The role of the pathologist and clinical implications in colorectal liver metastasis

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Abstract

Colorectal liver metastases (CRLM) affect about 50% of colorectal cancer patients. With the improvement of neoadjuvant chemotherapy and the introduction of targeted therapy, resectability of CRLM and survival rates have improved over time. However, 60-70% of patients still recur. Several pathological and molecular parameters have been described as prognostic factors after CRLM resection. These parameters encompass not only tumoral features, but also non-tumoral ones, such as chemotherapy related liver injury, or factors related to tumour environment, namely Immunoscore. This review summarizes these prognostic indicators to clarify which patho-molecular parameters should be addressed in the pathological report. (Acta Gastroenterol. belg., **2018**, 81, **419-426**).

Key words: tumour regression grading, histopathological growth pattern, chemotherapy related liver injury, Immunoscore, RAS, prognostic scoring system

Introduction

Colorectal cancer is the third leading cause of cancer death in the world and nearly 50% of patients develop colorectal liver metastasis (CRLM) (1). Despite advances in pre-operative treatment, 60-76% of patients recur after CRLM resection, and interestingly, 50% recur within the first two years (2, 3). However, there is currently no clear way to identify those patients at risk of recurrence.

To date, several pathological features have been described as prognostic factors, and these include tumour regression grading (TRG), and histopathological growth pattern (HGP) as an indication of efficacy of pre-operative treatment, and steatohepatitis, nodular regenerative hyperplasia (NRH), and sinusoidal obstructive syndrome (SOS), as chemotherapy related liver injury (CALI).

The aim of this review is to summarize these prognostic patho-molecular parameters to help pathologists provide an appropriate pathological report for CRLM patients and for the clinician to understand the importance of these parameters (Table 1).

Macroscopic evaluation of the resection specimen

Macroscopic evaluation and sampling of CRLM is a crucial and defining step and the surgical samples should be treated and examined accordingly.

The resection specimen should be macroscopically evaluated in sections of 5 mm thickness. Before cutting, the surgical margin (SM) can be inked. The macroscopic aspect should then be carefully evaluated and each lesion described with respect to aspect, size, and distance from the SM. The specimen should be fixed in formalin for a maximum of 24-48 hours. Fixing in excess of 48 hours should be avoided to preserve the quality of the DNA for molecular tests (4). Specimens from each CRLM, the SM with respect to the lesions, and the surrounding liver parenchyma (taken as far as possible from the lesion to avoid the mass-effect artefact) should be collected. It is desirable to sample the entire lesion for a precise assessment. Otherwise one sample per centimetre, including both the centre and the periphery of the lesion, should be collected (5).

Anatomical evaluation

Number of tumours

Up to 60% of CRLM patients present with multiple metastatic lesions (6) and the number of CRLMs is considered to be a negative prognostic factor (7). As such, the pathological examination is crucial, particularly to assess those lesions not clearly detectable by radiological evaluation (8). Survival rates decrease particularly in patients with more than three lesions (6,9,10) ; a possible explanation being the difficulty to achieve a negative SM when there are multiple lesions (11).

Size of the metastasis

The size of the CRLM, for example >5 cm, used to be considered a negative prognostic factor (9,12), especially when the SM is positive (10).

It is, however, important for the pathologist to know if the patient received any pre-operative treatment. Recent literature regarding patients who receive neoadjuvant therapy indicates that the size of the metastasis is not

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Table 1. — **Pathological and molecular parameters that should be included in histology report**

Parameters	Assessment	Clinical significance	Assessment in multiple lesions
Number of metastasis	Macroscopical evaluation	> 3 lesions negative prognosis if resection margin is positive [6]	Characterisation of each lesion
Size of metastasis	Macroscopic measurement before fixation	> 5 cm negative prognosis if resection margin is positive [7]	Measurement of each lesion
Surgical margin(SM)	Macroscopic measurement before fixation	If no neoadjuvant chemotherapy 1 cm If neoadjuvant chemotherapy 1 mm is sufficient 13,18	To mention for each lesion even if SM has an impact when is positive in the bigger lesion
Pathological response to chemotherapy	Tumour regression grading (TRG)[4]	TRG 3,4,5 associates with lower survival	Worst TRG among the lesions
Histological growth pattern (HGP) [34]	Desmoplastic HGP, Pushing HGP, Replacement HGP, Sinusoidal HGP, Portal HGP	Replacement HGP worse prognosis, desmoplastic HGP better prognosis	HGP for each lesion has to be described
Chemotherapy related liver injury (CALI) [22]	Sampling of the liver parenchyma far from the lesion.	Presence of NRH or steatohepatitis associates with shorter outcome; NRH independent predictive factor of postoperative liver failure	NA
RAS and BRAF	Specimen from the primary or liver lesion	KRAS NRAS and BRAF mutations associates with shorter overall survival; KRAS NRAS mutations lack of response to anti-EGFR therapy	The interlesion heterogeneity in RAS mutational status is observed in a negligible percentage of cases.

