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Revolutions in cancer treatment:
how can they be integrated?

Patient selection in oncology: the pathologist's perspective, using the example of colorectal cancer

Frédéric Bibeau
Department of Pathology, CHU de Caen
UNICAEN, Normandy University, Caen, France

Springer Healthcare

Abstract

Pathology is used to select non-metastatic colorectal cancers (CRCs) patients who are candidates for adjuvant chemotherapy, essentially on the basis of the pTNM classification. This selection process is not perfect, however, and it could be improved through possible refinements to the TNM classification system and validation of emerging morphological markers such as tumour budding, poorly differentiated clusters, stroma and the immune response. Digital pathology and artificial intelligence are also expected to contribute towards identifying these markers quickly and in a standardised way. In patients with metastatic CRC, treatment selection is largely based on molecular markers, which may be incorporated into treatment algorithms for rational decision-making. The aim is to develop an integrated approach that brings together factors from the fields of morphology, molecular biology and liquid biopsies.

Pathology involves analysing tissue lesions caused by disease, and is a critical specialty in the management of cancers. In response to advances in modern oncology, this morphological discipline has been enriched to include complementary investigations such as immunohistochemistry and *in situ* hybridization, creating interfaces with molecular biology, specifically in the analysis of nucleic acids from specimens fixed and embedded in paraffin. The contribution of pathology to oncology practice therefore covers a wide spectrum: precise diagnosis of malignant tumours, determination of many histoprognostic factors, including the pTNM classification,[1] and the identification of protein changes that indicate pathological processes and molecular abnormalities. The information obtained from pathological analysis may also help to predict response or resistance to a particular systemic or targeted treatment.

Patient stratification, however, needs to be refined in terms of both prognosis and prediction. This improved selection process may be based on improvements in the parameters mentioned above (pTNM classification, molecular markers etc.) and a so-called integrative approach, in which information from multiple factors are combined in rational ways. In addition, artificial intelligence can allow a more detailed histological analysis of tumours, creating opportunities to revisit "morphology": the cellular characteristics of the tumour, its architecture, stroma and immune microenvironment. Details of the tumour can be integrated synergistically into an assessment that considers cellular components that have not been adequately analysed until now. The role of pathology in patient selection and in so-called precision medicine is described here in an overview using the context of non-metastatic colorectal cancers (CRCs).

SELECTION OF PATIENTS WITH NON-METASTATIC COLORECTAL CANCER

The majority of patients with stage II CRCs, i.e. those without metastasis in regional lymph nodes (80%) are cured by surgery. Adjuvant chemotherapy is only beneficial in 5% of patients and about 15% of them have a recurrence despite adjuvant treatment. For patients with stage III CRC, which is defined by the presence of metastatic regional lymph nodes, the therapeutic window is even narrower: most patients (60%) benefit from surgery alone, 20% benefit from chemotherapy, and the relapse rate is 20%.

One of the roles of the pathologist today is to correctly determine conventional histoprognostic factors and also to identify additional markers, in order to improve the selection of patients who will truly benefit from adjuvant treatment.

CONVENTIONAL FACTORS CURRENTLY USED FOR PROGNOSTIC STRATIFICATION OF PATIENTS

The pTNM classification

The pTNM classification system is commonly used to define histoprognostic factors that are useful for treatment purposes.[1] In CRC, a clear schematic distinction is made between stage II without regional metastases and stage III with metastatic regional lymph nodes, irrespective of the tumour extent.[1] Serosal invasion, a histological parameter that is likely to alter the prognosis and the type of treatment, is an important morphological factor which must be analysed accurately. It may prove difficult to determine in some situations, which can potentially lead to underestimation.[2]

Regional lymph node involvement is an important factor that must be taken into account. In the past, adjuvant chemotherapy was only given if one or more positive lymph nodes were found; the most common regimen was FOLFOX, given for 6 months. This approach has now changed: patients are defined as being at low risk or high risk of recurrence, depending on the number of lymph nodes involved and the pT stage (pT1-3 and N1: low risk; pT4 and/or N2: high risk). A risk-based approach may change the duration of chemotherapy. This is extremely important in terms of neurotoxicity risk, which is linked to the duration of exposure to oxaliplatin.

