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Disrupted mucosal vascular barrier in eosinophilic esophagitis

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Esophageal barrier has been investigated until now on the epithelial side only. Gut vascular barrier dysfunction has been recently implicated in a number of immune-mediated gastrointestinal disorders. We here characterized the esophageal vascular barrier (EVB) in eosinophilic esophagitis (EoE). Probe-based confocal laser endomicroscopy (pCLE) was performed in two EoE and two reflux esophagitis (RE) patients. The vascular barrier marker plasmalemma vesicle-1 (PV-1) was investigated as a measure of barrier disruption, by both immunohistochemistry and qPCR, in esophageal biopsies of 16 patients with EoE, 15 with RE, and 15 healthy controls (HC). In EoE, but not RE, pCLE revealed leakage of the EVB, which was restored in one patient after dupilumab treatment. PV-1 was significantly increased in EoE in comparison to RE and HC, both in terms of protein and transcript levels, supporting vascular leakage. EVB is disrupted in active EoE. Further studies are needed to understand the diagnostic and pathogenic implications of this finding.

Keywords Confocal laser endomicroscopy, Dupilumab, Esophageal mucosa, Plasmalemma vesicle-1, Reflux esophagitis

Abbreviations

EREFS	Endoscopic reference score
EoE	Eosinophilic esophagitis
EVB	Esophageal vascular barrier
PV-1	Plasmalemma vesicle-1
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HC	Healthy control
pCLE	Probe-based confocal laser endomicroscopy
RE	Reflux esophagitis
RNA	Ribonucleic acid

The recent availability of advanced endoscopic techniques has made possible the evaluation of the gastrointestinal vascular barrier integrity. In particular, probe-based confocal laser endomicroscopy (pCLE) is able to provide detailed morphological and functional images at a subcellular level, including the microvasculature architecture[1,2]. pCLE has been applied to several inflammatory (e.g., inflammatory bowel disease, chronic gastritis) and neoplastic (e.g., pancreatic, colorectal, gastric) disorders, providing novel insights into the diagnosis, monitoring and pathogenic mechanisms of these conditions[1,2]. However, there are no studies addressing the esophageal vascular barrier (EVB) through pCLE in eosinophilic esophagitis (EoE), an immune-

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mediated disorder characterized by an abnormal eosinophil-rich infiltrate of the esophageal mucosa causing dysphagia and food impaction[3]. The pathogenesis of EoE is still elusive[4], and most of the studies investigating the esophageal mucosa barrier function in EoE are focused on evaluating the integrity of the epithelial layer[5]. The new concept that the permeability of the gastrointestinal mucosa is regulated not only at epithelial (intercellular junctions), but also endothelial level[6], and the discovery that the endothelial-specific protein plasmalemma vesicle associated protein-1 (PV-1) finely regulates the permeability of the mucosal vascular barrier[7], has led to recognize a dysfunction of the gastrointestinal vascular barrier as a pathogenic mechanism implicated in a number of chronic digestive disorders[8–11]. Starting from these premises, we aimed to investigate in vivo through pCLE the EVB in patients with EoE in comparison to patients with reflux esophagitis (RE) -the latter used as a non-immune-mediated form of esophagitis-. Moreover, we analyzed the expression of the vascular barrier marker PV-1 both at protein and transcript level, in esophageal biopsies from EoE patients in comparison to RE patients and healthy controls (HC).

Methods

pCLE (Cellvizio®) was performed in one patient (female, young adult) with active EoE before and after a 3-month treatment with the monoclonal anti-interleukin-4/-13 antibody dupilumab (300 mg weekly injection), and in one patient with active EoE (adult male), only before treatment. Two patients with Los Angeles grade A reflux esophagitis (two adult males) were also examined through pCLE, as disease controls. In order to assess the vascular leakage, a dose of 3 mL of sodium fluorescein (10% solution) was administered intravenously within 30–60 s before the procedure. The endoscopic examinations were performed by an experienced endoscopist (RC), using high-definition, white-light Olympus gastroscopes (EVIS EXERA III series).

