The tumor-stroma ratio in epithelial cancer types: towards implementation in diagnostic pathology

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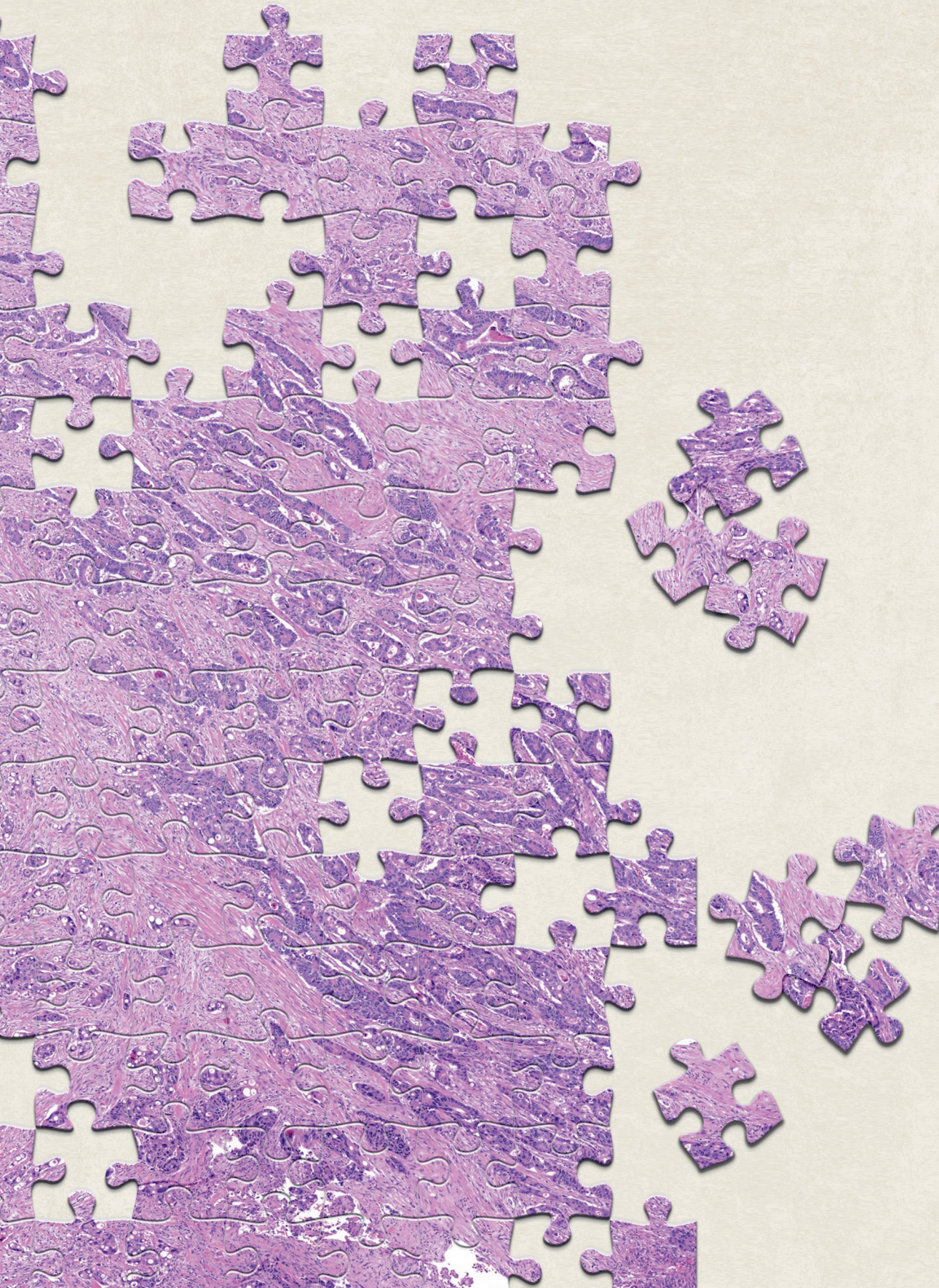
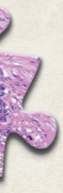
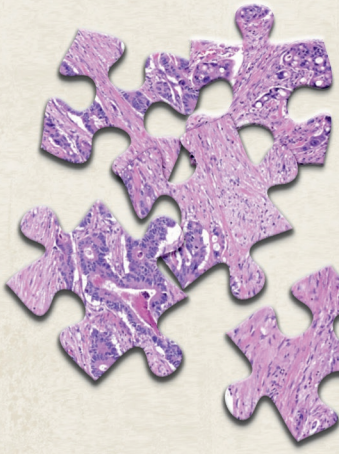
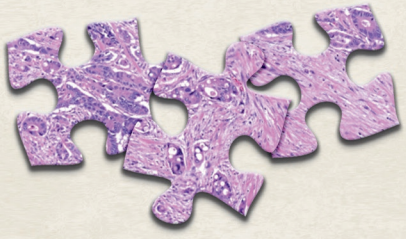