The IROCAS phase 3 randomised study, which is currently being conducted, is testing the usefulness of intensifying chemotherapy by comparing the effects on survival of treatment with FOLFIRINOX (oxaliplatin, folinic acid, irinotecan and 5-fluorouracil) and FOLFOX (oxaliplatin, folinic acid and 5-fluorouracil) in the adjuvant setting, in patients with stage III colon cancer and extensive lymph node invasion (N2).[3] This underlines the importance of rigorously determining the pTNM stage.

In the latest pTNM classification, tumour deposits classified as N1c correspond to nodules of tumour cells, with no invasion of regional lymph nodes.[1] When tumour deposits are associated with positive lymph nodes, the risk of peritoneal, pulmonary and hepatic metastatic recurrence is higher.[4] The presence of tumour deposits is severely underused as a prognostic marker at present and it seems logical for the pTNM classification to incorporate this morphological parameter in future to improve prognostic stratification of patients. The study by Delattre et al recently analysed the impact of tumour deposits in the IDEA (International Duration Evaluation of Adjuvant Chemotherapy) trial, which compares 3- and 6-month periods of adjuvant chemotherapy (FOLFOX or CAPOX) in stage III cancers.[5,6] This retrospective study shows that the presence of tumour deposits is an important independent prognostic factor for disease-free survival in patients with stage III CRC.[6] Their presence could be integrated as a factor to modify the duration of adjuvant chemotherapy. All these data therefore emphasise the importance of the pTNM classification for stratification of patients with CRC for current and future treatments.

Other histoprognostic factors

The presence of venous emboli is another important morphological parameter that must be assessed. This factor is linked to a poor prognosis and identifies patients with stage II CRC who are at higher risk of recurrence. This histoprognostic factor is, however, underestimated in 30% of cases.[7] Perineural infiltration is also a poor prognostic factor in stage II CRC. Either of these findings would justify discussing adjuvant chemotherapy, in the context of a multidisciplinary tumour board.[7]

In stage II CRC, the risk of recurrence is also based on the grade and the patient's microsatellite instability (MSI) status. Patients who present with high-grade tumours with MSI have been found to have a better prognosis than patients with high-grade tumours that do not have MSI. These differences can be attributed to the favourable immune environment associated with MSI, which allows the host to defend itself against the tumour. It must be emphasised that MSI status has to be determined using rigorous procedures at expert centres. A number of studies have found a non-negligible proportion of false positives (≈10%) mainly in the area of molecular biology.[8] This observation supports the importance of quality control in relation to the use of biomarkers.

EMERGING MARKERS

Immunohistochemical and molecular markers

Patients can be stratified using other simple markers based on immunohistochemistry and/or molecular biology, such as *BRAF* mutational status. In this context, a study by Toon et al.[9] revealed that the prognosis is better for patients with MSI, whether *BRAF* mutated or not mutated, compared with patients without MSI who are *BRAF* mutated based on immunohistochemistry. CDX2 is another promising marker that can be analysed using immunohistochemistry.[10] Loss of this marker is associated with a poor prognosis. This marker, which is now attracting more interest, could also be included in this relatively simple and inexpensive prognostic stratification method.

The Consensus Molecular Subgroup (CMS) molecular classification system comprises four groups (CMS1 to CMS4), each with distinct prognostic features.[11] The CMS1 "immune" group presents with a MSI-positive phenotype and is associated with better median recurrence-free survival (RFS) than any of the other groups, but has the most unfavourable median survival after recurrence (SAR). The CMS2 group, which is also called "canonical", has intermediate RFS, but the highest overall survival (OS) and SAR of all four groups. The CMS3 group, which is

referred to as "metabolic", is associated with RFS, OS and SAR that are all in the intermediate range. The CMS4 group has the lowest RFS and OS of the four groups, and an SAR in the intermediate range. This CMS classification, although it is appealing, cannot currently be used in clinical practice.[11]

Histological markers of the invasion front: tumour budding, poorly differentiated clusters and stroma

Tumour budding is a morphological marker of interest, since it corresponds to the presence of isolated tumour cells at the invasion front, often considered as the first step in the metastatic process.[12] A number of studies have shown that the presence of budding was linked to a poor prognosis in stage II CRC.[12] International studies have proposed a classification of this morphological parameter to improve its reproducibility.[13] Nevertheless, although it is mentioned as an additional prognostic factor in the TNM classification, tumour budding is not currently taken into account when stratifying patients for adjuvant chemotherapy.

