
- Accessory lacrimal glands of Krause and Wolfring - located in the region of conjunctival fornices

Eyelid

- Composed of multiple layers (in order from outermost to innermost):
  - Epidermis
  - Dermis
  - Subcutaneous connective tissue (including):
    - Thin layer of adipose tissue
    - Orbicularis oculi muscle
    - Orbital septum
    - Levator muscle
    - Tarsal plate
    - Muller's muscle
    - Palpebral conjunctiva
  - Accessory eyelid structures include the plica semilunaris and the caruncle

TUMOR ("T" of TNM)

- **Tumor Size and Focality:**

  - Provide three dimensions of each tumor or maximum diameter of any visible lesion.
  - State if unifocal or multifocal.

- **Tumor Site(s):** *(Figure 2)*

  - Specify laterality of the lesion.
  - State the orientation of the lesion if provided by the surgeon.
  - Document the anatomic location the tumor:
    - Conjunctiva
      - Bulbar
      - Palpebral
    - Lacrimal gland
    - Orbit
      - Anterior
      - Posterior
    - Nasolacrimal drainage system
    - Eyelid
      - Upper
      - Lower
      - Medial canthus
      - Lateral canthus

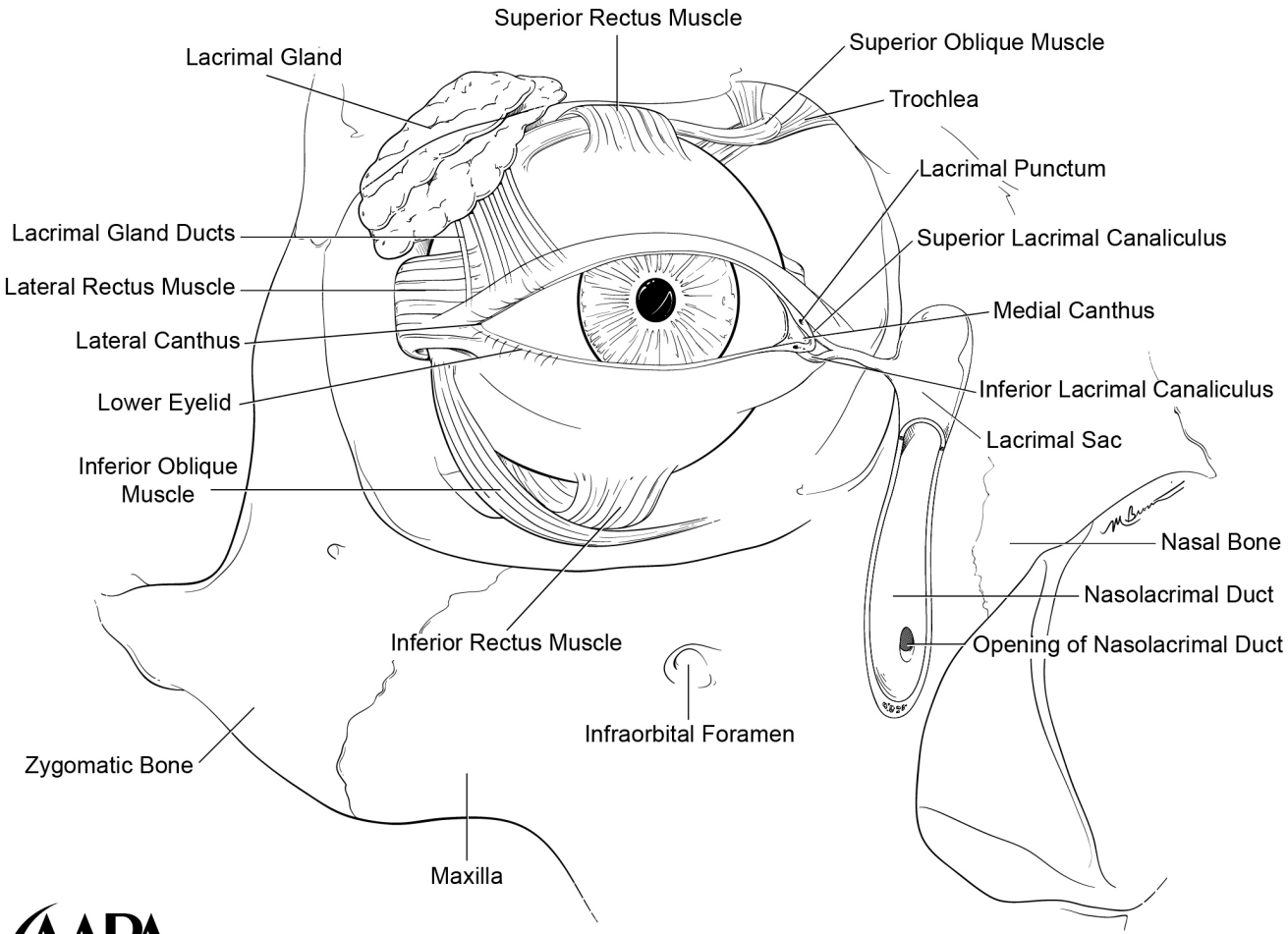

Figure 2: Ocular Adnexal Anatomy Anterior Right Orbit

- **Tumor Extent of Invasion and Relationship to Attached Organs / Structures:** *(Figures 3a and 3b)*

  The most significant prognostic factors are extent of invasion into surrounding tissues and metastatic spread. Eyelid involvement exists when the ocular adnexal lymphoma infiltrates preseptal tissues (dermis or orbicularis muscle of the anterior eyelid skin) or tissues anterior to the orbital septum.

  - Specify if tumor invades:
    - Nasopharynx
    - Paranasal sinuses
    - Intracranial structures
    - Bone