CHAPTER 1

General introduction and thesis outline







Colon cancer and prognosis

Colon carcinoma is one of the most common cancer types worldwide (1). Although survival rates are increasing as a result of population screening and new treatment strategies, there is still a need for improvement (2). In clinical practice, the Tumor Node Metastasis (TNM) classification in combination with the American Society of Clinical Oncology (ASCO) criteria containing high-risk factors, is used for staging. This stage subsequently forms the basis for disease prognosis and treatment (3, 4). Current guidelines advise adjuvant chemotherapy for stage III colon cancer and stage II colon cancer with one or more high-risk factors. However, there is still much over- and undertreatment: despite the selection criteria for adjuvant chemotherapy, there are still patients who do not benefit from this and are thus overtreated, while another subset of patients, who were not high risk, is undertreated and will develop a recurrence of disease or metastasis (5). This reflects the fact that disease progression is caused by more than staging alone, since there is enormous heterogeneity of colon cancer tumors within a single disease stage, emphasizing the need for better risk stratification and the development of additional criteria to select patients at risk for disease progression and/or recurrence.

Personalized medicine (a personalized approach for each patient based on multiple factors) is increasing in relevance to the field of medical oncology. Herein, not only the patient's current condition and medical history is taken into account, but also the tumor stage and other characteristics of the tumor, like genomics. This risk stratification strategy is subject to much research, and new biomarkers are being studied to aid in this. In addition to the development of new treatment strategies based on tumor characteristics for instance, new laboratory techniques have been developed as well. Over the past couple of years, DNA analysis and whole genome sequencing of tumors have become increasingly available for patients, resulting in better understanding of the tumor biology and improved subgroup classifiers. This categorization, in combination with the current TNM classification, in turn helps identify patients at risk for disease progression or who will benefit from adjuvant treatment. Not only in the area of molecular pathology new techniques for these classifiers have been developed, but in the field of histopathology (tissue examination) new strategies are rolled out as well. The tumor microenvironment and certain characteristics of the tumor microenvironment, for example, are increasingly being studied.

Biomarkers

Biomarkers are measurable factors of normal biological or pathogenic processes and are studied for a better understanding of biologic pathways in the tumor. Moreover, biomarkers can be studied for their prognostic value, i.e. prognosis of patient outcome like disease

recurrence or survival, and/or predictive value, describing the potential of therapy response (6, 7). A prognostic biomarker is not by definition a predictive biomarker, or vice versa. However, hurdles must be overcome and criteria must be fulfilled before a new biomarker can be implemented in clinical (pathology) practice. Adequate robustness, reproducibility and validity are required, for instance. A biomarker is preferably validated in a prospective setting, ideally multicenter, with quality control during the process (7, 8). Moreover, publication of an accurate description of the methods, including laboratory techniques and/or scoring protocols, is highly recommended (7).

Tumor-stroma ratio

The last decades, it has become more clear that the tumor microenvironment, or tumor-stroma, plays an important role in tumor development and progression. A novel biomarker which has been discovered, defined, and which takes the tumor microenvironment into account is the tumor-stroma ratio (TSR). This biomarker is based on the amount of stroma in the primary tumor and has shown to be an independent prognosticator for disease-free and overall survival in various epithelial cancer types, including colon cancer (9-11). The TSR scoring method is based on the amount of stroma in relation to the amount of tumor epithelial cells, is robust and has been is described in detail (12). The experienced pathologists can easily score the TSR in daily routine on diagnostic hematoxylin and eosin (H&E) stained tissue slide sections of the carcinoma in 1-2 minutes through conventional microscopy. The amount of stroma is scored in increments of 10 percent, and tumors are subsequently categorized in two groups based: stroma-low tumors, with 50% or less stroma, and stroma-high tumors, containing more than 50% stroma. This categorization is based on risk stratification, in which patients with stroma-high tumors have a worse disease-free and overall survival (11, 12).

To meet the requirements for implementation of the TSR as a new biomarker into clinical practice, the UNITED study (Uniform Noting for International application of the Tumor-stroma ratio as Easy Diagnostic tool) was initiated. In ***Chapter 2***, the published UNITED study protocol is described. To promote the UNITED study and to provide more information on the study to pathologists worldwide, a promotion article which has been published in 'The Pathologist' (the magazine for pathologists) can be found (in ***Chapter 3***). For the first part of the UNITED study, an E-learning was developed to confirm the reproducibility and easily acquired knowledge of the TSR scoring method. The second part of the UNITED study was developed to validate the prognostic value of the TSR in a prospective colon cancer cohort.

A biomarker should be reproducible. By a specialist him/her self, but also between colleagues. To measure this agreement within one observer, one looks at the intra observer agreement.

Between two (or more) observers the interobserver agreement is measured. Cohen's kappa is the statistical analysis which is often performed. It looks into the agreement of scoring, corrected for chance, a number between 0-1 is the result. 0 means no agreement and 1 means a perfect agreement (13). The intra- and interobserver agreements published for scoring the TSR are good (kappa between 0.6-0.9). These published observer agreements were determined between two or maximum four observers (12, 14). However, no data is available about how these pathologists were trained and how the learning curve is for the scoring method of TSR.

