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Use of intraoperative ex VIVO fluorescence confocal microscopy to identify ganglionic bowel in Hirschsprung's DISEASE: A case series

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ABSTRACT

Introduction: Hirschsprung's disease requires precise intraoperative identification of ganglionic bowel to ensure successful pull-through. Ex vivo fluorescence confocal microscopy (FCM) may offer a rapid, real-time diagnostic adjunct.

Case Presentations: Case 1: A 2-month-old male presented with delayed meconium passage, abdominal distension, and bilious vomiting. Contrast enema revealed a rectosigmoid transition zone, and rectal suction biopsy confirmed aganglionosis. He underwent laparoscopic Soave-Georgeson pull-through. Intraoperatively, seromuscular biopsies were stained with acridine orange and fast green and examined using FCM. Digital images were analyzed remotely by a pathologist, who confirmed the presence of ganglion cells within 5 minutes. The pull-through was completed based on this assessment. Postoperative recovery was uneventful, and at 18-month follow-up, the child had normal bowel function and growth. Case 2: A 3-month-old female presented with chronic constipation, vomiting, and failure to thrive. Imaging and rectal biopsy confirmed Hirschsprung's disease. She underwent laparoscopic pull-through guided by FCM, following the same protocol as in Case 1. Real-time identification of ganglion cells allowed for prompt selection of the pull-through segment. The postoperative course was uncomplicated. At 12-month follow-up, the child had normal stooling patterns and appropriate weight gain.

Conclusion: Intraoperative ex vivo fluorescence confocal microscopy seems to be a fast and reliable method for the identification of ganglionic bowel during the pull-through procedure for the management of Hirschsprung disease.

1. Introduction

Hirschsprung's disease is a congenital disorder characterized by defective intestinal innervation due to the absence of ganglion cells in the myenteric and submucosal plexuses. This defect results in functional bowel obstruction, manifesting clinically as severe constipation. Identifying and confirming the presence of ganglionic bowel segments intraoperatively is essential to ensure correct surgical

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treatment [1]. Currently, the gold standard for intraoperative diagnosis is frozen-section biopsy, recognized for its high precision and accuracy. Nevertheless, this method presents significant limitations, including prolonged processing time with extended anesthesia duration and susceptibility to artifacts [2]. Ex vivo fluorescence confocal microscopy (FCM) VivaScope® 2500M-G4 (MAVIG GmbH, Munich, Germany) has emerged as an innovative digital diagnostic tool offering rapid, non-invasive, and real-time microscopic evaluation of excised tissues. Unlike traditional histological techniques, FCM bypasses extensive tissue processing and provides immediate, diagnostic-quality images within minutes, significantly enhancing intraoperative decision-making [3,4].

The FCM system employs two diode lasers to visualize tissue in two distinct modes: reflectance mode utilizes a 785-nm wavelength laser to detect subcellular structures by identifying differences in tissue refractive indices, while fluorescence mode operates at a 488-nm wavelength to excite cellular fluorochromes, enabling detailed visualization of cellular architecture. This innovative method produces digital hematoxylin-and-eosin-like images, allowing real-time tissue assessment without conventional histopathological preparation [4,5]. The effectiveness of FCM has been well-documented across various medical specialties, including dermatology, urology, laryngeal surgery, pulmonary oncology, breast surgery, hepatology, colorectal surgery, pancreatic surgery, and recently in pediatric surgical oncology [2,6–16].