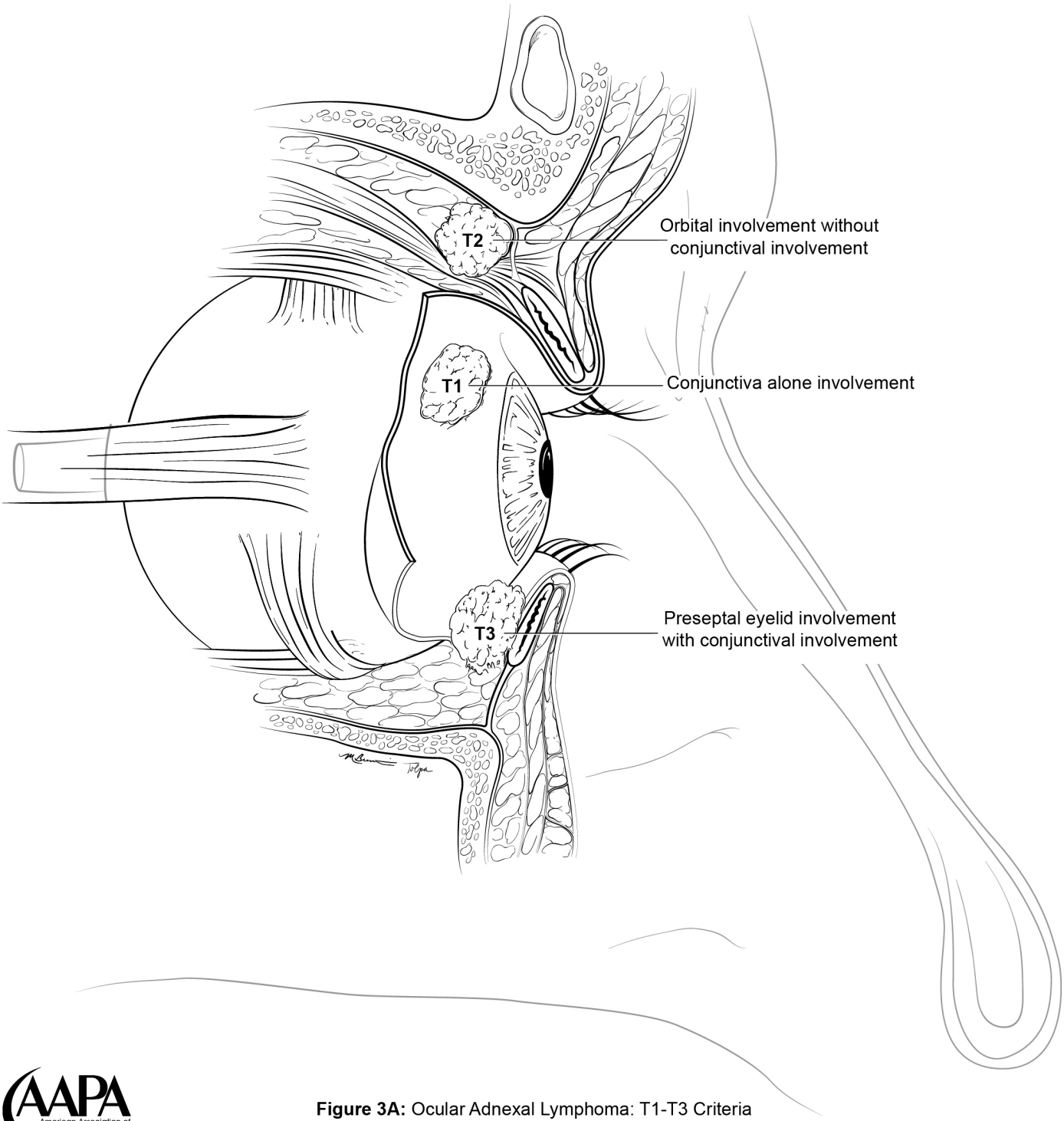

Figure 3A: Ocular Adnexal Lymphoma: T1-T3 Criteria

T1: Lymphoma involving the conjuctiva alone without eyelid or orbital involvement
T2: Lymphoma with orbital involvement with or without conjunctival involvement
T3: Lymphoma with preseptal eyelid involvement with or without orbital involvement and with or without conjunctival involvement

