Surgical Margins and Lymphoid Infiltrate in Cholangiocarcinoma: When a Surgical Technique “Pushes” Tumor Biology to Provide Answer

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INTRODUCTION

Mass-forming cholangiocarcinoma (MFCCC) is a disease at increasing incidence (1,2). Liver resection (LR) is the standard treatment, while chemotherapy has a limited effectiveness (3,4). Five-year survival rates after complete surgery range between 20 and 35% (5-9). Although surgery represents the unique curative treatment, the disease is characterized by low resectability and high post-surgical recurrences rates (1-4). To date, resectability is based on morphological features (number and size of lesions, vascular invasion), lymphnode metastases (N stage), and surgical radicality (5-12). If the surgical technique could play a role in improving resectability, molecular markers rather than gross tumor features could aid in defining and clarifying further prognostic predictors helpful to better select and manage the patients accordingly.

On a technical standpoint, the margin width, if negative, does not impact the outcome, while a positive margin (< 1 mm, R1 resection) is associated with higher local recurrence rate and worse survival (8-15). However, our group disclosed subcategories of R1 with a different prognostic impact in the event of surgery for colorectal liver metastases, which allowed to increase their resectability (16-17). Whether these subcategories play a role for other liver tumors is still matter of debate.

Prognosis prediction is of paramount relevance for patients management either for their selection, and for the therapeutic and follow up strategies. Reliable molecular markers for MFCCC are still lacking as well as an adequate assessment of tumor biology. The analysis of lymphoid infiltrate (LI) may work in this direction. The oncologic impact of LI has been analyzed in several tumors. The largest evidences concern colorectal cancers (18-23). Focusing on liver tumors, some data have been reported for HCC and colorectal liver metastases (24-32), while no prior study focused on MFCCC.

These two aspects will be discussed in this review article.

SURGICAL MARGINS

In surgical oncology, the definition of the adequate surgical margin is of para-

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mount importance influencing both resectability, and type of resection. During the last decades, the adequate width of the surgical margin passed from 10 mm to 1 mm (33-35). Although mostly referred to colorectal liver metastases, resection margin less than 1 mm, herein named R1par, was associated with higher local recurrence and worse prognosis (33-35). Several analyses confirmed these data also for MFCCC (8-15). However, some recent studies about colorectal liver metastases denied the negative prognostic impact of R1par resection (36-38). The effectiveness of modern chemotherapy regimens and the adoption of targeted therapies probably contributed to these results, especially in patients with multiple bilobar metastases (36-39). This is not the case for MFCCC, for which medical treatments have a limited effectiveness and the quality of surgery is still the main determinant of outcome (3,4).

Our group recently proposed a new type of R1 resection, the R1vasc resection in which the tumor is detached from major intrahepatic vessels (16, 17, 40-44). This technique relies on the hypothesis that vessels are a sort of boundary to the tumor diffusion and no margin is required if tumor is detachable. Inversely partial resection or division of hepatic vein (according to the degree of vessel wall involvement) and division of glissonean pedicles is recommended in case of invasion (17,41-43,45). The feasibility and safety of R1vasc resection have been previously reported for colorectal liver metastases (16,17,40), and promising data on HCC are going to be published.

MFCCC is usually diagnosed at an advanced stage as large centrally-located masses and often has wide contact with major intrahepatic vessels. In these conditions, the detachment of MFCCC from vessels could shift to resectability in otherwise unresectable patients (bilateral vascular contact). However, R1vasc resection for MFCCC in our initial experience based on more than 50 patients resulted in a poor local disease control (local recurrence in more than one fourth of patients), similar to that obtained for R1par resection, and largely inferior to R0 one (*unpublished data*). Even survival of the R1vasc group was lower than R0 group. The discrepancy between the results of R1vasc resection for colorectal liver metastases and R1vasc resection for MFCCC may have several explanations. First, different diseases have different local aggressiveness. Second, MFCCC are often diagnosed as large masses having a wide contact with intrahepatic vessels which results in more difficult tumor-vessel detachment and higher risk for tumor exposure out of the detachment area (mixed R1vasc-R1par resection). Finally, for

patients with MFCCC medical treatments provide a limited contribution to the local disease control (3, 4). However, just the latest point paradoxically provides rationale for the R1vasc approach in these patients. Indeed, although R1vasc resection in MFCCC seems having an outcome similar to R1par resection showing higher local recurrence risk and lower survival rate compared to R0 resection, it assumes clinical relevance once it becomes the only way for shifting it from surgically unresectable to resectable. This is the case because even a risk of local recurrence of one over four patients could be reasonable if compared with the impairment in patients prognosis associated by the exclusion from the surgical perspective.

