Evaluation of the diagnostic accuracy of ultra-fast ConfocAl imaging for rEtroareolar Limit in nIpple-sparing mAstectomy: CAMELIA project

Angelica Conversano[1,3], Neila-Ines Ben-Romdhane[2,3], Natacha Joyon[2,3], Anne Puchar[1], Anna Ilenko[1], Adeline Turpin[1], Alexia Alfaro[3,4], Marie-Christine Mathieu MC[2,3], Muriel Abbaci[3,4]

Gustave Roussy, 114 rue Édouard Vaillant - 94805 Villejuif Cedex - France

INTRODUCTION

Nipple-sparing mastectomy (NSM) followed by breast reconstruction is now widely recognized as a safe technique, including in patients with a history of radiotherapy or chemotherapy, regardless of tumor size or axillary status, except in cases of inflammatory breast cancer or direct involvement of the nipple-areola complex on imaging or clinical examination.

The main oncological concern is the potential persistence of tumor remnants in the residual subareolar breast tissue. At least 10% of patients undergo a second surgery to excise this tissue. Such reinterventions may increase patient anxiety, negatively impact body image, raise healthcare costs, and delay adjuvant treatments.

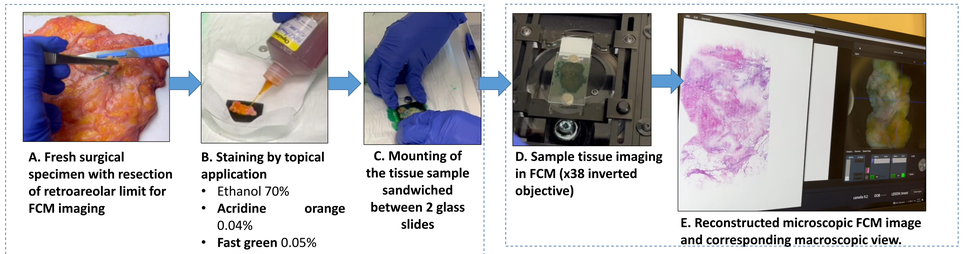
A new generation of ultra-fast confocal microscopes (UFCM) enables high-resolution imaging of fresh tissue in a shorter time than frozen section analysis, potentially guiding intraoperative assessment and avoiding reoperation [1-4].

OBJECTIVES

The CAMELIA project is a prospective, non-interventional, ex vivo clinical study analyzing retroareolar margins after NSM, imaged using UFCM and interpreted by pathologists and surgeons. The examined tissue is preserved for subsequent conventional histology (HES). At this stage, UFCM results do not influence surgical management.

The primary objective is to use UFCM to provide an accurate intraoperative diagnosis of retroareolar tissue. Accuracy is calculated based on the proportion of images correctly interpreted compared to definitive histology on corresponding HES slides, the reference standard.

Figure 1: From sample collection to UFCM images acquisition



A. Fresh surgical specimen with resection of retroareolar limit for FCM imaging

B. Staining by topical application
- Ethanol 70%
- **Acridine orange** 0.04%
- **Fast green** 0.05%
- NaCl 0.9% (rinsing)