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1.1. Cases presentation

1.1.1. Case 1

A female newborn diagnosed with Down's syndrome and congenital cardiac defects developed abdominal distension, bilious vomiting, and delayed passage of meconium, requiring rectal stimulation on the third day of life. Radiographic examination revealed dilated bowel loops throughout the abdomen, and a barium contrast enema demonstrated a narrow caliber with a clear transition zone between the sigmoid colon and rectum. Due to clinical suspicion of Hirschsprung's disease, a rectal suction biopsy was performed, confirming the absence of ganglion cells. The patient initially underwent rectal decompression through colonic irrigation and subsequently had an elective laparoscopic Soave-Georgeson pull-through procedure at two months of age. In the operating room, the surgeon obtained full-thickness biopsies during the laparoscopic procedure, while another surgeon managed the entire image acquisition process using the ex vivo FCM. An expert pathologist remotely reviewed and analyzed the digital images in real-time. Intraoperatively, fresh biopsy samples from the proximal intestinal segments were briefly immersed in 70 % ethanol to clean the tissue and facilitate staining. Tissue staining involved exposure to a 0.04 % acridine orange dye solution for 30 seconds to highlight nucleic acids, followed by immersion in a 0.067 % fast green dye solution for 20 seconds to enhance nucleus-to-cytoplasm contrast. Excess dye was removed by rinsing the samples in saline solution. The stained tissue was then positioned on a sponge and covered with magnetic-mounted glass slides and placed onto the microscope lens. After selecting the desired area and tissue depth, rapid confocal scanning was conducted, generating digital images within approximately 50 seconds. Upon detailed remote evaluation by the pathologist, the presence of ganglion cells in both submucosal and myenteric plexuses was confirmed. Subsequently, tissue samples were preserved in formalin and underwent conventional histopathological examination, which corroborated the intraoperative confocal microscopy findings (Fig. 1). At **18 months of follow-up**, the patient has **regular, spontaneous bowel movements, no need for laxatives, and no episodes of enterocolitis or fecal incontinence**.

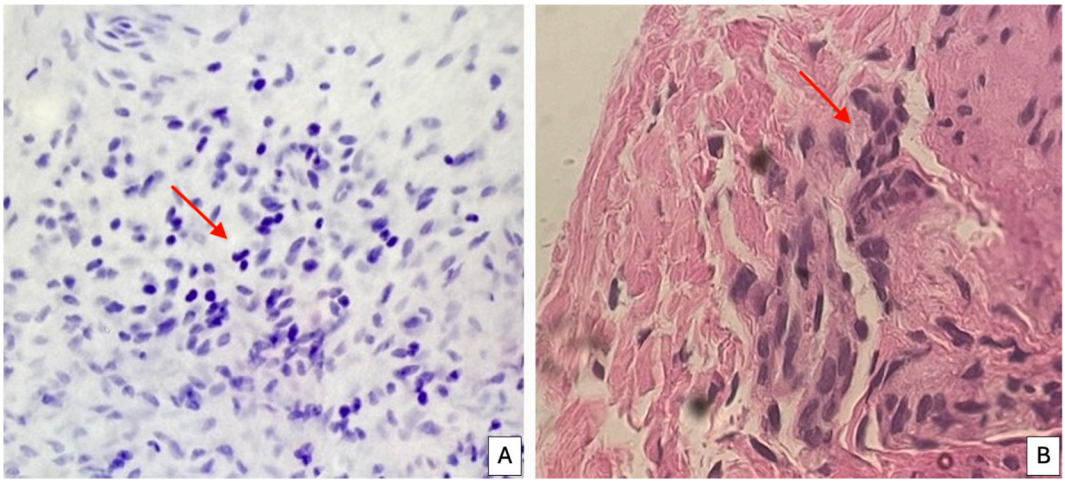

Fig. 1. Image showing immature neural elements characterized by small, hyperchromatic nuclei and scant cytoplasm, with occasional ganglion-like cells displaying larger nuclei and abundant pale cytoplasm (magnification scale 40×). A: Ex vivo fluorescence confocal microscopy (FCM). The pink background is due to slightly thicker tissue and more intense staining with acridine orange. Arrows indicate ganglion cells in the submucosal plexus. B: Corresponding hematoxylin and eosin (H&E) stained section confirming the presence of ganglion cells (arrow).

