Integrating AI-Based Orbital Ultrasound with Tear Proteomic Biomarkers for Precision Diagnosis of Orbital Inflammatory Disorder (OID), Including Graves' orbitopathy (GO)

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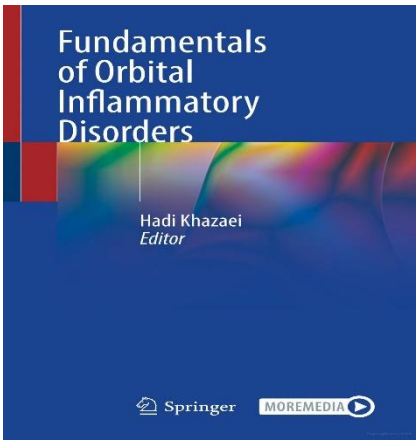

ABSTRACT

Graves' orbitopathy (GO) is a potentially blinding manifestation of Orbital Inflammatory Disorder (OID). Although characteristic clinical signs and imaging features (bilateral exophthalmos, extra-ocular muscle swelling) often guide diagnosis, there remain substantial diagnostic and prognostic challenges. Approximately half of patients with Orbital Inflammatory Disorder (OID) develop orbitopathy, but only a minority experience sight-threatening diseases. The inability to predict who will progress and the lack of biomarkers to distinguish inflammatory versus fibrotic stages hinder timely intervention. Recent research highlights two complementary advances: (1) tear proteomics can reveal disease-specific protein signatures and offers a non-invasive source of biomarkers; and (2) an AI-assisted orbital ultrasound proof-of-concept model has been developed, using Google Vertex AI Platform, that can differentiate orbital inflammatory disease (OID) from non-inflammatory orbitopathy (NIO) with high precision. This proposal aims to integrate tear proteomic biomarkers with AI-based orbital ultrasound to create a hybrid diagnostic workflow that enhances sensitivity, specificity, and prognostication in OID. Longitudinal tear sampling from OID, GD without orbitopathy and other inflammatory controls will be coupled with mass–spectrometry–based proteomic profiling and ELISA validation. Concurrently, an updated AI model will be retrained on new OID cases and used as a confirmatory imaging layer. The hybrid algorithm will cross-validate proteomic and imaging signals to provide a precision diagnostic framework for early detection and staging of OID.

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Understanding Orbital Inflammatory Disorders

Understanding Orbital Inflammatory Disorders Orbital inflammatory disorders encompass a diverse group of conditions characterized by inflammation in the tissues surrounding the eye. These disorders can lead to a myriad of symptoms, including proptosis, pain, diplopia, and visual impairment. While the etiology of OIDs can vary, encompassing autoimmune, infectious, and idiopathic causes, the heterogeneity of presentations often poses a challenge for clinicians in determining the most appropriate and effective treatment strategies.

The Role of Precision Medicine

Precision medicine in OIDs involves a comprehensive analysis of the patient's genetic makeup, molecular profiles, and other relevant factors to identify targeted therapeutic interventions. Genetic testing, advanced imaging techniques, and biomarker analysis play pivotal roles in this approach, allowing clinicians to pinpoint specific molecular pathways involved in the inflammatory process.

For instance, identifying specific genetic markers associated with autoimmune OIDs can guide the selection of immunomodulatory agents tailored to the patient's genetic predisposition. Precision medicine also enables the identification of potential side effects and helps predict treatment response, thereby minimizing adverse reactions and optimizing therapeutic outcomes.

Evidence-Based Approaches in Ophthalmology

The foundation of evidence-based medicine involves integrating the best available scientific evidence with clinical expertise and patient preferences. In the context of OIDs, this means relying on well-designed clinical trials, systematic reviews, and meta-analyses to inform treatment decisions.

Randomized controlled trials (RCTs) evaluating different treatment modalities, including corticosteroids, immunosuppressive agents, and biologics, have provided valuable insights into their efficacy and safety profiles. Understanding the level of evidence supporting each intervention is crucial for clinicians to make informed decisions about the most appropriate course of action for individual patients.