C. Mounting of the tissue sample sandwiched between 2 glass slides

D. Sample tissue imaging in FCM (x38 inverted objective)

E. Reconstructed microscopic FCM image and corresponding macroscopic view.

METHODS AND MATERIALS

UFCM images acquisition

60/195 surgical specimens from NSM (with and without neoadjuvant chemotherapy or hormonotherapy NACT/NAHT before surgery or reoperation after lumpectomy) were processed fresh in the pathology department. The retroareolar margin (RAM) was resected either by the pathologist, guided by a Vicryl wire placed by the surgeon on the specimen (Fig. 1A), or directly by the surgeon and submitted with the surgical specimen. The RAM was then placed on foam supports, rinsed with saline, and topically stained with sequential applications of acridine orange (0.04%) and fast green (0.05%) (Fig. 1B). The tissue-laden foam was sandwiched between glass slides (Fig. 1C, D). Fluorescence confocal microscopy (FCM) imaging was performed using a Vivascope 2500M-G4 (Vivascope GmbH, Munich, Germany) confocal microscope with 488/638 nm lasers. This system generates reconstructed images (34 × 25 mm max), allows z-plane imaging from 0 to 200 µm with an axial resolution of 4 µm. The resulting fluorescence images resemble hematoxylin and eosin staining, with nuclei appearing purple and cytoplasm/extracellular structures pink (Fig. 1E). Following UFCM imaging, the samples were fixed in formalin and embedded in paraffin for conventional histopathological processing.

Data base design

The CAMELIA database, implemented through REDCap, includes clinical data, acquisition data (sample dimensions, processing and acquisition duration, and number of images), final histopathological reports (detailing pamectomy procedures when applicable), digital hematoxylin-eosin-saffron slides, and UFCM images. In a blinded assessment, 3 pathologists and 3 surgeons will independently review all UFCM images at distance of UFCM image acquisition. Their interpretations and the duration of their reviews will be recorded in the REDCap database. Concordance will be determined by comparing UFCM image classifications with the final histological findings of the RAM. Major discrepancies will be defined as disagreements between normal/benign and malignant classifications.

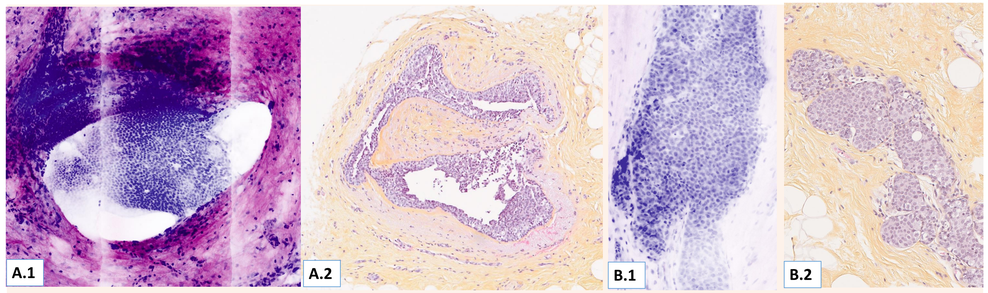


Figure 2: UFCM image of A/DCIS and B/LCIS and corresponding HES image.

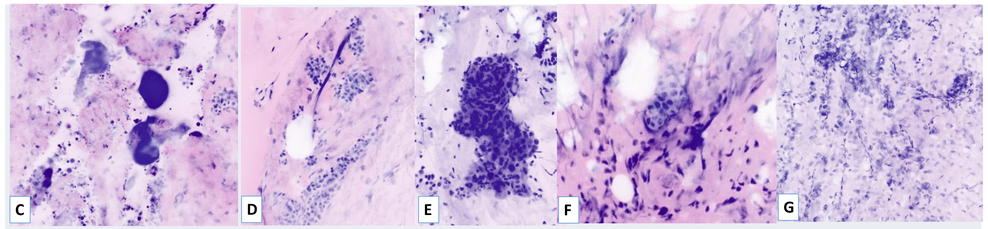


Figure 3: Challenging UFCM images: C/clusters of staining (artifact), D/lobular atypia, E/cluster of cells mimicking cancer, F/cluster of cancerous cells (invasive ductal BC), G/ cluster of cancerous cells (invasive lobular BC)

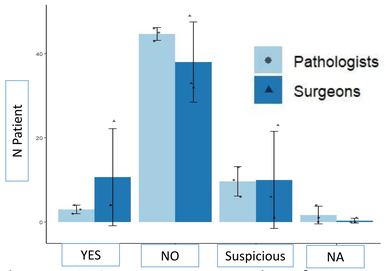


Figure 4: UFCM Image Interpretation of RAM Status by Pathologists (n=3) and Surgeons (n=3) with: yes: positive margin, no: negative margin, Suspicious and NA: don't know

