THE IMPACT OF LIVER SURGERY AND MARGIN STATUS ON SURVIVAL ACCORDING TO KRAS MUTATIONAL STATUS IN COLORECTAL LIVER METASTASES

O IMPACTO DA MUTAÇÃO KRAS NAS METÁSTASES HEPÁTICAS DO CANCRO COLORRETAL NAS MARGENS CIRÚRGICAS E NA CIRURGIA HEPÁTICA

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ABSTRACT

Introduction: The impact of kirsten rat sarcoma viral oncogene homolog (KRAS) mutational status on surgery planning for colorectal liver metastases (CRLM) remains unknown. The aim of the study was to evaluate the impact of type of liver surgery and margin status in recurrence free survival (RFS) of patients with CRLM, according to KRAS mutational status. **Materials and methods**: Retrospective review of all patients consecutively submitted to CLRM surgery between January 2011 and December 2016 with KRAS determination. Exclusion criteria were 2-stage hepatectomy strategy, loss to follow up and non-anatomical and anatomical resections performed simultaneously. **Results**: 114 patients were included, with a median age of 61 [31-80] years old. 67.5% of patients were male. KRAS mutation was present in 46.5% of patients, 58.8% had non-anatomical resections and R0 surgery was obtained in 69.3%. With a median follow up of 43 [4-105] months, recurrence rate was 86.8%, median overall survival and RFS were 53 and 11 months, respectively. In the mutated KRAS (mKRAS) group, the detection of R1 margins was the only predictor of worse RFS (31 versus 13 months, p=0.022). In the wild-type KRAS (wtKRAS) group a similar difference was not observed (24 versus 19 months, p=0.310). The most common form of recurrence after R1 resections in the mKRAS group was extra-hepatic, while in the wtKRAS was isolated hepatic recurrence. **Conclusion**: In patients with mKRAS, R1 resection was associated with a decreased RFS, mainly due to extra-hepatic recurrence. These findings were not replicated in the wtKRAS group. KRAS mutational status should be considered while planning liver resection for CRLM, namely when deciding optimal margin width.

Key-words: *colorectal liver metastasis, KRAS status, liver margin, recurrence free survival.*

RESUMO

Introdução: O impacto do estado mutacional do *kirsten rat sarcoma viral oncogene homolog* (KRAS) no planeamento da cirurgia por metastização hepática de carcinoma colorretal permanece desconhecido. O objetivo do estudo foi avaliar o impacto do tipo de cirurgia hepática e do *status* das margens de resseção hepática na sobrevivência livre de recidiva (SLR) em doentes com metastização

hepática de carcinoma colorretal, de acordo com o estado mutacional do KRAS. **Material e métodos**: Revisão retrospetiva de todos os doentes consecutivamente submetidos a cirurgia hepática por metastização de carcinoma colorretal entre janeiro de 2011 e dezembro de 2016, com determinação do estado mutacional do KRAS. Os critérios de exclusão foram estratégia de hepatectomia em 2 tempos, perda de seguimento e resseção anatómica e não-anatómica no mesmo tempo cirúrgico. **Resultados**: Foram incluídos 114 doentes na análise, com mediana de idade de 61 [31-80] anos e 67.5% de doentes do sexo masculino. KRAS mutado estava presente em 46.5% dos doentes, 58.8% realizaram uma resseção não anatómica e uma cirurgia R0 foi obtida em 69.3%. Com uma mediana de tempo de seguimento de 43 [4-105] meses, a taxa de recidiva foi de 86.8%, a mediana de sobrevivência global e de SLR foi de 53 e 11 meses, respetivamente. No grupo com KRAS mutado (mKRAS), as margens R1 foram o único fator preditor de pior SLR (31 *versus* 13 meses, p=0.022), o que não se verificou no grupo KRAS *wild-type* (wtKRAS) (24 *versus* 19 meses, p=0.310). A forma mais comum de recidiva após resseção R1 no grupo mKRAS foi extra-hepática, enquanto que no grupo wtKRAS foi a recidiva hepática isolada. **Conclusão**: Em doentes do grupo mKRAS, a resseção R1 associou-se a diminuição da SLR, sobretudo à custa de recidiva extra-hepática. Estes achados não foram replicados no grupo wtKRAS. O estado mutacional do KRAS deve ser tido em consideração aquando do planeamento da resseção hepática em doentes com metastização de carcinoma colorretal, nomeadamente na decisão da margem cirúrgica ótima.