always an important prognostic factor. This hypothesis may be as a result of the efficiency of neoadjuvant chemotherapy, or to down staging secondary to chemotherapy, which does not reflect the initial size of the tumour (13).

Pathological assessment

Surgical margin (SM)

The SM is well known as an independent prognostic factor, which, when positive, strongly associates with higher intrahepatic recurrence rates (14,15).

Previously, a 1 cm margin was widely considered as the gold standard (12,16), however, recent studies have demonstrated that a 1 mm margin is adequate for a favourable prognosis (17-19).

The situation is different in multiple lesion patients, because recent literature describes that a positive SM has an impact on OS when the margin is positive in the larger lesion (20) and this may be because micrometastases and satellite nodules may be more frequently seen within 2-4 mm of the main tumour, and this distance increases relative to the size of the nodule. Therefore, a detailed and extended evaluation of the surgical margin for each individual lesion is recommended.

Pathological response to neo-adjuvant treatment

Pathological evaluation of the surgical specimen is the most precise method to evaluate tumour regression, compared to radiological examinations that may overestimate the response (21).

To date, there are three main methods to assess tumour regression. The first is a semi-quantitative method described by Blazer at al. (22), the second involves evaluating tumour thickness at the tumour-normal interface (TNI) (23), and the third is tumour regression grading (TRG) (5).

The first method, described by Blazer *et al.*, is based on the percentage of viable tumour cells remaining (complete response, i.e., no residual cancer cells, major response, i.e., 1% to 49% residual cancer cells, and minor response, i.e., >50% residual cancer cells). For patients with multiple tumoral lesions, the response was based on the mean value of all the tumour nodules. The major limitation of this system is that it is based on an estimation of the initial tumoral area and could be subject to variable interpretation.

The second predominant method to evaluate the tumour response to preoperative treatment is tumour thickness at the tumour-normal interface (TNI), proposed by Maru *et al.* (23). This system assesses the maximum thickness of uninterrupted layers of tumour cells, measured perpendicularly. For patients with multiple lesions, the average tumour thickness at the TNI is calculated. The greater the thickness, the shorter the predicted recurrence-free survival (23).

Finally, TRG is a semi-quantitative five-grade system, proposed by Rubbia-Brant *et al.* (5), that assesses the relative proportion of tumoral cells and fibrosis. It is scored as follows : TRG 1 indicates a complete response to treatment with maximal fibrosis ; TRG 2 indicates a good response with only rare neoplastic cells scattered throughout the fibrosis ; TRG 3 indicates more residual tumour cells but predominant fibrosis ; TRG 4, is indicated when residual cancer cells predominate over fibrosis ; and TRG 5 indicates no signs of regression (Fig. 1). Importantly, necrosis should not be considered a response to treatment. TRG 1-2 is associated with a major or complete pathological response and is an

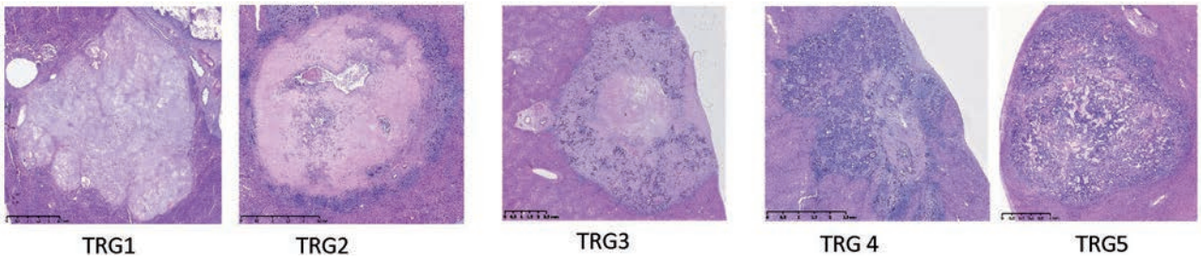

Fig. 1. — Tumour regression grading (TRG) is a grading system based on a quantitative morphological assessment of fibrosis and residual tumour cells. These figures show colorectal liver metastases (haematoxylin and eosin-stained sections) illustrating TRG 1 to 5. TRG 1 indicates complete response to the treatment with maximal fibrosis; TRG 2 indicates a good response with only scattered neoplastic cells in a fibrotic context; TRG 3 shows more residual tumour cells but fibrosis predominates; TRG 4 shows residual cancer cells predominating over fibrosis; and TRG 5 shows no signs of regression.

