Doctoral Dissertation
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Sentinel Lymph Node in Rectal Cancer: Role of Transanal Endoscopic Microsurgery

Carlo Alberto Ammirati
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Supervisors
Prof. A. Arezzo, Supervisor
Prof. M. Morino, Co-Supervisor

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Summary

This thesis represents the synthesis of the work carried out over three years of PhD study and focuses on the exploration of the concept of sentinel lymph node applied to colorectal pathology and more specifically, to rectal cancer.

In recent years, significant technological and therapeutic innovations, as well as the increasing implementation of prevention strategies, have led to a radical transformation in the approach to oncological pathology. Concepts such as personalized therapy and tailored surgery have also provided new impetus for a therapeutic proposal that is entirely patient-centered, in a continuous effort to progressively reduce the undesirable effects and complications often associated with oncological treatment.
In colorectal surgery, screening programs have shown a shift towards earlier staged cancers[1-3], thus broadening the range of therapeutical chances and granting a space to therapeutic proposal for minimally invasive surgery and endoluminal strategies. Indeed, **Early Rectal Cancer** (ERC) represents a heterogeneous group mainly due to the different risk of loco-regional lymph nodes metastases[4], which gradually increases with submucosal invasion, and impacts significantly on the approach to these patients, as metastatic spread to regional lymph nodes is one of the most important prognostic factors and determines the need for adjuvant chemotherapy.

The standard of care for radical surgery in rectal cancer is low anterior resection (LAR) with total mesorectal excision (TME) or abdominoperineal resection (APR). Both procedures are worsened by high operative morbidity rate and significant negative impact on functional outcome[5-7]. Furthermore, a huge number of patients after TME should face with stoma related difficulties, morbidity and subsequent hazards from stoma reversal.
In case there are not adverse features for lymph node metastatic involvement, local excisional procedures such as **Transanal Endoscopic Microsurgery** (TEM) are appropriate[8]. In order for TEM to be curative, the tumor must be limited to the rectal wall and able to be resected with negative margins. In this regard, accurate staging is paramount to selecting appropriate patients for local excision. Among several endoluminal proposal, TEM surely stands as standard of care. This technique allows more accurate en bloc, full-thickness excision than other techniques, and can provide similar oncological results in patients without lymph node involvement (clinical cN0)

compared with TME, without compromising anorectal function[1, 9]. In this setting TEM could therefore represent both a curative technique, and an accurate staging technique.
Nonetheless, the lack of adequate lymphadenectomy this technique entails so far, represents the main concern to this approach. As the risk of lymphatic metastases in ERC ties in with submucosal invasion, lymph drainage and its pathway appear to be crucial aspects to be assessed in this setting.

The lymphatic vasculature is a system draining the organs through vessels and lymph nodes that act as filters. The **Sentinel Lymph Node** (SLN) is the first lymph node that receives lymphatic drainage from a tumor, and its identification and analysis for tumor involvement should predict the status of the remaining lymph nodes, providing key information in defining a proper oncological therapeutic strategy. The SLN procedure is actually regarded as the standard of care in the treatment of breast cancer and melanoma. However, its added value in colorectal cancer has not yet been established. Among the reason for this uncertainty, lack of deep understanding of the complexity of lymphatic drainage in the anatomical area of mesorectum certainly plays a central role.

In this scenario, the implementation of imaging diagnostic techniques has contributed to the refinement of radiological lymph node staging in a reliable and specific manner[10-12]. As magnetic resonance imaging (MRI) is considered to be the standard of care for the assessment of lymph node involvement and local staging in rectal cancer, the first step of our research was to evaluate the chance and feasibility to identify a preferential lymphatic drainage pathway in the mesorectal space in patients with rectal cancer, through the analysis of preoperative MRI scans. The positive results of this analysis were then summarized in a recent published **retrospective radiological study**[13], which highlights a lymphatic drainage preference lane as well as its correlation with primary rectal tumor location, although a clear node distribution pattern was not established.
The recent improvement of imaging prognostic tools that correlate with and are validated by final histological examination[14, 15], along with some authors' attempt to propose different techniques for the identification of metastatic lymph node based on histological specimens from patients undergoing rectal resection with TME[16] acknowledged from literature, synergised with the need of an external validation of these preliminary radiologic results.

These efforts led to the development of an on-going protocol designed for a **prospective pathological study** aiming for a pathological validation of previous results. Specifically, the main focus of this analysis will be the ability to recognize the preferential drainage lymphatic pathway of rectal cancer through the analysis of biological samples from patients underwent rectal surgery.

Despite the experimental nature of our analyses and the lack of robust supportive literature on this topic still constraint our results to speculations, the promising results from our first study and the expected ones from the ongoing prospective analysis pave the way for future clinical applications. A **prospective operative protocol** will be set up as third step of this multidisciplinary project, once the results from feasibility radiological and validation pathological study will be assessed. It will focus on the operative and clinical validation of TEM navigation and SLN removal on ex-vivo TME specimen of patients underwent rectal surgery. The meaning will be to assess the possibility to surgically perform proper SLN removal with TEM, the result being confirmed by pathologist.

In this pioneering setting, that perfectly aligns with increasingly widespread concepts such as tailored surgery and organ preserving strategies, accurate patient selection appears to be mandatory. Despite endoscopic biopsy is considered to be the standard of care for tumor diagnosis, it could be inaccurate in up to 20%[17] of rectal polyps, as rectal cancers often commence as adenomatous lesions and superficial biopsies may miss a malignant focus.
The spread of Artificial Intelligence (AI) and its applications in many areas of modern medicine already offers a complementary rather than an alternative proposal. As a matter of fact, it has been already demonstrated, through the use of fluorescent indocyanine green (ICG) and near-infrared (NIR) imaging, that perfusion is visibly different between cancerous, benign and healthy tissues and that this discovery can be exploited for clinical use by the application of AI methods[18]. This idea, combining computer vision and machine learning has significant clinical implications, and represents the core of **CLASSICA**: validating artificial intelligence in classifying cancer in real time surgery[19], a Horizon project in which Università di Torino is actively involved as partner centre among other European Universities and Hospitals.

The research areas explored in this thesis serve as foundational stepping stones for future applications, emphasizing the importance of continued exploration and multidisciplinary collaboration in oncology. By building upon these insights, we can further enhance our understanding of colorectal cancer, ultimately leading to better diagnostic tools and treatment options that will improve survival rates and quality of life for patients facing this challenging diagnosis.