1.1.2. Case 2

The second case involved a full-term male newborn referred from another center. He presented with delayed passage of meconium on the third day of life after rectal stimulation, accompanied by feeding difficulties, frequent regurgitation, and vomiting with intermittent stooling. Radiographic examination showed dilated bowel loops throughout the abdomen, while a barium contrast enema indicated a reduced-caliber rectum and dilated sigmoid colon, with a clear recto-sigmoid transition zone. A rectal suction biopsy confirmed the diagnosis of Hirschsprung's disease. Initial management included colonic irrigation for rectal decompression, followed by an elective laparoscopic Soave-Georgeson pull-through performed at one month of age. Similar to the first case, full-thickness biopsies were analyzed intraoperatively using FCM with remote real-time evaluation by the pathologist. The staining procedure followed the previously described methodology. The surgical team managed the entire staining and imaging process autonomously, demonstrating the practicality of FCM even in a surgical setting. Following remote image analysis, the pathologist was able to identify the presence of ganglion cells within both the submucosal and myenteric plexuses (Fig. 2). The specimens were subsequently placed in formalin and processed through conventional histopathology, which validated the intraoperative observations made using confocal microscopy. At **12 months of follow-up**, the child has **age-appropriate bowel function, regular stooling without medication, and no complications such as constipation, incontinence, or enterocolitis**.

2. Discussion

The application of confocal microscopy in pediatric surgery remains relatively novel and limited, with selective applications within specific contexts [15,16]. At our center, the FCM was introduced approximately one year ago, initially targeting pediatric oncology [15]. This study represents one of the initial explorations of its potential use for diagnosing Hirschsprung's disease in pediatric patients. Although our experience is still preliminary, the results indicate promising diagnostic capabilities of FCM for assessing colonic tissues and accurately identifying ganglion cells, crucial elements in the diagnosis of Hirschsprung's disease. One of the key advantages of FCM in the surgical management of Hirschsprung disease lies in its ability to deliver real-time, high-resolution imaging of fresh, unstained tissue, enabling rapid intraoperative decision-making. This is particularly valuable in neonatal surgery, where minimizing anesthesia duration and reducing operative time are critical. In fact, it is now known that anesthesia time, especially in younger patients, can be considered a risk factor for future neurodevelopment [17]. Several studies have evaluated this without reaching significant results, but the authors agree that shortening anesthesia time is nonetheless an important element [18]. In our experience, the total time from biopsy collection to FCM-based diagnosis was approximately 5 minutes, significantly shorter than the average 30–40 minutes typically required for conventional frozen-section analysis, thus offering a meaningful reduction in intraoperative waiting time. The possibility of having a diagnosis in real time, during Georgeson-Soave surgery, is in this sense decisive in significantly reducing the anesthesia time. which otherwise risks lengthening while waiting for histological evaluation.

In addition to ex vivo applications, recent studies have explored the use of confocal laser endomicroscopy (CLE) for in vivo evaluation of the enteric nervous system in Hirschsprung's disease. CLE enables real-time imaging of mucosal and submucosal structures during endoscopic procedures using biocompatible fluorescent dyes such as fluorescein. Notably, Kobayashi et al. and Shimojima et al. demonstrated the feasibility of CLE for identifying ganglion cells and nerve trunks in vivo, suggesting a potential role for this technique in preoperative or intraoperative diagnosis [19,20]. However, CLE requires endoscopic access, specialized probes, and specific training for image interpretation, which may limit its widespread use. In contrast, ex vivo FCM offers digital, high-resolution, hematoxylin-and-eosin-like images without requiring extensive infrastructure or patient exposure to dyes, making it a practical and safe tool for intraoperative decision-making in surgical settings where rapid histological assessment is essential.