important prognostic factor for relapse and survival after CRLM resection (24). In cases with multiple CRLM, TRG should be assessed for each lesion. The worst TRG of the multiple lesions appears to correlate with the patient's prognosis (24).

We highly recommend using the TRG score as it is the most widely used method, the least subjective, and is strongly correlated with survival rates. In addition, TRG not only takes into account residual tumour cells, but also fibrosis, which is strongly correlated with improved survival (25,26).

Histopathological growth pattern (HGP)

HGP was described initially by Vermeulen *et al.* (27), and then Van den Eynden *et al.* (28).This method assesses the histological features of the tumoral lesion at the interface with the liver parenchyma. Four patterns are described: desmoplastic HGP, characterized by the presence of a desmoplastic rim between the liver and metastatic tissue; pushing HGP, where the liver is pushed aside by metastatic tissue; replacement HGP, the tumour cells replace normal hepatocytes with minimal alteration in the architecture; and mixed HGP, where growth at the tumour margin shows more than one type of expansion (Fig. 2). The prognostic impact varies depending on the history of treatment. In patients who have not received neoadjuvant treatment, desmoplastic HGP indicates the best outcome, while a pushing HGP indicates more aggressive tumour behaviour compared to the others. This may be due to the pushing effect of the lesion on the surrounding parenchyma, which may stimulate angiogenesis (27). In contrast, in patients who have received pre-operative treatment, mixed and replacement HGP are associated with a significantly poorer prognosis than desmoplastic HGP, and those with pushing HGP tend to have earlier recurrence. This difference in HGP seems to be related to its dynamic nature (29). For example, after systemic treatment with an anti-angiogenetic agent, liver metastases can switch from desmoplastic HGP (an angiogenetic pattern) to replacement HGP (a non-angiogenetic pattern) (30, 31).

A recent consensus report made some changes to the pathological classification of HGP (32). They divided replacement HGP into two types : type 1, liver cell plates used by the cancer cells are perpendicular to the tumour-liver interface; and type 2, liver cell plates are pushed away whilst cancer cells replace the hepatocytes. Moreover, they added two patterns : sinusoidal HGP, where cancer cells are present as emboli within the lumens of the sinusoidal blood vessels, and/or grow in the peri-sinusoidal space ; and portal HGP, where the cancer growth is restricted to the connective tissue areas of portal tracts, liver septa, and the liver capsule.

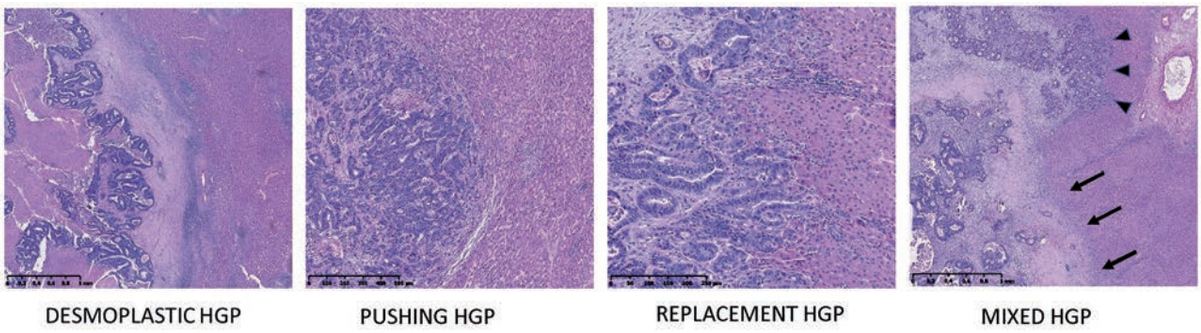

Fig. 2. — Colorectal liver metastases (haematoxylin and eosin-stained sections) showing the different histopathological growth patterns (HGP). Desmoplastic HGP (metastasis is separated from the surrounding liver parenchyma by a desmoplastic rim), pushing HGP (metastasis grows by compressing the liver parenchyma), replacement HGP (metastases growth preserves the architecture of the hepatic tissue), and mixed HGP (a mix of two or more patterns. Desmoplastic HGP (arrow) and replacement HGP (arrowhead) are shown here.