LYMPHOID INFILTRATE

The initial studies of LI explored its association with the tumor pathology data and TNM stages. In colorectal cancers, Galon et al. demonstrated that CD3+, CD4+, and CD8+ infiltrates are inversely associated with microvascular, lymphatic, and perineural infiltration into the tumors (21). Conversely, no association between the LI and microscopic vascular infiltration was reported for HCC by Sun et al. (26) and for MFCCC in our initial analyses (*unpublished data*). However, we found an association between the LI and the MFCCC tumor size (the larger the tumor size, the lower the LI), and an inverse association between the CD8+ infiltrate with T and N stages. This is in line with similar data reported in colorectal cancer (20, 46).

The prognostic impact of LI is the most relevant issue. In 2011, a meta-analysis considering different tumors reported a prognostic value for CD3+ and CD8+ infiltrates (47). Colorectal cancer, patients with high CD3+ and CD8+ tumor infiltrates had superior survival and lower recurrence risk in comparison with patients with low infiltrates (21-23). The survival benefit was reported for every tumor stage (21). These data were the basis for the elaboration of an “immunoscore” (23), i.e., an immunological staging system based on the CD3+ and CD8+ presence. In the proposers’ opinion, that score could integrate or even replace the TNM staging. For liver tumors, only limited data are available to date. In 2003, Okano et al. suggested an association between the LI and prognosis in 41 colorectal cancer patients with liver metastases (48). Subsequent analyses in the same disease showed that CD3+ and CD8+ infiltrates have an inverse association with tumor doubling time (49), and a positive association with survival (29, 31). The same positive prognostic value of CD3+ and CD8+ cell infiltrates was described in HCC patients

(25,26). Due to the consistency of data with those reported for colorectal cancers, some authors proposed an immunoscore for HCC patients as well (24, 26). In our exploratory studies in MFCCC, CD3+ and CD8+ infiltrates were associated with a superior prognosis. A large survival advantage was observed when a relevant infiltrate was abundant. Furthermore, CD3+ and CD8+ refined prognosis prediction in T1 and N1 tumors. Collectively, these results support the notion that LI could complement TNM staging.

Furthermore, Foxp3+ T regulatory cells have been rarely analysed in previous studies. Data from primary tumors and liver metastases in colorectal cancer are inconsistent (28, 32, 48-51). In our experience it seems that a Foxp3+ infiltrate has a strong negative prognostic impact in MFCCC, being associated with significantly lower 5 year survival rate when is evident (*unpublished data*). Another potential element of interest would be the macrophages (CD69+) infiltrate. Zhou et al. reported a positive association between macrophages and prognosis in colorectal metastases (52). This does not appear to be the case in MFCCC according with our experience which showed no impact on survival of the macrophages infiltrate. Finally, some recent papers investigated the prognostic impact of circulating neutrophils (neu), lymphocytes (lymph) and platelets (PLT), and their ratios. In MFCCC patients, neu/lymph and PLT/lymph ratios have been associated with survival (53-56). However, we are not having confirmatory results in this sense, since neu/lymph and the PLT/lymph seem associated neither with LI at pathology nor with prognosis. The LI at pathology and not the ratios of circulating cells acts as prognosticator.

Finally, in colorectal cancer, the analysis of the LI at the tumor margin together versus the intratumoral LI improved prognosis prediction (23), but this was not confirmed in studies in liver tumors (24, 25, 30). Our current experience although based only on data from a limited number of patients, provides some preliminary evidence concerning the prognostic value of intratumoral LI. These should be the starting point for further analyses in larger studies in MFCCC, which should also investigate to the LI at the tumor margin.

CONCLUSIONS

MFCCC was confirmed as a rather aggressive disease since the R1vasc approach is clearly not as successful as for colorectal liver metastases or the HCC. However, considering that surgery still remains the only potentially curative treatment for this tumor, R1vasc surgery in case of otherwise unresectability should be an option

to be considered. To better foresee for which patient this policy could be more or less appropriate, markers of tumor biology could potentially be very helpful. Thus, there is renewed interest in studying potential pathological prognostic factors for MFCCC. The intratumoral LI showed preliminary association with patients' prognosis, and could potentially be useful to estimate the post-surgical risk of recurrence. This could aid the surgeons in retailing their surgical attitude accordingly. While the data on intratumoral LI is validated in larger studies, there is growing interest in evaluating the peritumoral LI which could provide additional prognostic or predictive information for the risk of tumor recurrence after vascular detachment.

Conflicts of Interest

No conflicts of interest were declared.

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