AI-assisted orbital ultrasound

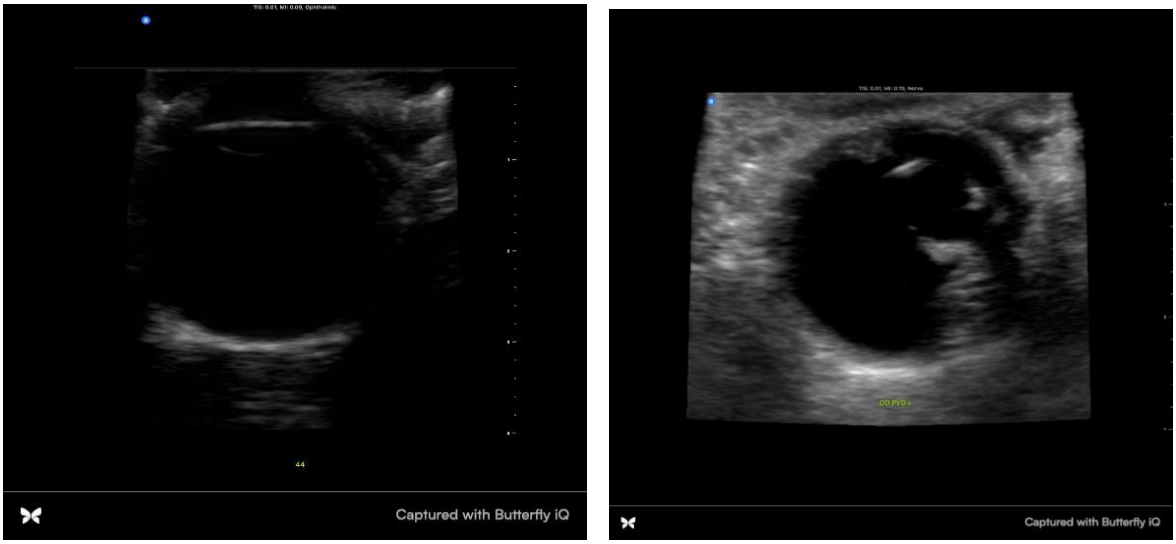


Figure 1: Normal/Abnormal Orbital Ultrasound Images.

Vertex AI, a cloud-based machine learning platform developed by Google, supports both customized model development and automated (AutoML) training approaches. In the "OIDvsNIO-NoAn" case study, a point-of-care B-scan ultrasound dataset was systematically curated to enable the differentiation of Orbital Inflammatory Disease (OID) from Non-Inflammatory Orbitopathy (NIO). A balanced dataset of 140 non-annotated images (112 Training, 14 Validation, 14 Test) was used; the AutoML classification trained model achieved a PR AUC of 0.986 with 92.9 % precision and 92.9 % recall. Notably, 100 % of OID images and 86 % of NIO images were

correctly identified, emphasizing high sensitivity for inflammatory disease. The study highlighted that balancing the dataset and removing annotation noise markedly improved performance. These findings show that AI-assisted orbital ultrasound has the potential to be a reliable tool for real-time confirmatory diagnosis of orbital pathology.