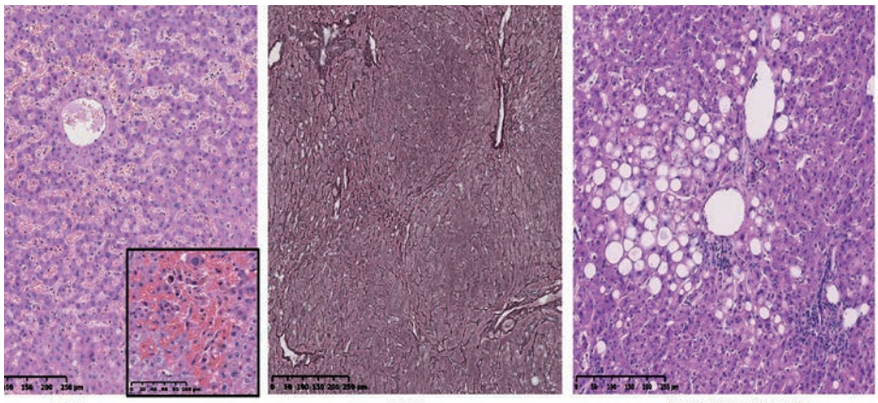

Fig. 3. — Chemotherapy related liver injury (CALI) demonstrated in patients with colorectal liver metastases. Sinusoidal Obstructive Syndrome (SOS) (shown in a haematoxylin and eosin-stained section) is characterized by varying degrees of endothelial damage; Nodular Regenerative Hyperplasia (NRH) (shown by reticulin staining) is indicated by a nodularity aspect of the liver parenchyma without fibrosis; and Steatohepatitis (shown in a haematoxylin and eosin-stained section) is indicated by steatosis, ballooning and lobular inflammation.

In this study, each lesion was assessed based on the predominant HGP (present in more than 50% of the total length of the interface). Sinusoidal HGP seems to occur in patients with rapidly progressive CRLM, however, both sinusoidal and portal HGP are quite rare. In addition, they categorized HGP on the basis of the predominant pattern, rather than using the mixed HGP classification. Finally, in cases of multiple lesions in single patients, describing the HGP of each lesion separately is recommended.

Chemotherapy-related liver injury (CALI)

Recently, CALI has gained attention with an increase in its incidence due to increased administration of neoadjuvant chemotherapy. Three different types of CALI have been described: SOS, NRH and steatohepatitis (Fig. 3**)**, and to assess these lesions histologically, reticulin staining is required (especially to assess NRH). The type of CALI corresponds with the regimen of neoadjuvant chemotherapy, for example oxaliplatin treatment correlates with a high occurrence of SOS (33, 34). It is therefore very important for pathologists to be aware of the type of pre-operative treatment prior to making pathology protocols.

However, it remains unclear whether CALI influences survival. Tamandl *et al.* (35) observed that the presence of moderate to severe SOS impairs long-term outcome in CRLM, while others argue that SOS correlates with a lower response to chemotherapy, as assessed by TRG, probably due to sinusoidal vascular damage (36), without any impact on prognosis (24, 37). Nevertheless, it is well known that there is a correlation between SOS and NRH, and severe forms of SOS are associated with NRH, which is an independent predictive factor of post-operative liver failure (38) and is associated with idiopathic portal hypertension. SOS and NRH tend to regress after nine months without chemotherapy (39).

Although an association between irinotecan and steatohepatitis has been described (40,41), the presence of a metabolic condition, such as high body mass index (BMI), and obesity, and the interval between the end of treatment and operation, may also contribute to this pathology (42,43). Therefore, liver biopsy would be an option prior treatment especially in patient with metabolic condition because steatohepatitis is associated with increased 90-day mortality after hepatic surgery.

Generally CALI is clinically diagnosed only in its advanced stages, therefore, a pathological evaluation in the surgical specimen is important and should be included in the pathological protocol.