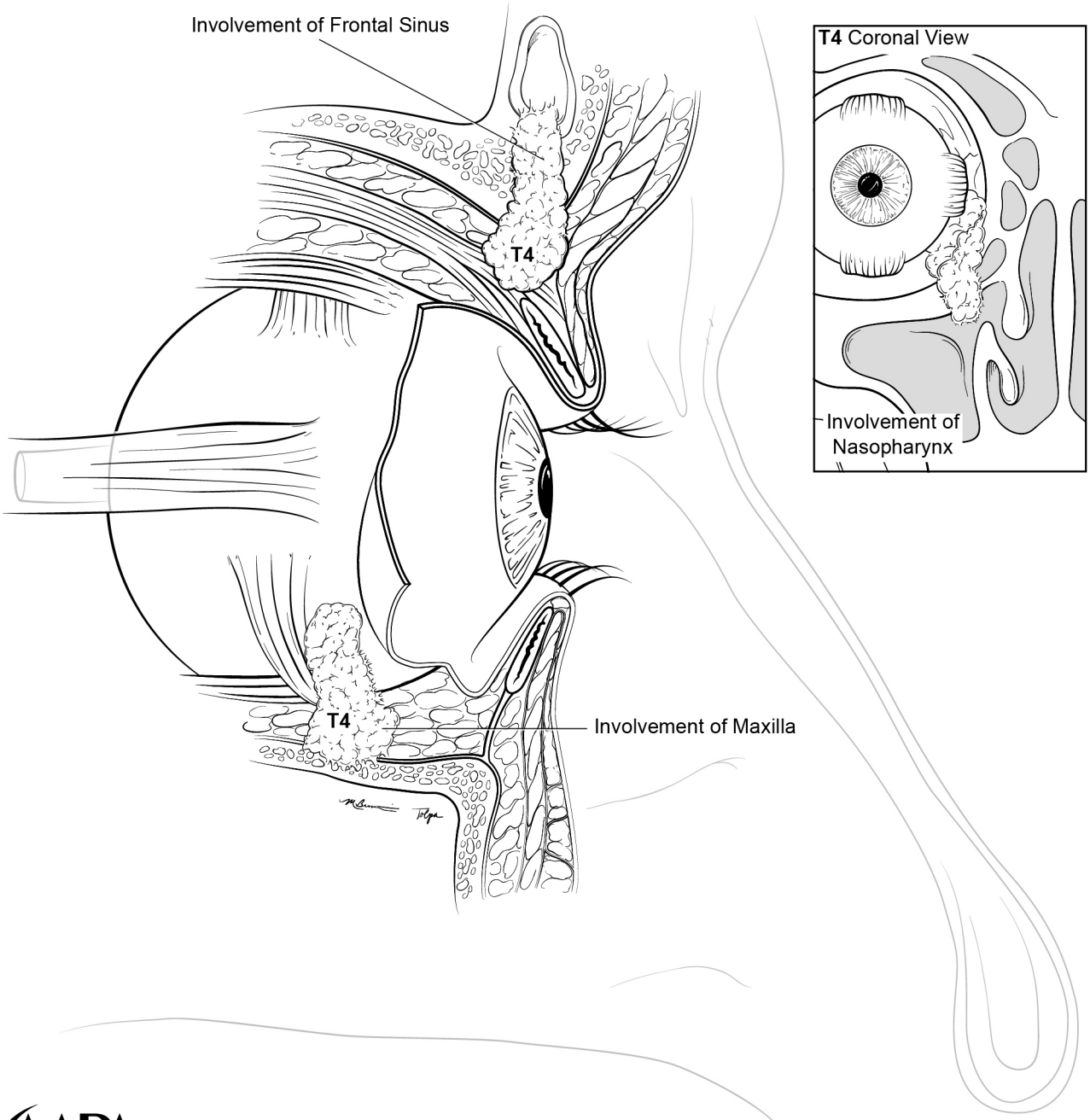

Figure 3b: Ocular Adnexal Lymphoma: T4 Criteria

T4: Orbital adnexal lymphoma and extraorbital lymphoma extending beyond the orbit to adjacent structures, such as bone, maxillofacial sinuses, and brain.

Definition of Primary Tumor (pT)

pT Category	pT Criteria
pTX	Lymphoma extent not specified
pT0	No evidence of lymphoma
pT1	Lymphoma involving the conjunctiva alone without eyelid or orbital involvement
pT2	Lymphoma with orbital involvement with or without conjunctival involvement
pT3	Lymphoma with preseptal eyelid involvement with or without orbital involvement and with or without conjunctival involvement
pT4	Orbital adnexal lymphoma and extraorbital lymphoma extending beyond the orbit to adjacent structures, such as bone, maxillofacial sinuses, and brain.

TNM Descriptors:

For identification of special cases of TNM or pTNM classifications, the "m" suffix and "y," "r," and "a" prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis. The "p" indicator refers to classification based on gross and microscopic exam.

The "m" suffix indicates the presence of multiple primary tumors in a single site and is recorded in parentheses: pT(m)NM.

The "y" prefix indicates those cases in which classification is performed during or after initial multimodality therapy (i.e., neoadjuvant chemotherapy, radiation therapy, or both chemotherapy and radiation therapy). The cTNM or pTNM category is identified by a "y" prefix. The ycTNM or ypTNM categorizes the extent of tumor actually present at the time of that examination. The "y" categorization is not an estimate of tumor before multimodality therapy (i.e., before initiation of neoadjuvant therapy).

The "r" prefix indicates a recurrent tumor when staged after a documented disease-free interval and is identified by the "r" prefix: rTNM.

The "a" prefix designates the stage determined at autopsy: aTNM.

The "b" prefix indicates bilateral lymphoma involving ocular adnexal structures.