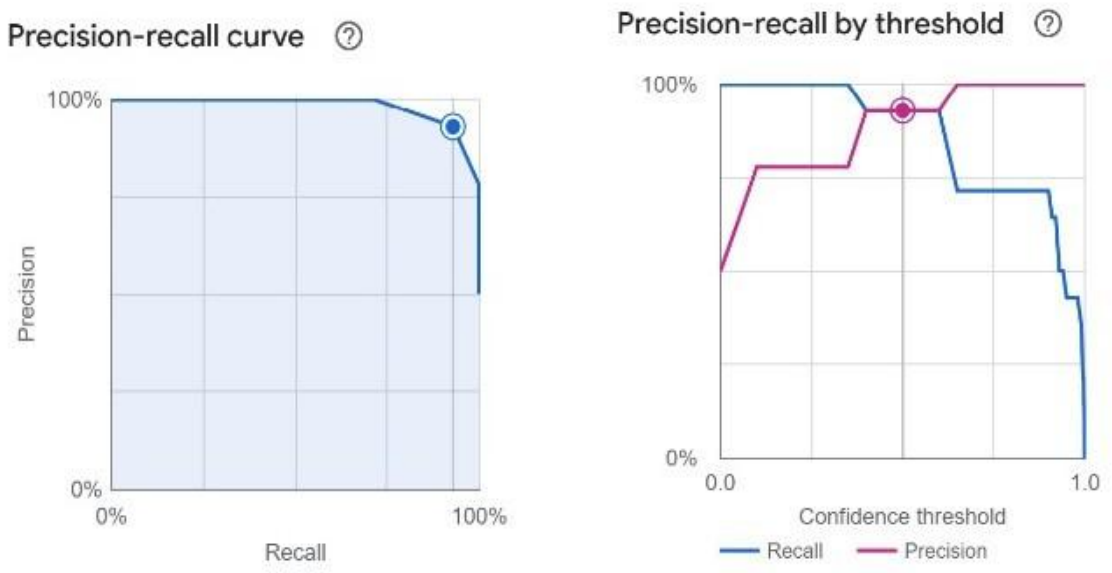


Figure 2: Precision-Recall Curve/ by Threshold OIDvsNIO-NoAn Project.

DISCUSSIONS

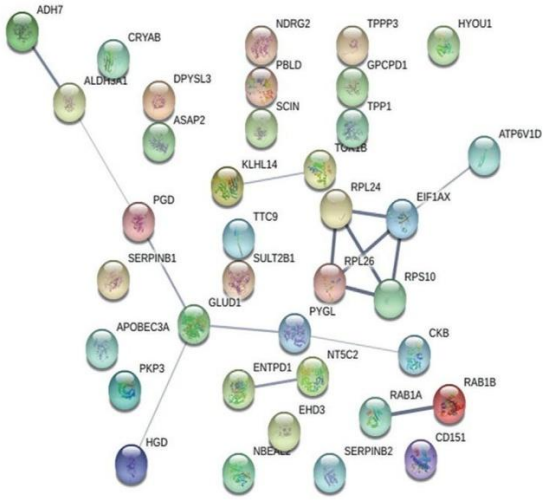
Despite advances in clinical assessment, imaging, and novel therapies, accurate diagnosis and staging of Graves' orbitopathy remain difficult. Current reliance on clinical features and serum thyroid-stimulating immunoglobulin (TSI) involves subjective grading and imaging that do not reliably capture molecular activity. No biochemical marker currently distinguishes the active inflammatory phase from the chronic fibrotic phase or predicts progression to vision-threatening disease.