Unlike traditional frozen section analysis, which requires time-consuming preparation, sectioning, and staining, FCM allows for

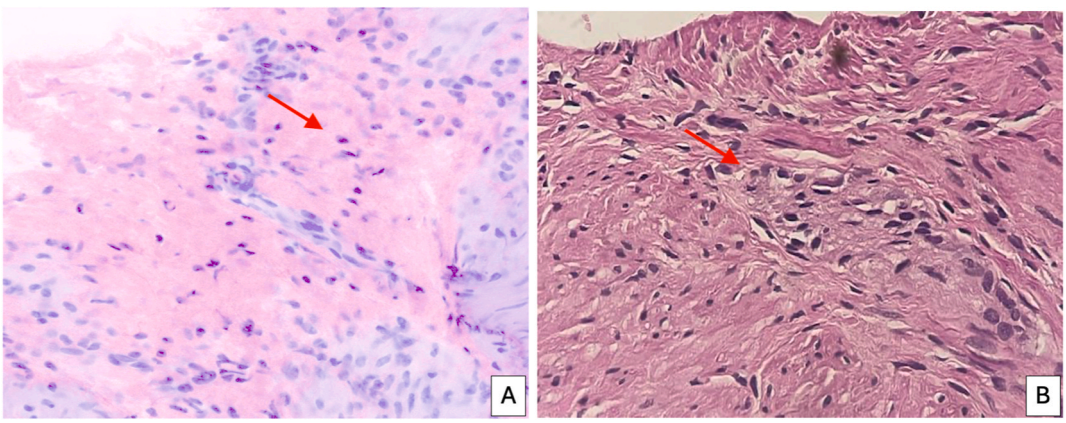

Fig. 2. Immature neural cells with occasional ganglionic element with larger nucleus and abundant pale cytoplasm (magnification scale 40×). A: Ex vivo fluorescence confocal microscopy. The lighter background compared to Fig. 1 results from thinner tissue and more homogeneous staining. Arrows point to ganglion cells identified in the submucosal and myenteric plexuses. B: Corresponding H&E-stained section confirming the presence of ganglion cells (arrow).

immediate, high-quality visualization of histological features such as ganglion cells and hypertrophic nerve trunks, without the need for tissue preparation [3]. A major benefit of this technique is that an entire specimen can be examined directly under the microscope, with no tissue lost to sectioning. This is especially important in patients where biopsy samples are often small and preservation of the entire specimen for further analyses is essential. Since the tissue is not frozen, it can later be processed as a standard formalin-fixed, paraffin-embedded sample, preserving its integrity for definitive diagnosis [15]. Additionally, the digital histological-like images produced by FCM can be stored indefinitely without the degradation seen in traditional glass slides, ensuring preservation of diagnostic data and rapid retrieval [12,21]. In settings where immediate access to pediatric pathology expertise is limited, FCM can be particularly transformative, streamlining surgical workflow and optimizing patient outcomes [15].

Training is essential for the effective use of FCM in the operating room. In our center, pediatric surgeons received focused sessions from microscopy specialists to become proficient in staining, image acquisition, and navigation of the confocal interface. **Identifying the correct area and depth relies on knowledge of ganglion cell location** and familiarity with adjusting imaging parameters accordingly.

Despite its many advantages, FCM presents some limitations. First, the technology requires a dedicated equipment, specifically the VivaScope® 2500M-G4, which costs approximately €200,000–€250,000. Currently, its use in pediatric surgery is limited to Hirschsprung's disease and select oncology cases, raising concerns about cost-effectiveness in lower-volume centers. Second, a technical limitation is the phenomenon of photobleaching, where prolonged exposure to laser light can degrade the fluorescent signal, reducing image quality. This issue can be mitigated by optimizing the scanning protocol to minimize exposure time and by ensuring that the image acquisition process is carried out quickly and efficiently [15]. Additionally, the need for proper tissue staining and sample preparation requires a trained and coordinated team in the operating room [16]. To address this, we established in our center standardized protocols and initial training sessions, allowing surgical staff to become proficient in handling both the technical aspects of staining and image acquisition.

Future larger-scale studies involving diverse pediatric patient populations are essential to fully validate and establish the diagnostic effectiveness of the FCM in pediatric conditions. Ongoing advancements in confocal microscopy technology suggest a growing potential for its application in clinical practice, enhancing both diagnostic accuracy and patient management [6].

3. Conclusion

Intraoperative ex vivo fluorescence confocal microscopy seems to be a fast and reliable method for the identification of ganglionic bowel during the pull-through procedure for the management of Hirschsprung disease.