In various medical specialisms, an E-learning has been shown to be a good and easy method to learn and introduce a new technique (15-19). One of the advantages of an E-learning is that many potential participants, e.g. specialists and trainees, can be reached simultaneously and globally. Moreover, learning curves can be monitored in the meanwhile. Before pathologists (or trainees) participate in the UNITED prospective validation study, the E-learning for scoring the TSR should be completed with a good intra- and interobserver agreement (Cohen's kappa at least >0.7). The main goal of this E-learning is to study the reproducibility of TSR scoring. ***Chapter 4*** describes how this UNITED E-learning is set up, the results and the conclusions.

Digital pathology

Over the last years, pathology is becoming more and more digitalized and automated. This provides opportunities for automation of the TSR method, including optimization and standardization of the quantification of stroma. Through eyeballing, scoring the TSR may be challenging in the tumors containing 50 to 60 percent stroma, the so-called "grey area". These cases are where discrepancies arise in interobserver analysis. Quantifying the absolute stroma percentage by automation could provide more certainty for cases around the cut-off value. In order to implement the TSR as extra high-risk factor in addition to the TNM classification, a dichotomized biomarker based on the quantified stroma percentage would be best for application. In ***Chapter 5***, the results of deep learning algorithms compared to scoring the TSR by eyeballing are described and discussed.

Worldwide, the focus is shifting towards machine learning and artificial intelligence. For example, Zhao et al. described how to quantify the amount of tumor-stroma in colorectal cancer on whole slide images by artificial intelligence (20). Moreover, the independent prognostic value of the TSR in this study cohort was proven as well. In ***Chapter 6***, our opinion regarding this research article in the field of quantifying the TSR in colorectal cancer is given in a commentary. Herein, the importance of research stimulating a more digitized pathology workflow is advocated and two main subjects of discussion are reviewed. First,

the quantification of the amount of stroma on a whole slide image instead of in a field similar to a microscopic view is analyzed, and second, the importance of stain normalization due to variation in colors of H&E stained sections and the sensitivity of deep learning algorithms.

Tumor budding

In addition to the TSR, many other biomarkers have been studied over the last couple of years, including tumor budding. Patients with a tumor with high-budding have a worse survival compared to patients with a tumor with low-budding. Tumor budding can, like the TSR, be scored during pathology routine diagnostics on H&E stained tissue slides. A tumor bud is defined as a single tumor cell or small cluster (up to 4 tumor cells) at the invasive front of the tumor. For stages I and II in colon cancer it is recommended to report tumor budding, whereas for stage III, there is no clear need (21). Tumor budding can be scored in different ways, thus a wide spread of interobserver agreements is reported. Sometimes only a weak correlation (Cohen's kappa 0.2-0.4) was described at times between two observers, despite the fact that a consensus agreement of scoring protocol has been published. ***Chapter 7*** looks into the association between tumor budding and the TSR in colon cancer stages II and III and the degree of reproducibility.

Other tumor types

The TSR has shown to be a prognostic biomarker in various epithelial cancer types, including breast cancer. The scoring method is similar in all cancer types. Moreover, with stroma-high tumors leading to a worse disease outcome (9-11). However, for breast cancer, a molecular test, the 70-gene signature (70-GS) also known as the MammaPrint®, aids in identification of patient subgroups likely to benefit from adjuvant chemotherapy after curative surgery (22, 23). This panel of 70 genes includes tumor related genes as well as stroma related genes (22, 23). The TSR and the 70-GS have both shown to be independent prognostic biomarkers, and have shown to be better prognostic biomarkers in specific subgroups. For example, the TSR has more prognostic value in triple negative and estrogen receptor negative breast cancer, compared to other subtypes (24, 25). In ***Chapter 8***, the association between the 70-GS and the TSR in breast cancer is investigated.

Another, less favorable type of cancer, but prevalent type of cancer is lung cancer. Moreover, patients with lung cancer have a poor prognosis with 5-year overall survival rate of approximately 15%, thus, much is to be improved. Lung cancer can be divided in two main groups: the small cell lung carcinomas (SCLC) and the non-small cell lung carcinomas (NSCLC). The NSCLC group is mostly comprised of adenocarcinomas and the squamous cell

carcinomas (SqCC). SqCC of the lung are nearly always the result of a history of tobacco smoking, whereas adenocarcinomas of the lung have a much wider variety of origins (26). In the case of adenocarcinoma of the lung, immunotherapy is the new treatment strategy after surgery, whereas for SqCC, no specific treatment or tests are available to select patients at risk for recurrence, disease progression or cancer related death. In lung cancer the TSR has not been investigated often and the prognostic effect of the TSR has not yet been validated (27, 28). In ***Chapter 9***, the prognostic value of the TSR in squamous cell lung cancer is investigated.

A summary and a discussion on the results of this thesis are provided in ***Chapter 10***. Topics for future research and next steps of implementation of the TSR in routine pathology are addressed in the future perspectives section, also in ***Chapter 10***.

This thesis focusses on the steps for implementation of the TSR as biomarker into clinical practice, following the route from laboratory development to clinical implementation. During this process, the relationship of the TSR to other biomarker techniques is investigated, as well as the prognostic value of the TSR in other cancer types.

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