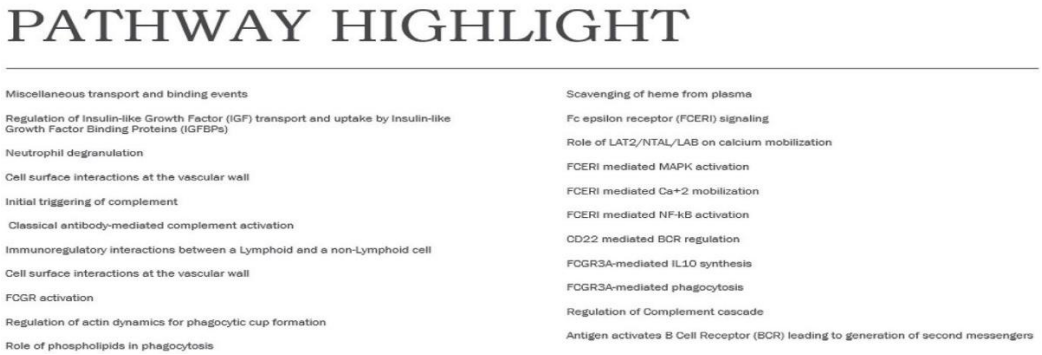
Imaging plays an essential role in assessment. Computed tomography (CT) and magnetic resonance imaging (MRI) can localize orbital changes and assess disease activity, but they are expensive and may involve radiation exposure or contrast administration. There is a lack of predictive biomarkers to identify which patients with OID may develop orbitopathy or transition from inflammatory to fibrotic phases. Meanwhile, AI models trained to distinguish OID from NIO have demonstrated high accuracy, but these models have yet to be applied to OID-specific cohorts or integrated with molecular biomarkers. Orbital ultrasonography offers a more accessible and safer alternative, though its interpretation can be highly operator-dependent and subjective. That's why we propose a combined approach to integrate tear proteomics to identify disease-specific proteins using AI-enhanced orbital ultrasound to assess inflammatory changes. This approach may offer a robust, non-invasive approach for diagnosing and staging Graves' orbitopathy. Using tear proteomics to detect disease-specific proteins and AI-driven orbital ultrasound to confirm inflammatory changes could provide a robust, non-invasive framework for diagnosing and staging OID. Such integration may enable precision medicine, matching therapy to disease activity, predicting progression, and reducing unnecessary interventions.

RESEARCH & METHODOLOGY

Tear Proteomic Biomarkers Validation

Validate tear proteomic biomarkers for differentiating OID from other orbital inflammatory diseases. Conduct longitudinal mass spectrometry profiling of tear samples from patients with GO, Graves' disease without orbitopathy, and control groups, including sarcoidosis, IgG4-related orbitopathy, granulomatosis with polyangiitis, Sjögren's syndrome, and nonspecific orbital inflammation. Identify individual proteins or protein panels that distinguish GO from these conditions and evaluate their correlation with clinical activity scores.





Proteomic Analysis and Quantification of Human Tears in Graves Orbitopathy (GO)…

AI Imaging Integration as a confirmatory diagnostic layer for tear proteomic findings. Integrate an AI-based imaging model as a confirmatory diagnostic layer for tear proteomic findings. Retrain and refine the Vertex AI model training methods using newly acquired orbital B-scan ultrasound images from the study cohort. Assess the model's ability to differentiate GO from non-inflammatory orbitopathy and to evaluate disease activity or fibrosis. Use imaging outcomes to validate tear-based molecular signatures and provide anatomical confirmation.

