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# MORPHOLOGICAL EXAMINATION METHODS OF TISSUE MATERIAL OBTAINED FROM SURGERY FOR OTOSCLEROSIS

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***Abstract.*** *Otosclerosis is a pathological remodeling process of the endochondral bone of the inner ear capsule, often manifesting as fixation of the stapes footplate and conductive or mixed-type hearing loss. Clinical otosclerosis is more common in early adulthood, is noted relatively more frequently in women, and familial cases and bilateral involvement are observed in many cohorts. Surgical treatment, particularly stapedotomy and in some cases stapedectomy, allows not only for functional rehabilitation but also for the acquisition of tissue material for morphological analysis. Current literature indicates that the stapes suprastructure, footplate fragments, and, less commonly, larger otic capsule samples are important sources for morphological evaluation.*

***Keywords:*** *otosclerosis, stapedotomy, stapedectomy, morphological examination, histology, immunohistochemistry, electron microscopy, SEM, micro-CT, stapes footplate.*

**Introduction.** Otosclerosis is a localized bone disease of the human otic capsule in which the normal endochondral bone undergoes a pathological cycle of resorption and reossification. This results in stapes fixation, foci of dense or porous remodeled bone around the oval window, and occasionally cochlear spread. Clinically, it most often manifests as conductive or mixed hearing loss. The literature indicates that the prevalence of clinical otosclerosis in some Caucasian populations is approximately 0.3-0.4%, while histological otosclerosis is considerably more common than the clinical form. In a British cohort, women constituted 65% of cases, and a family history was noted in approximately 40% of patients; some reviews state that bilateral involvement can reach up to 80%. A population-based study in the USA showed that the incidence of the disease in the period after 2000 decreased significantly compared to previous decades. Sufficient standardized population data for Uzbekistan could not be found in open indexed sources, which further increases the local relevance of this topic.

Although audiological and radiological criteria are important in diagnosing otosclerosis, histological examination remains the gold standard for confirming the disease and assessing its true morphological scope. In particular, stapes material obtained during surgery helps determine the active or inactive stages of the disease, the inflammatory and vascular components in the tissue, and the degree of bone remodeling. Therefore, standardizing morphological methods in otosclerosis is of both scientific and practical importance.

**Objective.** The objective of this article is to analyze the methods for morphological examination of tissue material obtained from otosclerosis surgery, evaluate their informativeness, and propose a practical pathomorphological algorithm.

**Materials and Methods.** The main focus was on studies dedicated to surgically obtained material, the histology of the stapes suprastructure and footplate fragments, as well as immunohistochemical and ultrastructural methods that aid in a deeper interpretation of this material. Original research, cohort and case series studies, and methodologically significant reviews were included.

**Results and Discussion.** Types of surgically obtained material

The primary material obtained for morphological examination during otosclerosis surgery consists of the stapes suprastructure, stapes footplate fragments, and, in some cases, the entire stapes or adjacent bone fragments. Although the suprastructure removed during a stapedotomy is often regarded as "surgical waste," several studies have demonstrated its diagnostic and scientific value. Footplate fragments are particularly important for clinical-histological correlation, as this specific zone reflects the main fenestral localization of the otosclerotic focus.

Due to the small size, high mineralization, and fragility of the surgical material, the pre-analytical stage directly impacts the quality of the result. The literature recommends fixation in 10% buffered formalin, followed by careful decalcification. For example, one study on the stapes suprastructure used 24-hour fixation, followed by 12-hour decalcification in 4% formic acid, paraffin embedding, sectioning to a thickness of 3 μm, and staining with hematoxylin and eosin. Other works also describe fixation in buffered formaldehyde, decalcification, and standard H&E staining as the fundamental steps. This approach is considered sufficient to preserve the osteoid, osteocytes in lacunae, fibrous stroma, and inflammatory infiltrate within a small bone fragment.

Immunohistochemistry is crucial for elucidating the mechanisms of chronic inflammation, neoangiogenesis, and bone remodeling in otosclerosis. In surgical fragments, the use of CD3, CD4, CD8, and CD20 markers has shown that the main part of the infiltrate consists of T-lymphocytes, while B-lymphocytes are less common. Furthermore, staining with CD31 and CD34 has enabled the identification of hyperemic capillaries and newly forming small vascular networks. These findings provide a basis for viewing otosclerosis not merely as "passive sclerosis," but as a dynamic bone restructuring process involving inflammatory and vascular components.