Poorly differentiated clusters (PDCs) are another characteristic of tumour cells present at the invasion front.[14] These are groups of tumour cells, more numerous than those seen in tumour budding (more than five tumour cells in the case of PDCs, compared with isolated cells or fewer than five cells for tumour budding). PDCs are associated with a poor prognosis. Their use has not yet, however, been validated in the adjuvant setting.[14]

The fibrous stroma that accompanies the tumour is another emerging prognostic factor that could improve patient selection. Non-metastatic CRCs accompanied by a strong stromal component have a poorer prognosis than those without this characteristic. A number of research programmes have been set up to evaluate this factor in a way that is reproducible and rigorous, and to establish its validity. A prospective, multi-centre cohort study has been proposed,[15] and a training programme dedicated to the evaluation of stroma (watchstroma.com)[16] has been developed by Leiden University and is accessible online.

It should be emphasised that the morphological characteristics of tumours often reflect underlying molecular changes. Therefore, it seems logical to develop correlations between phenotype and genotype. Further progress in the use of morphology and the assessment of validated or emerging markers may involve the use artificial intelligence (AI) and machine learning. In this process, computers could 'learn' to recognise areas of interest (tumour budding, PDCs, stroma, immune response etc.) and rapidly assess them in a standardised way that could be integrated into clinical practice. Eventually, algorithms incorporating morphological biomarkers may be used for both prognosis and prediction.

Immune microenvironment markers

In addition to morphological features of the tumour, host-related factors also have a prognostic role. The host's immune system has an important influence on the control of tumours.[17] When a localised CRC is accompanied by an adaptive, protective lymphocytic infiltrate, the tumour is associated with fewer vascular emboli, less perineural infiltration, and fewer metastatic lymph nodes, and ultimately the prognosis is better, with fewer recurrences.

A number of methods, such as the Immunoscore® test, have been developed to evaluate this inflammatory immune reaction,[18] but none has yet been validated for use in clinical practice. These methods could make it possible to define the place of chemotherapy more effectively in patients with stage II and III CRC.

We should also mention tertiary lymphoid structures, which are morphological indications of adaptive immunity and associated with a good prognosis. These structures are easily visible morphologically or after immunohistochemical multiplexing, but they are also not currently taken into account in clinical practice.

SELECTION OF PATIENTS WITH METASTATIC COLORECTAL CANCER

Tumour sites are not always histologically documented at the metastatic stage because of the inoperability of metastatic sites and the natural history of CRC (metastatic disease can most commonly be linked to a colorectal

primary). In a number of specific cases, however, a biopsy is necessary to be sure of the diagnosis. The pathologist is usually called upon to determine the molecular abnormalities, usually on the basis of the histologically documented primary tumour. In most cases, the molecular abnormalities of the primary tumour match those of the metastatic site. The molecular abnormalities that are looked for most frequently are MSI and *RAS* and *BRAF* status.

Others are not often determined, such as *HER2* amplification or translocations of *NTRK, ALK* and *ROS*.[19] The latter may be a target for screening using immunohistochemistry and in situ hybridization, but a decision-making algorithm for patient selection has not yet been defined.

The morphological characteristics of resected metastatic sites after preoperative treatment, particularly from the liver, may provide useful prognostic information. It is now known that the interface between the metastasis and the healthy liver is an important prognostic indicator,[20] with a desmoplastic interface associated with a better prognosis.

The histological response of resected CRC metastases is no longer used in clinical practice, but it may come back into use, particularly in the era of immunotherapy, as it has been shown in melanoma.[21] Indeed, a complete histological response translates into an excellent prognosis, potentially indicating that patients can be managed by monitoring, rather than maintenance treatment.[21] In lung cancer, in the neo-adjuvant setting, a major histological response is often associated with an adaptive immune environment, which suggests a favourable outcome.[22] All these data must be taken into consideration in future trials in gastrointestinal oncology.

CONCLUSION

It is possible to select patients with non-metastatic CRC on the basis of simple morphological criteria but these have to be determined rigorously. It is vital to respect existing fundamental parameters of morphology, such as the pTNM classification, although this classification needs to be updated. An integrated approach that brings together factors from the fields of morphology, molecular biology and liquid biopsies is an approach that should be pursued in the future. Digital pathology and AI will allow us to make better use of emerging morphological markers and "see what the eye does not see".[23]

REFERENCES (Underlined references link to PubMed abstracts)