CRediT authorship contribution statement

Donatella Di Fabrizio: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Irene Tavolario:** Writing – original draft, Data curation. **Francesca Mastroberti:** Data Curation. **Edoardo Bindi:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis. **Alessandra Filosa:** Writing – review & editing, Resources. **Gaia Goteri:** Writing – review & editing, Validation, Supervision, Resources. **Giovanni Cobellis:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition.

Informed consent

Informed consent was obtained from the patient or guardian.

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Informed consent attestation

The authors attest that informed consent was obtained from the patient's parents or guardian.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Montalva L, Cheng LS, Kapur R, et al. Hirschsprung disease. Nat Rev Dis Primers 2023;9(1):54. https://doi.org/10.1038/s41572-023-00465-y. Published 2023 Oct 12.

[2] Nackenhorst MC, Kasiri M, Gollackner B, Regele H. Ex vivo fluorescence confocal microscopy: chances and changes in the analysis of breast tissue. Diagn Pathol 2022 Jun 28;17(1):55. https://doi.org/10.1186/s13000-022-01240-5. PMID: 35765032; PMCID: PMC9238046.

[3] Krishnamurthy S, Cortes A, Lopez M, et al. Ex vivo confocal fluorescence microscopy for rapid evaluation of tissues in surgical pathology practice. Arch Pathol Lab Med 2018;142(3):396–401. https://doi.org/10.5858/arpa.2017-0164-OA.

[4] Schüürmann M, Stecher MM, Paasch U, Simon JC, Grunewald S. Evaluation of digital staining for ex vivo confocal laser scanning microscopy. J Eur Acad Dermatol Venereol 2020 Jul;34(7):1496–9. https://doi.org/10.1111/jdv.16085. Epub 2020 Feb 6. PMID: 31732988.

[5] Ragazzi M, Longo C, Piana S. Ex vivo (fluorescence) confocal microscopy in surgical pathology: state of the art. Adv Anat Pathol 2016;23(3):159–69. https://doi.org/10.1097/PAP.0000000000000114.

[6] Razi S, Ouellette S, Khan S, Oh KS, Truong TM, Rao BK. Role of VivaScope 2500 ex vivo confocal microscopy in skin pathology: advantages, limitations, and future prospects. Skin Res Technol 2023 Jun;29(6):e13388. https://doi.org/10.1111/srt.13388. PMID: 37357649; PMCID: PMC10250963.

[7] Eissa A, Puliatti S, Rodriguez Peñaranda N, Resca S, Di Bari S, Vella J, Maggiorelli S, Bertoni L, Azzoni P, Reggiani Bonetti L, Campobasso D, Ferretti S, Micali S, Bianchi G. Current applications of ex-vivo fluorescent confocal microscope in urological practice: a systematic review of literature. Chin Clin Oncol 2024 Aug;13(4):52. https://doi.org/10.21037/cco-23-150. Epub 2024 May 11. PMID: 38769791.

[8] Bertoni L, Puliatti S, Reggiani Bonetti L, et al. Ex vivo fluorescence confocal microscopy: prostatic and periprostatic tissues atlas and evaluation of the learning curve. Virchows Arch 2020;476(4):511–20. https://doi.org/10.1007/s00428-019-02738-y.

[9] De Benedetto L, Moffa A, Baptista P, Di Giovanni S, Giorgi L, Verri M, Taffon C, Crescenzi A, Casale M. Potential use of vivascope for real-time histological evaluation in endoscopic laryngeal surgery. J Personalized Med 2023 Aug 12;13(8):1252. https://doi.org/10.3390/jpm13081252. PMID: 37623502; PMCID: PMC10455566.

[10] Kamm M, Hildebrandt F, Titze B, Höink AJ, Vorwerk H, Sievert KD, Groetzner J, Titze U. Ex vivo fluorescence confocal microscopy for intraoperative examinations of lung tumors as alternative to frozen Sections-A Proof-of-Concept study. Cancers (Basel) 2024 Jun 14;16(12):2221. https://doi.org/10.3390/cancers16122221. PMID: 38927926; PMCID: PMC11202023.