Although extended molecular immunohistochemistry has been primarily performed on archival temporal bone materials, it defines the prospects for evaluating surgical material. Hodge et al. studied TGF-β1, bone sialoprotein, nidogen-1, collagen-IX, β2-laminin, and ubiquitin markers, demonstrating a more specific expression of TGF-β1 in otosclerotic tissue compared to control samples. From a practical standpoint, while a "basic panel" of markers like CD3, CD20, CD31, and CD34 is sufficient for a typical pathology laboratory setting, it is advisable to include the TGF-β signaling pathway and matrix proteins for scientific research.

Electron microscopy offers an additional advantage for studying the otosclerotic stapes at the ultrastructural level. Lim et al. described cellular, fibrotic, and sclerotic types in stapes specimens obtained during stapedectomy, using light microscopy, histochemistry, immunochemistry, and electron microscopy, and demonstrated that macrophage involvement is one of the early phenomena. Recent

SEM studies have shown load-related surface remodeling, microrelief changes, and articular surface topography in the head and suprastructure of the stapes.

Micro-CT, on the other hand, allows for obtaining a three-dimensional image without decalcification and sectioning. In a 2022 study, a 3D reconstruction of an otosclerotic cochlea was performed using micro-CT without decalcification, revealing intense tissue remodeling, capillary dilation, fibrosis, and spongiotic changes. Although this method has not yet been widely implemented for small surgical samples, it holds great promise as the next stage of morphological research.

At the same time, studies examining the stapes suprastructure indicate that classic signs of active otosclerosis are not always present in this zone, but subtle changes such as woven bone, cement lines, fibrous ligament, and cartilage degeneration can be detected. Therefore, the diagnostic value of each piece obtained during surgery is not uniform: the footplate fragment is of primary importance, while the suprastructure has more of an auxiliary and scientific significance.

**Table 1. Key Methods for Morphological Examination of Surgical Material in Otosclerosis**

| Method | Primary Objective | Detectable Features | Practical Significance |
|---|---|---|---|
| Light Microscopy | Baseline morphological assessment | Osteoid, osteon, sclerosis, fibrosis, infiltrate | Mandatory initial method |
| H&E staining | Viewing the overall tissue architecture | Osteoblasts, osteocytes, new bone, edema | Diagnostic minimum |
| Masson's Trichrome | Differentiating collagen and fibrosis | Fibrous stroma, cement lines | Useful for differentiating stages |

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|---|---|---|---|
| Giemsa | Identification of mast cells | Perivascular mast cells | Assessment of the inflammatory component |
| Immunohistochemistry | Cellular and vascular phenotype | CD3, CD4, CD8, CD20, CD31, CD34 | Determination of pathogenesis and activity |
| Electron Microscopy | Ultrastructure | Changes at the cellular and matrix level | In-depth scientific analysis |
| SEM | Surface topography | Microrelief, articular surfaces, remodeling | Useful in suprastructure analysis |
| SEM-EDS | Elemental composition | Ca, P, O, C and other elements | Indirect assessment of metabolic activity |
| Micro-CT | 3D morphology | Internal structure without decalcification | Experimental and translational method |

**Table 2. Key Morphological Features in Surgical Material and Their Interpretation**

| **Morphological Feature** | **Most Common Stage** | **Clinical Interpretation** |
|---|---|---|
| Cellular, spongy bone change | Otospongiotic | Likelihood of active remodeling |
| Fibrous stroma | Fibrotic | Intermediate stage or transitional state |
| Dense sclerotic trabeculae | Sclerotic | Relatively inactive or late stage |

| | | |
|---|---|---|
| Osteoid lined by osteoblasts | Active stage | New bone formation |
| Vascular proliferation | Active stage | Neoangiogenesis and metabolic activity |
| T-lymphocyte predominant infiltrate | Focus with an active/inflammatory component | Chronic immune-inflammatory involvement |
| Mast cells | Active/inflammatory component | Mediator activity of the microenvironment |
| Woven bone and cement lines | Reconstruction zone | Remodeling trace |
| Endosteal spread | Widespread focus | Risk of cochlear damage |

The table was compiled based on various histological and immunohistochemical studies.

**Conclusion.** Tissue material obtained during surgery for otosclerosis, particularly stapes footplate fragments, is the most crucial substrate for the morphological confirmation of the disease. The basic examination algorithm should consist of fixation in buffered formalin, careful decalcification, paraffin block preparation, H&E staining, and light microscopy. Masson's trichrome and Giemsa staining increase diagnostic accuracy, while immunohistochemistry reveals the inflammatory and neoangiogenesis components. SEM, SEM-EDS, and micro-CT methods are more useful for in-depth scientific analysis than in practical pathology. The most optimal approach is to supplement basic histology with targeted immunohistochemistry and, where necessary, ultrastructural methods. The histological conclusion achieves its maximum diagnostic value only when integrated with clinical and audiological data.

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