Residual Tumor (R) Category

The absence or presence of residual tumor at the primary tumor site after treatment is denoted by the symbol R. The R categories for the primary tumor site are as follows:

R	R Definition
RX	Presence of residual tumor cannot be assessed
R0	No residual tumor
R1	Microscopic residual tumor
R2	Macroscopic residual tumor at the primary cancer site or regional nodal sites

- **Margins:**
    - **Conjunctival and Eyelid Biopsy:**
        - Provide a measurement to the closest margin (minimum clearance).
        - Some surgeons will identify surgical margins of interest by applying a suture to an edge of the specimen, by painting certain margins with dyes, or attaching the specimen to a piece of filter paper and making notations on the specimen mount.
            - Consider providing measurements to these additionally designated margins.

LYMPH NODES ("N" of TNM)

- **Lymph Nodes:**

Definition of Regional Lymph Node (pN)

pN Category	pN Criteria
pNX	Involvement of lymph nodes not assessed
pN0	No evidence of lymph node involvement
pN1	Involvement of lymph node region or regions draining the ocular adnexal structures and superior to the mediastinum (preauricular, parotid, submandibular, and cervical nodes)
pN1a	Involvement of a single lymph node region superior to the mediastinum
pN1b	Involvement of two or more lymph node regions, superior to the mediastinum
pN2	Involvement of lymph node regions of the mediastinum
pN3	Diffuse or disseminated involvement of peripheral and central lymph node regions

- **Regional lymph nodes include:**
  - Preauricular (parotid)
  - Submandibular
  - Cervical
- **Distant lymph nodes include:**
  - "Central" nodes, defined as those located in the trunk
    - Mediastinal
    - Para-aortic
  - "Peripheral" nodes refer to lymph nodes from distant sites not draining the ocular adnexa
    - Popliteal lymph nodes

- **Explanatory Note:**

Lymphatic drainage from the medial conjunctiva and medial eyelids is to the submandibular nodes with lateral areas of these tissues draining to the preauricular lymph nodes and then into the deeper cervical nodes.

Lymph Node Submission:

No lymph nodes exist in the ocular adnexa, however, if lymph nodes are encountered or submitted, follow the recommendations below:

- Specify site of lymph nodes identified or as submitted.
- Measure lymph nodes in three dimensions or provide a range in size of greatest dimension if multiple.
- Describe the cut surface of the identified lymph nodes.

- **For macroscopically positive lymph nodes**:
    - Give a precise size of the lymph node and tumor implant.
    - Representative sampling of these lymph nodes is adequate.
    - Sample areas where extranodal extension is seen or cannot be excluded.
    - If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.
    - Take steps to ensure that an accurate lymph node count can be rendered.

- **For small lymph nodes or macroscopically negative larger lymph nodes**:
    - Submit small lymph nodes in toto.
    - Section larger lymph nodes at 2 mm intervals and entirely submit.
    - Submit the entire capsule of the lymph node for possible extranodal extension.
    - If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.
    - If the lymph node is large but macroscopically negative, submit sequentially so that a size calculation can be established
    - Take steps to ensure that an accurate lymph node count can be rendered.

METASTASIS ("M" of TNM)

- **Metastasis:**

Definition of Distant Metastasis (pM) (required only if confirmed pathologically)

pM Category	pM Criteria
M0	No evidence of involvement of other extranodal sites
pM1	Evidence of involvement of other extranodal sites
pM1a	Noncontiguous involvement of tissues or organs external to the ocular adnexa (e.g., parotid glands, submandibular gland, lung, liver, spleen, kidney, breast)
pM1b	Lymphomatous involvement of the bone marrow
pM1c	Both M1a and M1b involvement

Specify site, if known.

The most common metastatic sites of ocular adnexal lymphoma are other extranodal tissues not contiguous with the ocular adnexa. These include organs such as the salivary glands, gastrointestinal tract, lung, and liver. Bone marrow infiltration may be micronodular, paratrabecular, or diffuse interstitial.

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