Applications of the tumor-stroma ratio: Towards clinical implementation

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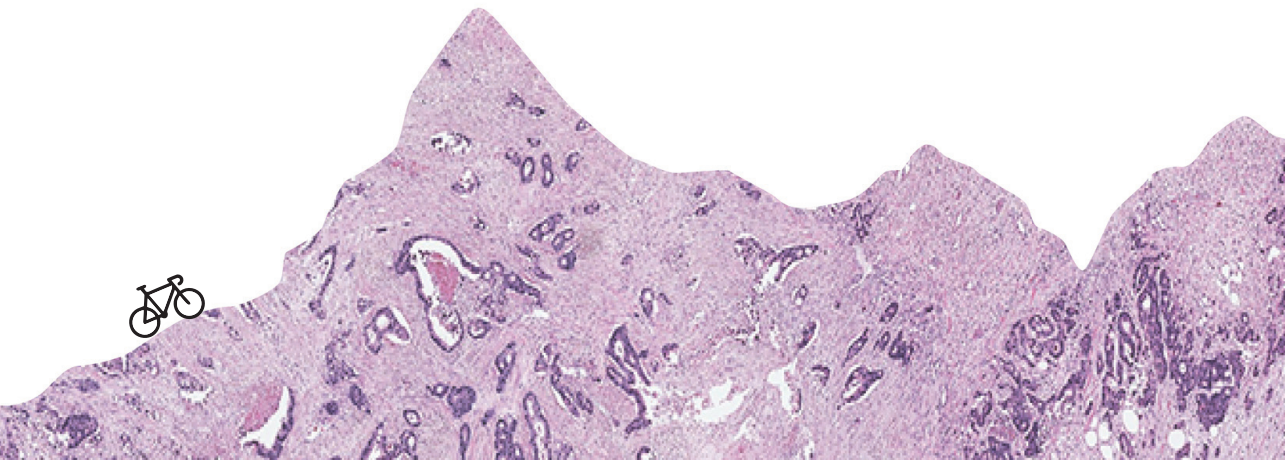
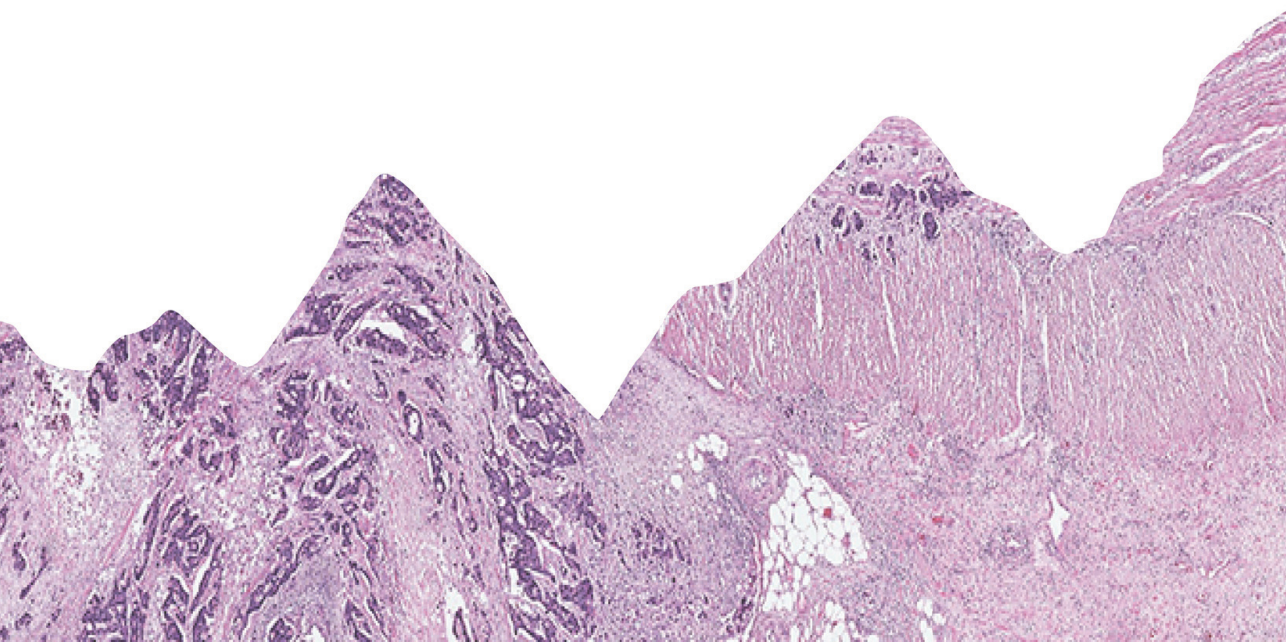


CHAPTER 1

General introduction and thesis outline



The tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC) provides prognostic information and aids in clinical decision making [1-3]. It is developed to define the anatomical extent of tumor spread, making epidemiological studies comparable, and is commonly used for clinical decision making since it has prognostic impact. However, it's becoming more evident that additional prognostic factors are needed [4]. For example, 5-25% of stage II colorectal cancer patients will develop recurrence of disease within 5 years. In addition, patients with stage IIB have a worse prognosis compared to stage IIIA colorectal cancer patients, which could lead to undertreatment (stage II) or overtreatment (stage III) [5]. Previously proposed additional biomarkers have been based on tumor cell characteristics, like tumor cell morphology, molecular pathways, gene expression and the tumor immune response [6-8]. A disadvantage of some of these is the high costs of generating genetic and transcriptomic data.