RAS and BRAF mutation analysis

The European Society of Medical Oncology (ESMO) and the American society of Clinical Oncology (ASCO) strongly recommend the evaluation of RAS and BRAF mutation status in all stage IV colorectal cancers (44,45). Around 50% of CRLM present with a RAS mutation; this is associated with more aggressive disease, poorer survival, and lack of response to anti-EGFR treatment (46,47). These correlations are especially seen with mutations in KRAS exon 2, 3, 4 and NRAS exons 2, 3, 4.

BRAF mutations (V600E) are seen in around 8-12% of CRLM and they never overlap with RAS mutations (48). BRAF mutations are a significant negative prognostic factor (49,50), and are described in some studies as a negative predictive biomarker for anti-EGFR treatment.

Theoretically, RAS mutational status should not differ among the primary and metastatic sites as RAS mutations occur in the early stages in a multistep genetic model of CRC. However, studies show conflicting results. While some studies have found high concordance rates between primary and metastatic sites (51,52), others have shown

Table 2. — **Summary of prognostic scoring systems in patients with colorectal liver metastasis**

Name of the Author	*Year*	*Prognostic factors*	*Stratification of the score*	*Prognosis*	
				OS at 5y (%)*	*DFS** at 1y (%)*
Nordinger et al.[62]	1996	≥60 years	0-2	(2y) 79	NA
		Serosal invasion of primary lesion	3-4	60	NA
		Node positivity in primary lesion	5-7	43	NA
		Interval colorectal/liver resection <2 years			
		Metastasasis larger than 5 cm			
		≥4 liver metastases			
		Margin ≤1 cm			
Fong et al. [13]	1999	Surgical margin positif	0	60	NA
		Extrahepatic disease	1	44	NA
		Bilobar distribution of metastases	2	40	NA
		Liver metastasis in the firts year	3	20	NA
		Multiple liver metastases	4	25	NA
		Metastasasis larger than 5 cm	5	14	NA
		CEA >200 ng/ml			
Iwatsuki et al. [71]	1999	Multiple liver metastases	1	48	NA
		Metastasasis larger than 8 cm	2	34	NA
		Bilobar distribution of metastases	3	18	NA
		Interval colorectal/liver resection ≤30 months	4	6	NA
		Surgical margin positif	5	1	NA
		Extrahepatic disease	6	0	NA
Ueno et al. [63]	2000	Tumor budding in primary lesion	A (no risk or a or b)	55	(6 mo) 93.3
		or node positivity in primary lesion (a)	B (a or b+c)	14	69.6
		Liver metastasis in the first year (b)	C (a+b+c)	0	55.6
		≥3 liver metastases (c)			
Lise et al. [64]	2001	>30% liver invasion	A (0-2)	NA	(3y) 80
		Node positivity in primary lesion	B (3-5)	NA	55
		Multiple liver metastases and size (>3cm)	C (≥6)	NA	
		GPT° levels ≥55 U/l			
		Non anatomical resection			
		Preoperative gamma-GT ≥65 U/l			
		Positive surgical margin			
		Dukes' stage			
		Non anatomical resection			
Nagashima et al. [65]	2004	Serosal invasion of primary lesion	A	85	NA
(matematical formula)		Node positivity in primary lesion	B	56	NA
		Resectable extrahepatic disease	C	0	NA
		Multiple liver metastases			
		Metastasasis larger than 5 cm			
Schindl et al. [66]	2005	Duke's stage C	Good (0-10)	48	NA
(matematical formula)		CEA level	Moderate (11-25)	15	NA
		Alkaline phosphatase	Poor (>25)	0	NA
		Albumin			
		>3 liver metastases			
Malik et al. [67]	2007	Inflammatory response to the tumor	0	49	NA
		≥ 8 liver metastases	1	34	NA
			2	0	NA
Zakaria et al. [68]	2007	Liver metastasis in the first 30 months	1	55	(5y) 54
		Metastasasis larger than 8 cm	2	39	33
		Blood transfusion	3	20	5
		Positive hepatoduodenal nodes			
Lee et al. [69]	2008	Surgical Margin ≤5 mm	Low (0-1)	46	NA
		CEA > 5 ng/ml	Intermediate (2)	41	NA
		Node positivity in primary lesion ≥4	High (3-4)	11	NA
		Multiple liver metastases			
Rees et al. [7]	2008	Multiple liver metastases	0	64	NA
		Node positivity in primary lesion	1-5	49	NA
		Poorly differentiated primary lesion	6-10	34	NA
		Extrahepatic disease	11-15	21	NA
		Metastasasis size ≥ 5 cm	>15	2	NA
		CEA >60 ng/ml	(Different points are assigned		
		Surgical Margin positive	for each variable)		
Knopke et al. [70]	2009	≥4 liver metastases	Low (no factors)	57	89
		Synchronous liver metastases	Intermediate (one factor)	38	83
		CEA >200 ng/ml	High (more than one factor)	0	46