For the study of the vascular barrier marker PV-1, perendoscopic esophageal biopsies were collected from 16 consecutive EoE patients (median age 32 years, IQR 23–48, F: M ratio = 1:3), 15 patients with Los Angeles grade A or B reflux esophagitis (median age 44 years, 24–62, F: M ratio = 1:1), and 15 patients undergoing upper gastrointestinal endoscopy with no esophageal diseases (median age 60 years, IQR 48–75, F: M ratio = 2:1). The diagnosis of EoE was made according to internationally agreed criteria; i.e., the presence of at least ≥ 15 eosinophils peak per high power field in esophageal biopsies[3]. The exact peak eosinophil count was determined. Biopsies were obtained from at least three segments of the esophagus (6 biopsies). Proton pump inhibitors were withdrawn at least 4 weeks before endoscopy, if taken prior to endoscopy. Some biopsy samples were immediately fixed in a 3% buffered formalin, embedded in paraffin within 24 h and processed according to standard methods for diagnostic purposes and for PV-1 detection through immunohistochemistry. Three-millimeter-thick paraffin sections were used for immunohistochemistry by the Dako Omnis automatic platform (Agilent, Santa Clara, CA). Slides were incubated with the anti-PV-1/PLVAP antibody (clone 174/2; LSBio, Seattle, WA) at a 1:100 dilution for 1 h at room temperature. An HRP-conjugated secondary antibody and DAB chromogen were used for visualization, followed by hematoxylin counterstaining. All staining procedures were performed under standardized conditions to minimize batch effects. As already reported[10], PV-1 expression was assessed in esophageal specimens through a semiquantitative manner by using the score 0 when there were no visible PV1-positive vessels, score 1 in case of rare PV-1-positive vessels, score 2 in case of numerous PV-1-positive vessels, but weak staining, and score 3 in case of numerous PV-1-positive vessels and intense staining. The staining evaluations were independently performed by two experienced pathologists who were blinded to the sample origin and clinical data. In cases of discordance, a consensus was reached after joint review.

Other biopsies were stored in RNALater solution and used for assessing PV-1 transcript mucosal levels, as previously described[10]. Briefly, total RNA was extracted from biopsies using the Direct-zol RNA Microiprep kit (Zymo Research, Irvine, CA). RNA was reverse transcribed with random primers and ImProm-II Reverse Transcriptase (Promega, Milan, Italy). cDNA expression was detected using QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher) and PV-1 primers (genome wide bioinformatically validated primers sets) were provided using Qiagen (QuantiTect Primer Assays). Real-time PCR reactions were performed using the Luna Universal qPCR Master Mix (New England Biolabs, Ipwsich, MA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous reference control. Transcript quantification was performed with the 2-ΔCT method, and the results are expressed as "fold induction" in reference to the expression of the housekeeping gene.

Data are presented with median and interquartile ranges (IQR). Fisher's exact test analysis was applied for comparison of variables; a $p < 0.01$ was considered as statistically significant. Pearson's correlation between peak eosinophil count per high power field and intensity of PV-1 immunohistochemical expression and PV-1 transcript levels was made. Written informed consent was obtained in all cases, and the research was approved by the local ethics committee (Protocol No. 0057625/23). We confirm that all experiments in this study were performed in accordance with the relevant guidelines and regulations. All relevant data are reported in the paper; additional data can be shared upon request to the corresponding author. No patients or members of the public were involved in the design of the study.

Results

Figure 1 shows a representative endoscopic (A) and pCLE image (B) of one of the two cases with RE, the endoscopic and pCLE image of one of the two cases with EoE before (C, D, respectively) and after (E, F, respectively) a 3-month treatment with dupilumab, and the endoscopic and pCLE image of another patient with active EoE (G, H, respectively). In patients with RE, despite the macroscopic erosion(s) of the third lower segment of the esophagus, at pCLE no disruption of the epithelium, nor disruption of the EVB after fluorescein injection were noticed. On the contrary, the active EoE female patient displayed endoscopic signs of EoE, including edema, rings, furrows, and mild exudates, with an endoscopic reference score (EREFS) of 5 (C), and with a marked