[11] Di Matteo FM, Stigliano S, Frasca L, et al. New instant digital pathology for EUS/EBUS samples: the last advance in bedside diagnostics for lung carcinoma. Cancers (Basel) 2024;16(23):4015. https://doi.org/10.3390/cancers16234015. Published 2024 Nov 29.

[12] Titze U, Sievert KD, Titze B, et al. Ex vivo fluorescence confocal microscopy in specimens of the liver: a Proof-of-Concept study. Cancers (Basel) 2022;14(3):590. https://doi.org/10.3390/cancers14030590. Published 2022 Jan 25.

[13] Guerrero JA, Pérez-Anker J, Fernández-Esparrach G, et al. Ex vivo fusion confocal microscopy of colorectal polyps: a fast turnaround time of pathological diagnosis. Pathobiology 2021;88(6):392–9. https://doi.org/10.1159/000517190.

[14] Stigliano S, Crescenzi A, Taffon C, Marocchi G, Di Matteo FM. Fluorescence confocal microscopy for rapid evaluation of EUS fine-needle biopsy in pancreatic solid lesions. VideoGIE 2023;8(3):113–4. https://doi.org/10.1016/j.vgie.2022.11.010. Published 2023 Mar 6.

[15] Di Fabrizio D, Bindi E, Ilari M, Filosa A, Goteri G, Cobellis G. Ex vivo fluorescence confocal microscopy meets innovation and revolutionary technology, for "Real-Time" histological evaluation, in pediatric surgical oncology. Children 2024 Nov 23;11(12):1417. https://doi.org/10.3390/children11121417. PMID: 39767846; PMCID: PMC11674610.

[16] Gretser S, Kinzler MN, Theilen TM, Wild PJ, Vogler M, Gradhand E. Fluorescence confocal microscopy for evaluation of fresh surgical specimens and consecutive tumor cell isolation in rare pediatric tumors. Virchows Arch 2025;486(3):585–93. https://doi.org/10.1007/s00428-024-03861-1.

[17] Sun LS, Li G, Miller TL, Salorio C, Byrne MW, Bellinger DC, Ing C, Park R, Radcliffe J, Hays SR, DiMaggio CJ, Cooper TJ, Rauh V, Maxwell LG, Youn A, McGowan FX. Association between a single general anesthesia exposure before age 36 months and neurocognitive outcomes in later childhood. JAMA 2016 Jun 7;315(21):2312–20. https://doi.org/10.1001/jama.2016.6967. PMID: 27272582; PMCID: PMC5316422.

[18] Bartels DD, McCann ME, Davidson AJ, Polaner DM, Whitlock EL, Bateman BT. Estimating pediatric general anesthesia exposure: quantifying duration and risk. Paediatr Anaesth 2018 Jun;28(6):520–7. https://doi.org/10.1111/pan.13391. Epub 2018 May 2. PMID: 29722100; PMCID: PMC6291204.

[19] Kobayashi M, Sumiyama K, Shimojima N, et al. Technical feasibility of visualizing myenteric plexus using confocal laser endomicroscopy. J Gastroenterol Hepatol 2017;32(9):1604–10. https://doi.org/10.1111/jgh.13754.

[20] Shimojima N, Kobayashi M, Kamba S, et al. Visualization of the human enteric nervous system by confocal laser endomicroscopy in hirschsprung's disease: an alternative to intraoperative histopathological diagnosis? Neuro Gastroenterol Motil 2020;32(5):e13805. https://doi.org/10.1111/nmo.13805.

[21] Au M, Almeida-Magana R, Al-Hammouri T, Haider A, Shaw G. Accuracy of Ex-vivo fluorescence confocal microscopy in margin assessment of solid tumors: a systematic review. J Histochem Cytochem 2023;71(12):661–74. https://doi.org/10.1369/00221554231212948.