1. Sobin LH, Gospodarowicz MK, Wittekind C (2017). TNM Classification of Malignant Tumours, 8th edn. Wiley-Blackwell: New York.
2. Frankel WL, Jin M. Serosal surfaces, mucin pools, and deposits, oh my: challenges in staging colorectal carcinoma. Mod Pathol 2015; 28 Suppl 1:S95-108.
3. Bennouna J, André T, Campion L, Hiret S, Miglianico L, Mineur L, et al. Rationale and design of the IROCAS study: multicenter, international, randomized Phase 3 trial comparing adjuvant modified (m) FOLFIRINOX to mFOLFOX6 in patients with high-risk stage III (pT4 and/or N2) colon cancer-A UNICANCER GI-PRODIGE Trial. Clin Colorectal Cancer 2019; 18:e69-e73.
4. Nagtegaal ID, Knijn N, Hugen N, Marshall HC, Sugihara K, et al. Tumor deposits in colorectal cancer: improving the value of modern staging - A systematic review and meta-analysis. J Clin Oncol 2017; 35:1119-1127.
5. André T, Vernerey D, Mineur L, Bennouna J, Desrame J, Faroux R, et al. Three versus 6 months of oxaliplatin-based adjuvant chemotherapy for patients with stage III colon cancer: disease-free survival results from a randomized, open-label, International Duration Evaluation of Adjuvant (IDEA) France, Phase III Trial. J Clin Oncol 2018; 36:1469-1477.
6. Delattre JF, Cohen R, Henriques J, Falcoz A, Emile JF, Fratte S, et al. Prognostic value of tumor deposits for disease free survival in patients with stage III colon cancer: A post hoc analysis of IDEA France phase III trial (PRODIGE-GERCOR). J Clin Oncol 2020; Mar 13:JCO1901960.
7. Nagtegaal ID. Current concepts of colorectal cancer resection pathology. Histopathology 2015; 66:102-111.

8. Cohen R, Hain E, Buhard O, Guilloux A, Bardier A, Kaci R, et al. Association of primary resistance to immune checkpoint inhibitors in metastatic colorectal cancer with misdiagnosis of microsatellite instability or mismatch repair deficiency status. JAMA Oncol 2019; 5:551-555.
9. Toon CW, Chou A, DeSilva K, Chan J, Patterson J, Clarkson A, et al. BRAFV600E immunohistochemistry in conjunction with mismatch repair status predicts survival in patients with colorectal cancer. Mod Pathol 2014; 27:644-650.
10. Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 2016; 374: 211-222.
11. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. PLoS Nat Med 2015; 21:1350-1356.
12. Wang LM, Kevans D, Mulcahy H, O'Sullivan J, Fennelly D, Hyland J, O'Donoghue D, Sheahan K. Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol 2009; 33:134-141.
13. Lugli A, Kirsch R, AjiokaY, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 2017; 30:1299-1311.
14. Konishi T, Shimada Y, Lee LH, Cavalcanti MS, Hsu M, Smith JJ, et al. Poorly differentiated clusters predict colon cancer recurrence: an in-depth comparative analysis of invasive-front prognostic markers. Am J Surg Pathol 2018; 42:705-714.
15. Smit M, van Pelt G, Roodvoets A, Meershoek-Klein Kranenbarg E, Putter H, Tollenaar R, et al. Uniform noting for international application of the tumor-stroma ratio as an easy diagnostic tool: protocol for a multicenter prospective cohort study. JMIR Res Protoc 2019; 8:e13464.
16. UNITED E-learning. http://watchstroma.com/the-stroma-research/. Last accessed 9 April 2020.
17. Galon J, Fridman WH, Pagès F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 2007; 67:1883-1886.
18. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018; 391:2128-2139.
19. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. Arch Pathol Lab Med 2017; 141(5):625-657.
20. Eefsen RL, Vermeulen PB, Christensen IJ, Laerum OD, Mogensen MB, Rolff HC, et al. Growth pattern of colorectal liver metastasis as a marker of recurrence risk. Clin Exp Metastasis 2015; 32:369-381.
21. Tetzlaff MT, Messina JL, Stein JE, Xu X, Amaria RN, Blank CU, et al. Pathological assessment of resection specimens after neoadjuvant therapy for metastatic melanoma. Ann Oncol 2018; 29:1861-1868.
22. Cottrell TR, Thompson ED, Forde PM, Stein JE, Duffield AS, Anagnostou V, et al. Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small-cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC). Ann Oncol 2018; 29:1853-1860.
23. Kather JN, Pearson AT, Halama N, Jäger D, Krause J, Loosen SH, et al. Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. Nat Med 2019; 25:1054-1056.