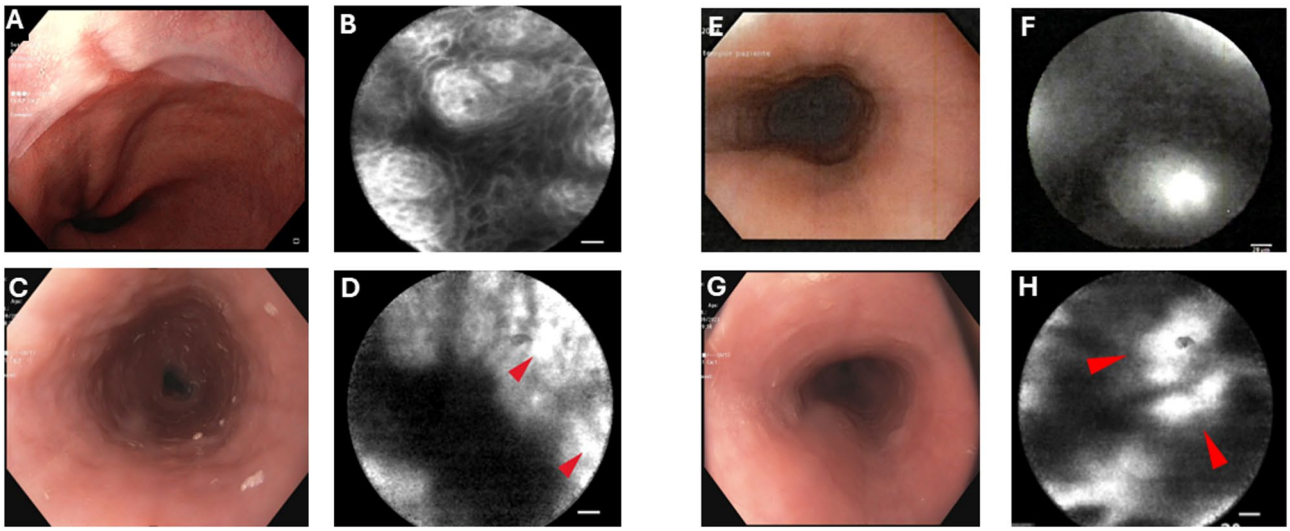

Fig. 1. Composite panel of images showing a representative case of reflux esophagitis (RE; **A**, **B**), one case of eosinophilic esophagitis (EoE) before (**C**, **D**) and after treatment with dupilumab (**E**, **F**), and another case with active EoE (**G**, **H**). Macroscopically, the endoscopy in this case showed reflux esophagitis Los Angeles grade A (**A**); at probe-based confocal laser endomicroscopy (pCLE) after fluorescein administration (**B**) no evident leakage is present, and the normal squamous epithelium is recognizable. In EoE, endoscopy showed some rings, furrows, and exudates in the middle esophagus (**C**, **G**); at pCLE, after fluorescein administration, leakage from small vessels is evident (red arrows), while the normal squamous mucosa is not recognizable (**D**, **H**). After a 3-month treatment with dupilumab, the macroscopic appearance of the esophagus is improved, with no visible rings, furrows, or exudates (**E**); at pCLE, after fluorescein administration, no leaky vessels were noticed (**F**). Scale bar = 20 µm.

leakiness of the EVB at pCLE, including dilated and tortuous vessels (D). After treatment with dupilumab, the EREFS was 1 (E), and pCLE revealed the complete resolution of the leakiness, as well as a normal appearance of the epithelium (F). In the other active male EoE patient with an EREFS of 6 (G), a marked leakiness of the EVB was noticed as well (H).

Figure 2A shows three representative images of PV-1 immunohistochemical staining in a patient with active EoE (numerous PV-1-positive vessels and intense staining), a patient with RE (rare PV-1-positive vessels), and a healthy control (absence of PV-1), respectively (A). In Fig. 2B, the PV-1 median intensity was significantly higher in EoE compared to both control groups. No significant difference was found between RE and HC. Moreover, a significant positive correlation (r_s=0.66, p = 0.006) was found between PV-1 expression and peak tissue eosinophil count in the EoE group. Figure 2C shows that PV-1 transcript levels were significantly higher in EoE compared to both control groups, without any significant difference between RE and HC. Moreover, a significant positive correlation (r_s=0.82, p = 0.0016) was found between PV-1 transcripts and peak eosinophil count in the EoE group.

Discussion

We have herein shown that EVB is disrupted in EoE as assessed in vivo by pCLE, and this damage seems to be reversible after therapy. The mucosal vascular leakage seems to be disease-specific, since in patients with a non-immune-mediated form of esophagitis, i.e., RE, the EVB is hardly affected. The *in situ* immunohistochemical and qPCR data, showing increased PV-1 mucosal expression in active EoE patients, further supports the in vivo pCLE findings of an altered EVB in this condition. The significant positive correlation between PV-1 expression and mucosal eosinophil infiltration, together with the evidence that EVB is restored after therapy in responder patients, support the concept that EVB dysfunction is not a primary phenomenon in EoE, and raise the possibility that PV-1 overexpression could be driven by the inflammatory microenvironment sustaining tissue damage.