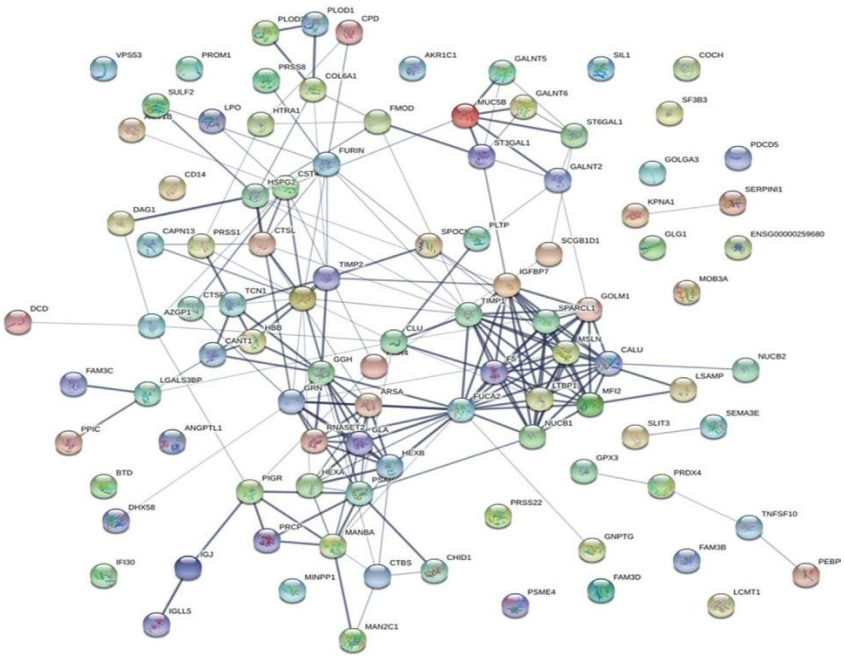


Figure 4: The data analysis flagged around 170 differential candidates between 6 pairs of OD-pinch (DPIN) and OS-proparacaine (SPRO) samples.

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Figure 5: Butterfly Handheld Ultrasound - Used in the OIDvsNIO-NoAn project.

Clinical Workflow

1. **Study design and participants:** Recruit three cohorts: (a) patients with clinically active GO; (b) patients with GD without orbitopathy; and (c) controls with other orbital inflammatory disorders (sarcoidosis, IgG4-related orbitopathy, GPA, Sjögren's syndrome, nonspecific orbital inflammation).
   Approach: Collect clinical activity scores, quality-of-life metrics, and imaging data at baseline and follow-up visits (e.g., 0, 6, and 12 months). Identify single proteins or multi-protein panels to differentiate GO from comparators.
2. **Tear sample collection and proteomic analysis:** Tear fluid will be collected using a standardized method (either Schirmer strips or cellulose sponges, per protocol) to ensure reproducibility and minimize sampling-induced variability in protein content. To control for technique-dependent biases, collection and extraction procedures will be carefully harmonized across centers. Extracted proteins will be labeled with tandem mass tags and analyzed by liquid chromatography-tandem mass spectrometry. Rigorous phased validation—including ELISA in independent patient sets and planned multi-center expansion—will bolster clinical adoption and generalizability. Only consistently differentially expressed proteins across replicates will be carried forward as diagnostic biomarkers.

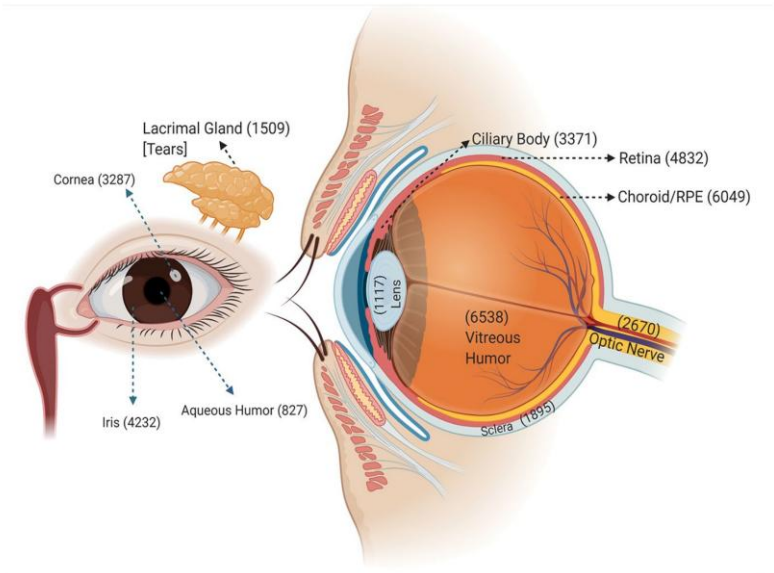

Figure 6: Tear protein digestion and tandem mass tag (TMT) labeling, mass spectrometric analysis,