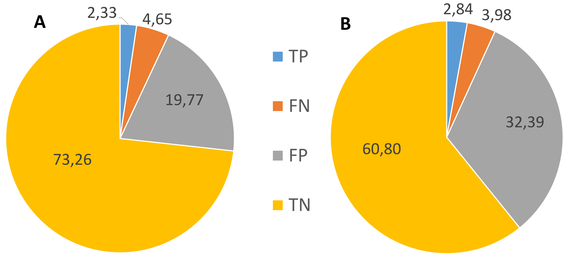


Figure 5: Concordance rate with final histology. A. UFCM images interpretation by pathologists. B. UFCM images interpretation by surgeons

RESULTS

Since October 2024, 56 out of 194 patients who underwent NSM have been included in this study corresponding to 60 RAM. Among them, 6 had prophylactic mastectomies and 50 had therapeutic interventions. 4 bilateral procedures were performed, and 25 patients underwent surgery after neoadjuvant chemotherapy. Importantly, four of these patients had positive margins. One RAM was not imaged due to the absence of the guide wire on the surgical specimen.

Three pathologists participated in the image analysis: two seniors (PT1 and PT2) and one junior (PT3). Both PT1 and PT3 have experience with UFCM. Additionally, an experienced UFCM surgeon along with two inexperienced surgeons interpreted all images. Images were categorized into four groups: don't know, cancer, no cancer, or suspicious. On average, the acquisition time for intraoperative images was 15 minutes. The image quality was rated 3.7/5 by the readers. The average interpretation time for all specialists was 1 minute and 44 seconds.

The concordance rate among surgeons for the first 59 RAM was 63.64% (60.80% true negative TN + 2.84% true positive TP), while that of pathologists was 75.59% (73.26% TN + 2.33% TP) compared with the final histology (Fig. 5). The surgeons showed a tendency to overestimate the presence of cancer on the images (Fig.4) resulting in a higher rate of false positives and a significantly greater error margin than that observed in pathologists (FP: 32.39% surgeons vs 19.77%).

One possible explanation could be the tendency to interpret areas of higher density and clusters of staining as positive, which can mimic cancer.

Various other causes can lead to errors, notably including the difficulty in identifying lobular carcinoma or atypia, which is intrinsically more challenging. Furthermore, the paraffin section is not exactly identical to the microscopic section due to the clearing process. The small number of real infiltrating cases—which may correspond only to clusters of cancerous cells—can escape detection by a less experienced eye (Fig.3).

CONCLUSION

The results aim to assess whether real-time cellular imaging of the retroareolar margin during NSM is suitable for large-scale use, in order to complete tumor resection at the same time if necessary and avoid reoperation. It remains to be determined to what extent this technique is suitable for interpretation by surgeons or whether it should be reserved for pathologists.

Contact :
Angelica Conversano, MD
Gustave Roussy
Email : angelica.conversano@gustaveroussy.fr

Presentation ID : PS5-09-13

References:

1. Conversano A. et al. BJS Open. 2023 May 5;7(3):zrad046
2. Mathieu MC et al. Virchows Arch. 2025 Feb;486(2):299-311
3. Mathieu MC et al. Life (Basel). 2024 Oct 28;14(11):1384
4. Spoor J et al. Eur J Surg Oncol. 2024 Jun;50(6):108320

AFFILIATION
1.Department of Breast and Plastic Surgery, Gustave Roussy, Université Paris-Saclay, Villejuif, France
2.Department of Medical Biology and Pathology, Gustave Roussy, Université Paris-Saclay, Villejuif, France
3.Surgery and Pathology Photonic Imaging Group, Gustave Roussy, Villejuif, France
4.UMS AMMICa 23/3655, Plateforme Imagerie Et Cytométrie, Gustave Roussy, Université Paris-Saclay, Villejuif, France

This presentation is the intellectual property of the author/presenter. Contact them for permission to reprint and/or distribute.