Although our study is very preliminary and suffers from a number of limitations (small sample size, lack of ex vivo experiments), the findings here shown might have potential diagnostic and pathogenic implications. On the diagnostic side, vascular leakiness assessed by pCLE could be a marker of EoE in those cases devoid of endoscopic findings, might anticipate the histological diagnosis and facilitate the collection of targeted biopsies, and could help in monitoring the mucosal recovery after therapy. On the pathogenic side, the altered permeability of the EVB could provide a pathophysiological explanation of the abnormal translocation of allergens from the respiratory tract to the esophageal mucosa, a mechanism supposed to be implicated in triggering an exaggerated Th2 response in the esophageal mucosa of EoE patients[4].

Further studies are needed to explore the significance of the EVB dysfunction in EoE and to verify whether it could be a potential therapeutic target, as already reported[11] in other chronic digestive disorders sustained by an altered mucosa vascular barrier.

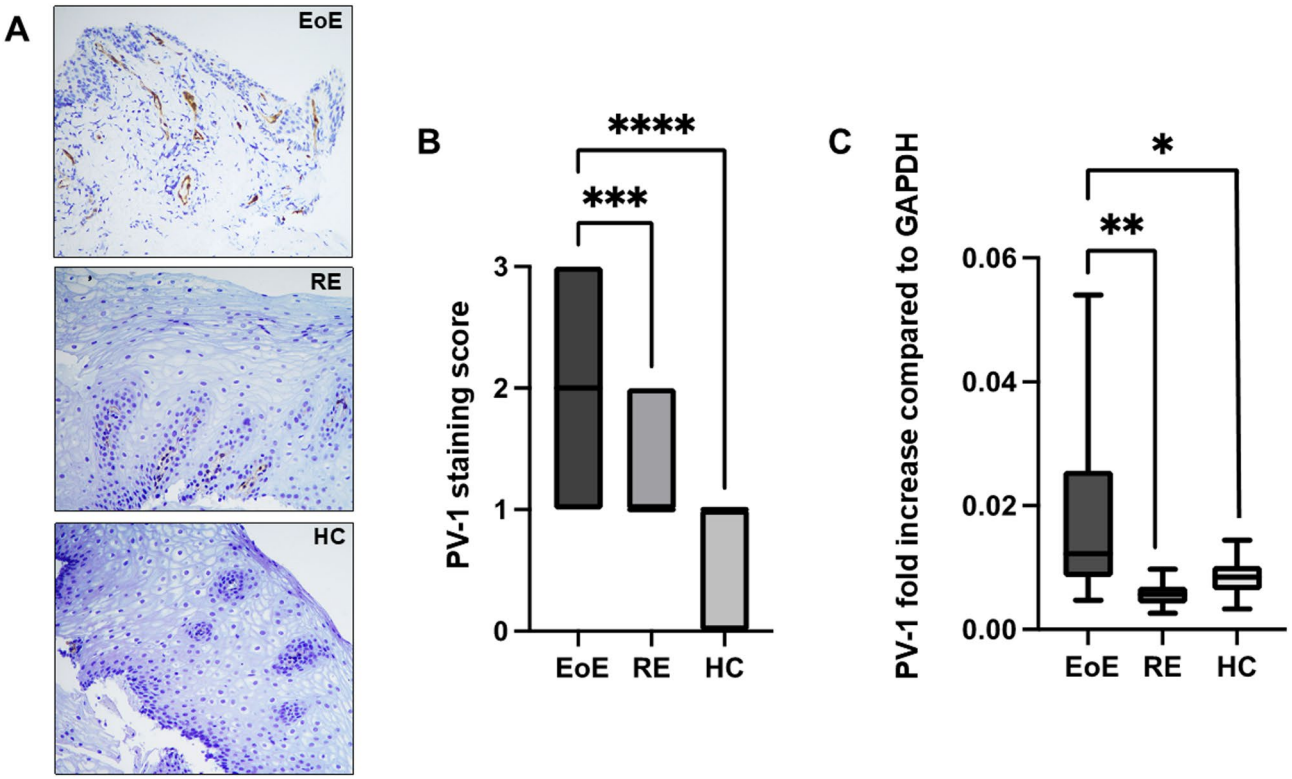

Fig. 2. (**A**) Representative images (40x) of esophageal biopsy specimens stained with plasmalemma vesicle-1 (PV-1) from 16 patients with eosinophilic esophagitis (EoE), 15 patients with reflux esophagitis (RE), and 15 healthy controls (HC). In EoE (upper image), PV-1 staining was well evident in numerous vessels, poorly recognizable –but still evident– in RE, and completely absent in HC. (**B**) PV-1 staining score was significantly higher in EoE compared to both RE and HC, with no significant difference between RE and HC (**C**) PV-1 mRNA was significantly more expressed in EoE compared to both RE and HC, with no significant difference between RE and HC. Legend: $^{*}p < 0.01$; $^{**}p < 0.001$; $^{***}p < 0.0001$; $^{****}p < 0.00001$. *GAPDH* glyceraldehyde 3-phosphate dehydrogenase.