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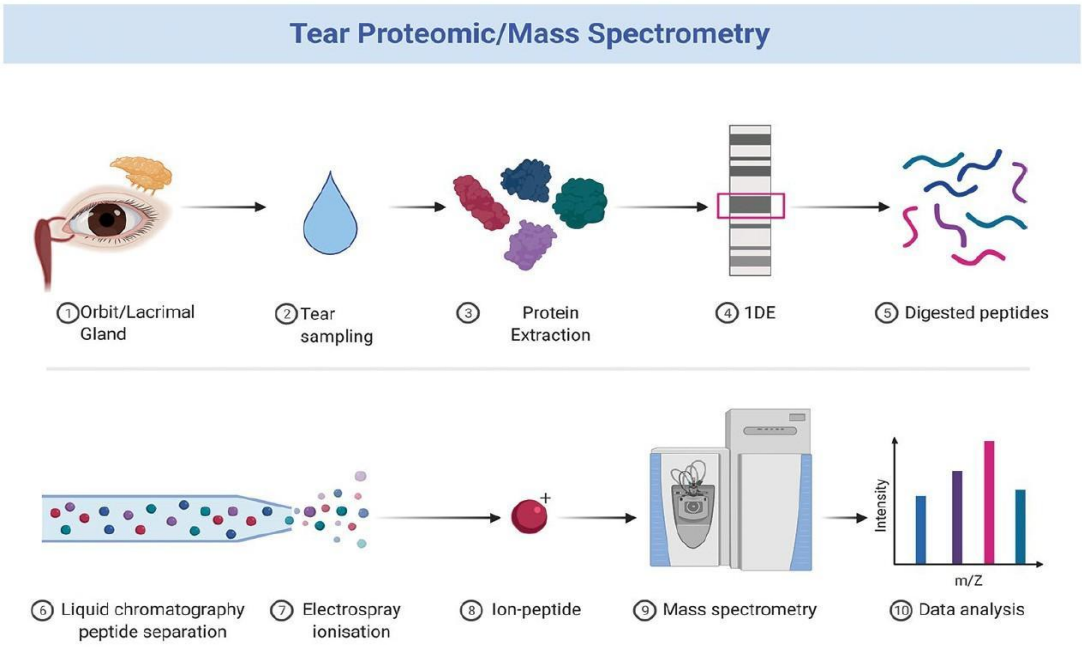

Figure 7: Tear protein digestion and tandem mass tag (TMT) labeling, mass spectrometric analysis.

3. **Orbital ultrasound imaging and AI model development:** Retrain and refine the Vertex AI model with new orbital B-scan images from the study cohort. using a portable handheld device analogous to the Butterfly iQ+ used in the OID vs NIO study. Images will include cross-sectional scans through the optic nerve and extra-ocular muscles. Data will be anonymized and uploaded to Vertex AI, a cloud-based platform designed for building machine learning models, for training model training methods to perform image prediction with minimal coding. Test the model's ability to distinguish OID from non-inflammatory orbitopathy.. Then differentiate active inflammation from fibrosis. Training, validation, and testing sets will be balanced, and hyperparameters optimized. Performance metrics (precision, recall, and PR AUC) will be compared with those reported for "OIDvsNIO-NoAn". The model will be evaluated for its ability to differentiate active OID from inactive OID and from other disorders.
4. **Hybrid diagnostic algorithm:** Develop a decision-support pipeline that integrates tear proteomic profiles (continuous variables or risk scores) with AI-derived imaging probabilities. Machine-learning techniques (e.g., logistic regression, random forest, or neural networks) will be employed to combine features. The primary endpoint will be diagnostic accuracy for OID; secondary endpoints include sensitivity to disease activity and prediction of progression. Cross-validation and external validation cohorts will ensure generalizability.
5. **Statistical analysis:** Proteomic data will be normalized and analyzed using multivariate statistics (principal component analysis, hierarchical clustering). Differential protein expression between groups will be assessed with false discovery rate control. AI-trained model performance will be assessed by confusion matrices, precision–recall curves, and ROC analysis. The hybrid model will be compared against individual modalities using the area under the curve (AUC) and decision-curve analysis.
6. **Ethical considerations:** Securing approval from an institutional review board and obtaining informed consent. Patient privacy must be protected through de-identification and secure data storage. Participants will be informed about the experimental nature of proteomic analysis and AI imaging. The process also includes registration on ClinicalTrials.gov, and compliance with GDPR/HIPAA standards for storage.