After years of focusing on the malignant cells to understand the role of tumor suppressors and oncogenic factors in the transformation to malignancy, nowadays the so-called tumor microenvironment (TME) is subject of investigation as well. It is increasingly known that the malignant cell relies on this stromal part of the tumor and therefore does not act alone. Intratumoral stroma within the TME is variable and different cell-types like infiltrating immune cells, cancer-associated fibroblasts, endothelial cells and pericytes all play a role in supporting malignant transformation, invasion of the tumor and metastasis [9, 10]. Moreover, intratumoral stroma has been associated with reduced chemotherapy delivery [11] and increased chemotherapy resistance [12], and consequently could play a role in patient treatment outcome.

A prognostic biomarker based on the TME is the tumor-stroma ratio (TSR), which is the main subject of this thesis. This biomarker is based on microscopic pathological analysis on conventional hematoxylin and eosin (H&E)-stained paraffin sections and assessment is fast, cheap and reliable. It has been shown to link patients with a high stromal content to worse prognosis. Since 2007, when Mesker et al. first published the TSR to be of prognostic value for survival of stage I-III colon cancer patients, many studies have validated this finding in different types of epithelial cancer [13-32].

An extensive literature overview on the background of tumor stroma and the tumor-stroma ratio is given in **Chapter 2,** followed by a detailed description of the assessment of the tumor-stroma ratio in **Chapter 3**, including possible pitfalls and recommendations.

Metastatic lymph nodes have important prognostic implications. In colorectal cancer, lymph node-negative patients have a more than 20% higher survival rate after 5 years compared to lymph node-positive patients [5]. Furthermore, research has shown that not only the number of positive lymph nodes is important for patient outcome, but also the composition of the microenvironment within the lymph node metastasis [33]. In **Chapter 4** we evaluated the TSR in metastatic lymph nodes of stage III colon cancer patients to investigate the possible additional prognostic value. Moreover, it was of interest whether the TSR in the lymph nodes was associated with the TSR in the primary tumor. These same research questions have also been investigated within a cohort of primary breast cancer patients. In **Chapter 5** the results of these studies on the prognostic importance of the TSR in metastatic lymph nodes have been summarized.

The search for prognostic and predictive biomarkers for implementation in routine clinical diagnostics has already resulted in several biomarkers which nowadays are used in the clinic to characterize colorectal tumors and determine specific treatment. Amongst them are the microsatellite instability (MSI) status and mutations in *BRAF* and *KRAS*. MSI status is the most consistently used biomarker for colorectal cancer prognosis in clinical practice, whereas *BRAF* and *KRAS* mutations are primarily used in the metastatic setting. However, in general, colorectal cancer patients are being treated in the adjuvant setting regardless of clinical or molecular characteristics, with heterogenous response to treatment. Patients with a *BRAF* mutation are known to have a poor prognosis and to not respond well to standard chemotherapy or *BRAF* targeted therapy [34, 35]. Studies showed the existence of different colorectal cancer subtypes [6], and even more specifically, two *BRAF* mutant subtypes [36]. Hence, the need for understanding the biology of *BRAF* mutated colorectal cancer and recognizing the different subtypes to select the most effective treatment for these patients. Unfortunately, most of the methods to detect these subtypes are based on gene expression arrays, which are, as stated before, difficult and expensive to implement clinically, whereas the TSR is easier to implement with little additional costs. Therefore, as described in **Chapter 6,** we investigated whether the TSR in combination with the *BRAF* mutation status could select for a subgroup of patients who might need a different treatment approach.

Due to changes in the tissue composition induced by neoadjuvant chemo- and/or radiotherapy, patients who are treated prior to surgery need to be excluded for TSR scoring [37-40]. As a consequence, rectal cancer and esophageal cancer patients, who often receive neoadjuvant therapy, are usually excluded from TSR studies. Analyzing the TSR in biopsies to predict the

prognosis of the patient might be an alternative, although the TSR cannot be determined at the most invasive front. However, in esophageal cancer, for instance, it has been shown that the TSR score assessed on biopsies could still be used as an independent prognostic factor for survival [41]. Especially for stroma-high cases it might be of interest to use the TSR as prognosticator, as the correlation between biopsies and primary tumors was found to be 100%. Another highly interesting approach to use the TSR in biopsies, is to see if it could aid in the prediction of the response to neoadjuvant treatment. This might prevent unnecessary neoadjuvant therapy for patients who are likely not to respond well to the treatment and therefore could continue directly with resection. This potential use of the TSR was investigated in both rectal cancer (**Chapter 7**) as well as in esophageal cancer (**Chapter 8**) patients.

Finally, the research published in this thesis is summarized and discussed in **Chapter 9**. The future perspectives on how to continue this line of research towards implementation for patient care are described here as well. **Chapter 10** describes the summary, discussion and future perspectives in Dutch.

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