Data availability

All relevant data are reported in the paper; additional data can be shared upon request to the corresponding author.

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References

1. ASGE Technology Committee. Confocal laser endomicroscopy. *Gastrointest. Endosc.* **80**, 928–938 (2014).
2. Bok, G. H. et al. The accuracy of probe-based confocal endomicroscopy versus conventional endoscopic biopsies for the diagnosis of superficial gastric neoplasia (with videos). *Gastrointest. Endosc.* **77**, 899–908 (2013).
3. Dhar, A. et al. British society of gastroenterology (BSG) and British society of paediatric gastroenterology, hepatology and nutrition (BSPGHAN) joint consensus guidelines on the diagnosis and management of eosinophilic oesophagitis in children and adults. *Gut* **71**, 1459–1487 (2022).
4. Santacroce, G. et al. Understanding tissue injury and remodelling in eosinophilic oesophagitis: development towards personalised medicine. *Gut* https://doi.org/10.1136/gutjnl-2024-333994 (2024).
5. Rochman, M., Azouz, N. P. & Rothenberg, M. E. Epithelial origin of eosinophilic esophagitis. *J. Allergy Clin. Immunol.* **142**, 10–23 (2018).
6. Di Sabatino, A., Santacroce, G., Rossi, C. M., Broglio, G. & Lenti, M. V. Role of mucosal immunity and epithelial-vascular barrier in modulating gut homeostasis. *Intern. Emerg. Med.* **18**, 1635–1646 (2023).
7. Brescia, P. & Rescigno, M. The gut vascular barrier: a new player in the gut-liver-brain axis. *Trends Mol. Med.* **27**, 844–855 (2021).
8. Spadoni, I. et al. A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* **350**, 830–834 (2015).
9. Carloni, S. et al. Identification of a choroid plexus vascular barrier closing during intestinal inflammation. *Science* **374**, 439–448 (2021).
10. Lenti, M. V. et al. Mucosal overexpression of thymic stromal lymphopoietin and Proinflammatory cytokines in patients with autoimmune atrophic gastritis. *Clin. Transl Gastroenterol.* **13**, e00510 (2022).
11. Mouries, J. et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J. Hepatol.* **71**, 1216–1228 (2019).

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Author contributions
Marco Vincenzo Lenti: conceptualization (lead); investigation (lead); data curation (lead); formal analysis (lead); writing—original draft (lead); writing—review and editing (lead). Carlo Maria Rossi: conceptualization (lead); investigation (lead); data curation (lead); formal analysis (lead); writing—original draft (lead); writing—review and editing (lead). Renato Cannizzaro: investigation (equal); writing—review and editing (equal). Giovanni Santacroce: investigation (equal); writing—review and editing (equal). Michela Lizier: investigation (equal); writing—review and editing (equal). Giulia Fornasa: investigation (equal); writing—review and editing (equal). Stefania Merli: investigation (equal); writing—review and editing (equal). Alessandra Pasini: investigation (equal); writing—review and editing (equal). Edoardo Vincenzo Savarino: investigation (equal); writing—review and editing (equal). Paola Spessotto: investigation (equal); writing—review and editing (equal). Michele Di Stefano: investigation (equal); writing – review and editing (equal). Aurelio Mauro: investigation (equal); writing—review and editing (equal). Alessandro Vanoli: investigation (equal); writing—review and editing (equal). Fabiana Zingone: investigation (equal); writing—review and editing (equal). Andrea Anderloni: investigation (equal); writing—review and editing (equal). Marietta Iacucci: investigation (equal); writing—review and editing (equal). Maria Rescigno: investigation (equal); writing—review and editing (equal). Antonio Di Sabatino: investigation (lead); data curation (lead); conceptualization (lead); writing—review and editing (lead); supervision (lead).

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
Approval of the research protocol by Fondazione IRCCS Policlinico San Matteo (Protocol No. 0057625/23). Written informed consent was obtained in all cases.

Additional information
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