Anticipated Outcomes

1. **Identification of tear biomarkers:** We anticipate discovering a panel of tear proteins whose expression distinguishes GO from GD without orbitopathy and other inflammatory orbitopathies. These may include inflammatory mediators, extracellular matrix proteins, or immune signaling molecules.
2. **Validated AI imaging Model:** By training on new OID cases, the Vertex AI model is expected to achieve precision and recall comparable to the "OIDvsNIO" study (PR AUC ~ 0.986). We expect the

model to accurately detect active versus inactive OID, identifying inflammatory changes such as muscle swelling while distinguishing non-inflammatory conditions.

3. **Hybrid diagnostic workflow:** Combining tear proteomic signatures with AI imaging probabilities should improve diagnostic sensitivity and specificity over either modality alone. The algorithm will generate a composite risk score and provide staging information (inflammatory vs fibrotic). We anticipate that this framework will detect subclinical OID before overt clinical signs and better predict disease progression.

Significance and Innovation

This study integrates two advanced diagnostics—omics-based tear biomarkers and AI-assisted orbital ultrasound—to meet an unmet clinical need in OID. Tear sampling is noninvasive and reflects ocular pathology, enabling early detection and personalized monitoring. Validating tear proteomic markers and correlating them with clinical activity addresses the lack of predictive indicators. AI-based orbital ultrasound is portable and radiation-free, improving accuracy in distinguishing inflammatory from noninflammatory disease. Together, these methods enhance diagnostic confidence, reduce misclassification, enable precise staging, and support earlier treatment, better selection for emerging therapies (such as IGF-1 receptor inhibitors), and robust response monitoring. The approach is adaptable to other orbital inflammatory diseases and advances precision ophthalmology.