*OS overall survival; **DFS disease free survival; CEA carcino embrionic antigen; °GPT glutamic pyruvic transamnase.

lower concordance rates (53). These low concordances are probably due to the small cohort of patients, or the evaluation of lymph node metastases as the metastatic site. High concordance rates are seen when the analysed metastatic site is the liver. No significant discordance has been described concerning BRAF mutational status between primary and metastatic sites.

In summary, based on these studies, tissue from either the primary lesion or liver metastases may be used for molecular RAF and BRAF testing; other metastatic sites may be used when these tissues are not available.

Immunoscore

Studies have shown the importance of the immune-environment in the development and diffusion of cancers. Some authors suggest that once a human cancer becomes clinically detectable, the adaptive immune response plays a role in preventing tumour recurrence. Following primary exposure to antigen, memory T-cells disseminate and are maintained for long periods. The trafficking properties and long lasting anti-tumour capacity of these T-cells result in long-term immunity in cancer (54). High densities of memory T-cells, in both the centre and the invasive margin of the primary tumour, are associated with long disease free survival (DFS), overall survival (OS), and low risk of relapse.

Immunoscore is a standardized scoring system created to grade densities of lymphocyte populations (CD3 and CD8) in the core and the invasive margin of the tumour. This score ranges from 0-4, as described by Galon at al. (54), and the higher the Immunoscore (thus higher infiltration), the longer the survival and recurrence rates.

It has been demonstrated that Immunoscore is not only an important prognostic marker in colorectal cancer, but it also plays a role in the metastatic setting; a high Immunoscore in brain metastases correlates with prolonged survival (55). Recent studies confirmed this with CRLMs (56), and, in a multiple lesion setting, the less the metastasis was infiltrated with lymphocytes the worse the prognosis (57). A possible explanation is that the lower the level of infiltration in the metastasis the less it will be affected by immune-based elimination, and metastatic progression may be further promoted. A comparison of Immunoscore and TRG show that a high immunodensity corresponds to high response of therapy, as assessed by TRG.

The importance of the Immunoscore is uncontested and in light of these results, its application has to be encouraged. The primary limitation on the use of the Immunoscore is that, for the quantification of immunodensities a morphometric evaluation is needed, and this is not available in all institutes.

Prognostic scoring system

A prognostic scoring system could help clinicians to integrate all the patient information and more precisely predict a prognosis.

Several scoring systems have been reported to predict the outcome of CRLM patients (9, 12, 58-67). However, the reported scoring systems comprise clinical and anatomical parameters, and do not include pathological factors such as TRG and/or CALI, and they are not always reliable in patients who have received neoadjuvant treatment. (Table 2)

The most used scoring systems proposed by Fong *et al.* (12) and by Iwatsuki *et al.* (67) appear to be fairly reliable, even in patients who received neoadjuvant chemotherapy (68). In addition, the scoring system created by Fong *et al.* has recently been used to select the patients who will benefit from neoadjuvant chemotherapy with good results (19).

Creating a new scoring system that incorporates histopathological parameters, such as tumour regression after chemotherapy, immune microenvironment, RAS mutational status, and the characteristics of the background liver parenchyma, may not only help clinicians to more precisely stratify a patient's prognosis, but may also identify those patients that could benefit from adjuvant chemotherapy, or a stricter follow-up.

Conclusion and prospects for future research

In this review, we summarize the useful patho-molecular and anatomical parameters that should be mentioned in the pathological report. These include the status of the surgical margin (R0 vs. R1), tumour regression grading (TRG 1 and 2 vs. TRG 3, 4, and 5), histopathological growth pattern (replacement HGP vs. others), chemotherapy related liver injury (steatohepatitis, NRH), and RAS mutational status. In addition, Immunoscore seems to be a very promising prognostic factor that has been validated in different types of cancers including colorectal cancer and CRLM (57,69).

To identify those patients with a high probability of recurrence after curative intent hepatectomy, a comprehensive scoring system, including all the described parameters, may be an option.

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