

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 01 del 21/02/2025 PO 20 Page 4 of 12</p>
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**PROJECT**

**Supervisor:** Federica Ungaro, PhD

**Title:** **Unraveling the spatial and temporal evolution of chronic esophageal diseases: profiling and potential overlap of eosinophilic esophagitis and Barrett esophagus**

**Curriculum:** Clinical and Experimental Medicine

Link to the personal page of the University or relevant hospital site website: <https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/units/experimental-gastroenterology-unit/federica-ungaro.html>

**Description of the Project (max 3,000 characters including spaces)**

**Background/gap of knowledge**

Eosinophilic esophagitis (EoE) is a chronic esophageal inflammatory disease, primarily activated by food antigens, with the concomitant accumulation of eosinophils within the esophageal mucosal layer [1]. Barrett Esophagus [BE] is a metaplastic conversion of esophageal epithelium to the chronic insult of gastric reflux, in Gastroesophageal Reflux Disease (GERD) patients [2]. BE is known to embody a neoplastic risk of developing esophageal adenocarcinoma (EA) [2]. Both EoE and BE have a suggested genetic basis but are influenced by multifactorial pathogenesis [2,3]. EoE symptoms include dysphagia, vomiting, chest pain, and food impaction [4], with a peculiar endoscopic appearance [5]. GERD, on the contrary, is characterized primarily by pirois and regurgitation, potentially causing distal erosive esophagitis, usually responsive to Proton Pump Inhibitors (PPIs) [6]. Due to the epithelial disruption and subversion, usually BE patients are asymptomatic. GERD has a direct correlation with BE development, consequently with a consistent increase in EA occurrence risk [7]. On the contrary, EoE inflammation recurs if untreated, leading to fibrosis and esophageal remodeling, without cancer risk [1]. EoE pathogenesis remains not completely understood, particularly concerning fibrogenesis and its apparent non-correlation with dysplastic epithelial degeneration [8]. Nonetheless, EoE and BE often overlap, being esophageal hypereosinophilia a phenotype present in both diseases. Microbiota dysbiosis is still an underinvestigated topic in both EoE and GERD/BE pathogenesis [10]. A recent systematic review [11] highlighted a high



abundance of *Proteobacteria*, *alfa-Streptococci*, and *Hemophilus Parainfluentiae* in the EoE esophageal mucosa and salivary samples by comparison with healthy samples [12]. Even in GERD and BE recent studies reported an increased abundance of *Proteobacteriaceae* and *Fusobacteriaceae*, particularly *Campylobacter* spp. niches in inflamed and metaplastic mucosa [13].

### **Rationale and hypothesis**

Our group recently developed and released the EoE Transcriptome and Metatranscriptome Meta-Analysis web app (EoETaMMA, <https://eoe-meta-analysis.herokuapp.com/> [14]), a complete survey of all public datasets generated for EoE-related studies, but including also comparative analysis of GERD patients. Similarly, we compiled another platform for the analysis of BE-affected patients. By exploiting the multi-omic factor analysis (MOFA) algorithm, we identified EoE/GERD and BE transcriptomic signatures and pointed out that microbiota dysbiosis was involved in EoE and GERD-to-BE transition. This project has the ultimate goal of validating molecular signatures identified by the computational analysis, both in EoE patients and in GERD with potential identification of overlapping characteristics between EoE and GERD/BE. Through Single-cell RNA sequencing, Spatial Transcriptomics, and Metatranscriptomics analysis, we will try to profile EoE and GERD/BE and identify the mechanisms of dysbiosis in inducing inflammation in the two diseases. Furthermore, by exploiting clinical features, we will investigate the different impacts of the microbiota environment in the development of EoE and GERD-related downsides (fibrosis versus BE/EA).

### **Objectives and specific aims**

- 1) To retrospectively validate, with either immunohistochemistry or transcriptomics, the main molecular pathways identified in the EoE TaMMA in FFPE and fresh-frozen biopsies from EoE and GERD patients (with or without BE) and healthy-derived esophageal mucosa. Fresh frozen samples have been already collected and stored at the San Raffaele EoE Biobank.
- 2) To identify the cellular subtypes and molecular pathways characterizing different EoE and GERD phenotypes and those impacted by specific bacterial factors by transcriptomics and metatranscriptomics analysis at single-cell resolution.
- 3) To define the spatial architecture and reconstruct the temporal sequence of events leading to chronic inflammation both in EoE and GERD (especially with the development of BE); To characterize specific bacterial niches within EoE inflamed tissues and in GERD tissues both with and without metaplasia, unveiling the molecular and cellular mechanisms potentially driving esophageal inflammation and dysplastic versus fibrotic degeneration.

### **Expected outcomes**

Identifying molecular signatures of different EoE and GERD phenotypes.

Unraveling the role of esophageal dysbiosis in EoE and GERD/BE pathogenesis

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**Skills that the student should acquire** (max. 600 characters including spaces):

- Isolation of primary cells from surgical specimens or endoscopic biopsies
- Competences in transcriptomics analysis (single-cell RNA sequencing and Spatial Transcriptomics and Meta-Transcriptomics)
- Competencies in molecular and cellular biology techniques (RNA extraction, reverse-transcription, RT-PCR, cells (co-)culture, plasmid production, and cell lentiviral transduction, cell sorting, cito-fluorimetry, and immunofluorescence analysis)
- Capability to critically discuss results
- Capability to write reports and the final manuscript, along with the final thesis
- Independence in coordinating experiments, under DoS's supervision

**References** (max. 15)

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8. Shoda T et al. Loss of Endothelial TSPAN12 Promotes Fibrostenotic Eosinophilic Esophagitis via Endothelial Cell-Fibroblast Crosstalk. *Gastroenterology.* 2022 Feb;162(2):439-453.
9. O'Shea KM, Aceves SS, Dellon ES, Gupta SK, Spergel JM, Furuta GT, Rothenberg ME. Pathophysiology of Eosinophilic Esophagitis. *Gastroenterology.* 2018 Jan;154(2):333-345.



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