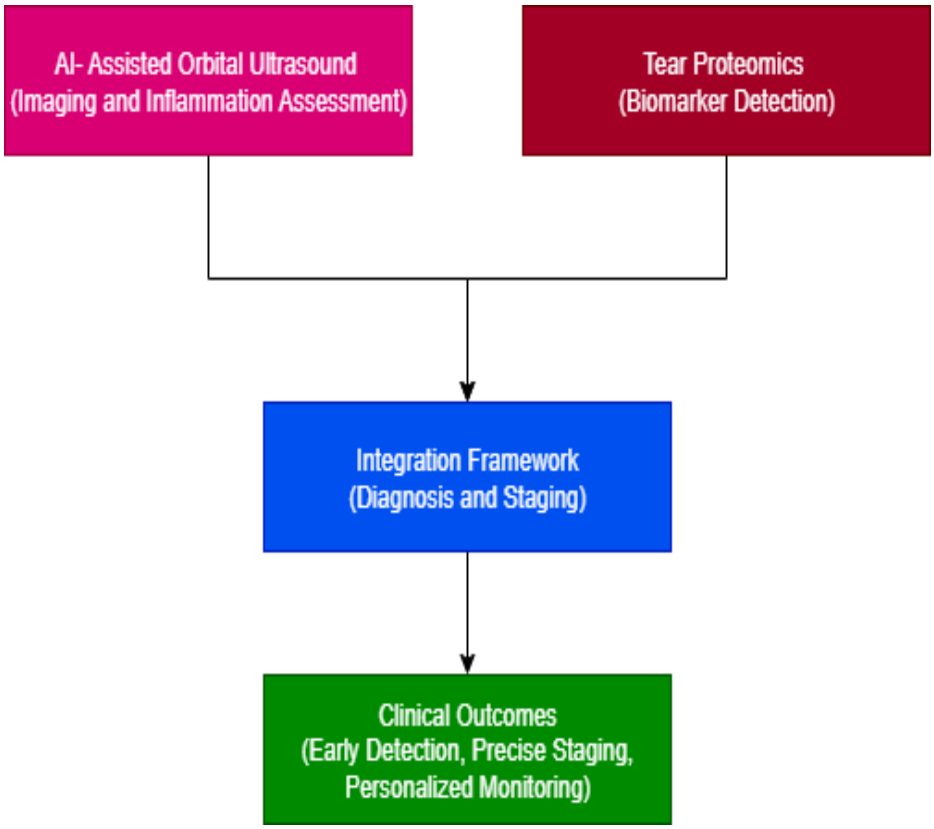

Figure 8: Integrated framework for diagnosing and staging Graves' Orbitopathy.

Strengths

1. **Non-invasive diagnostics:** Tear sampling avoids radiation or invasive procedures, while ultrasound is safe and repeatable.
2. **Complementary modalities:** Biomarker detection provides molecular specificity, and AI-ultrasound offers anatomical and functional insight.
3. **Clinical impact:** Together, these tools may enable earlier therapeutic intervention and more tailored treatment strategies.
4. **Broader applicability:** The framework could be extended to other orbital and autoimmune inflammatory diseases.

Limitations

1. **Validation required:** Tear proteomic biomarkers are still under investigation and require large-scale, multi-center validation before clinical adoption.
2. **Operator dependency:** While AI improves ultrasound interpretation, variability in acquisition and image quality may still affect diagnostic accuracy.
3. **Resource constraints:** Implementation may be limited in low-resource settings without access to advanced omics platforms or AI integration.
4. **Dynamic disease course:** Biomarkers and imaging findings may change over time, requiring repeated assessments to remain clinically useful.

Future research :

1. Biomarker validation will be phased, progressing from carefully controlled pilot cohorts to large, multi-center studies, with external validation at each stage.
2. Operator dependency in ultrasound will be minimized via standardized protocols, centralized training, and certification.
3. For low-resource settings, platform scaling and biomarker panel streamlining will be actively investigated.
4. Dynamic and longitudinal data analysis will be integrated to reflect the real-world course of OID, with advanced statistical modeling for time-dependent biomarkers and image changes.
5. Diverse, external validation cohorts will be prioritized to establish broad clinical applicability and robustness of the diagnostic pipeline.

CONCLUSIONS

Orbital Inflammatory Disorder (OID) remains a clinically challenging disorder to diagnose and stage due to its heterogeneous presentation, unpredictable disease trajectory, and the absence of reliable biomarkers that distinguish active inflammatory from chronic fibrotic phases. While orbital ultrasound is accessible, there is still operator dependency, variability, and interpretive subjectivity. These diagnostic shortcomings contribute to delays in appropriate treatment and complicate patient selection for emerging therapies. The proposed integration of tear proteomics and AI-assisted orbital ultrasound represents a significant step toward overcoming these limitations. Tear proteomics, as a non-invasive and easily repeatable approach, offers a unique opportunity to capture disease-specific molecular signatures reflective of orbital pathology. By validating proteomic biomarkers and correlating them with disease activity, it is possible to establish predictive and prognostic indicators that have long been missing in OID management. Complementing this, AI-assisted orbital ultrasound provides a radiation-free portable imaging modality that enhances diagnostic precision by reducing operator variability and enabling reliable differentiation between inflammatory and non-inflammatory disease stages. This approach also aligns with the broader movement toward precision ophthalmology, where individualized risk stratification and treatment monitoring can improve both short- and long-term patient outcomes and provide early diagnostic interventions.

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All the above materials are intellectual properties of the authors with the original Datasets and publications.

Corresponding Author Prof. Hadi Khazaei is the author of the Springer Nature textbook titled:

Fundamentals of Orbital Inflammatory Disorders. Springer Nature Switzerland; 2025:15-29. doi:10.1007/978-3-031-85768-3_3

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