Palavras chave: *Cancro coloretal, margens cirúrgicas, mutação KRAS, cirurgia hepática.*

INTRODUCTION

Colorectal liver metastases (CRLM) will occur in up to 50% of patients with colorectal adenocarcinoma[1, 2] and, at this moment, are the leading cause of morbidity and mortality in these patients[3]. Despite being a significant event, treatment is currently not standardized and individualized strategies have been established in dedicated multidisciplinary meetings[4, 5]. Decision in such setting relies on determining the potential for resectability and on prognostic factors to determine the need of systemic therapy, the efficacy of ablative and/or percutaneous therapies and, if more than one option is to be taken, the timing by which each intervention should be performed[6, 7]. In suitable patients, upfront surgery is considered the best curative treatment[8].

Multiple factors have been described either of prognostic and/or therapeutic value[9, 10]. However, these factors are considered surrogate markers of the underlying tumor biology and therefore there has been growing interest in molecular profiling and integrating biomarkers into prognostic nomograms and therapeutic decisions[11]. Neoplastic transformation results from a series of genetic alterations involving activation of protoncogenes and inactivation of tumor suppressor genes. Activation of RAS protoncogenes by point mutations is frequent[12]. Currently kirsten rat sarcoma viral oncogene homolog (KRAS), BRAF and microsatellite instability have clinical relevance in treatment selection but are mostly restricted to guide systemic therapy[13]. KRAS however has been identified as a potential marker to guide surgery. These reports have either shown a necessity for wider margins in KRAS mutated disease, even considering abandoning wedge resection in this circumstance or simply demonstrating an increased difficulty obtaining R0 margins in cases of mutated KRAS metastasis[14, 15].

Therefore, the aim of this study was to evaluate the impact of the type of liver surgery performed and margin status in recurrence free survival (RFS) in patients with liver metastasis of resected colorectal adenocarcinoma, according to KRAS mutationalstatus.

MATERIALS AND METHODS

All patients consecutively submitted to liver surgery for CRLM between January 2011 and

December 2016 at Instituto Português de Oncologia do Porto, in Portugal, were identified and data retrospectively collected. All adult patients that had their first liver surgery during this period and had a KRAS determination were included. KRAS status could be determined either in the primary tumor or in the hepatic metastasis. Patients were excluded if they had a 2-stage hepatectomy strategy upfront, were lost to follow up and if both types of resection (non-anatomical and anatomical) were performed simultaneously at the first surgery.

The type of liver surgery was classified as anatomical or non-anatomical resection according to the Brisbane terminology[16] and the primary tumor was classified according to site of origin as right colon, left colon, or rectum. R1 resection was defined as the presence of tumor on the liver margin. Patients were stratified according to Fong criteria[17], namely metastases were classified as synchronous or metachronous using a 12 months cut-off, node status of the primary tumor as negative or positive, number of hepatic metastasis as one or more, size of largest metastasis as up to 5 cm and over 5 cm and carcinoembryonic antigen (CEA) as up to 200 ng/ml and over 200 ng/ml.

Variables concerning preoperative patient data, surgical procedure and survival were collected and statistical analysis was performed using the version 24 of SPSS®, with a $p<0.05$ considered significant. Continuous data were presented as median and range. Categorical variables were analyzed using $\chi 2$ test or Fisher's exact test, as appropriate. Overall survival (OS) and RFS were estimated using the Kaplan-Meier curves and compared using the log rank test. A univariate analysis was first performed to test the association of potentially predictive factors (independent variables) with the outcome of interest (dependent variable), which in this case was RFS. The factors that achieved a $p<0.1$ in the univariate analysis were then used to build a Cox proportional hazard regression model and hazard ratios as well as 95% confidence intervals were reported.

RESULTS

During the study period 210 liver surgeries for CLRM were performed and, after application of inclusion and exclusion criteria, a total of 114 patients were included in the analysis. The median age was 61 [31-80] years old and 67.5% (n=77) of patients were of male gender.

Colon cancer was the primary tumor in 61.4% (n=70) of patients (corresponding 18.4% (n=21) to the right colon and 43% (n=49) to the left colon) and 38.6% (n=44) had rectal cancer. Most patients had pT3-4 (87.7%; n=100) and node positive tumors (69.3%; n=79).

Liver metastasis were synchronous in 53.5% (n=61) of cases, bilobular in 26.3% (n=30), with a median number of lesions of one [1-10] and a median size of largest metastasis of 3 cm [0.5-16.5]. Regarding Fong groups, distribution was as follows: 8.8% (n=10) of patients in Fong 0, 28.1% (n=32) in Fong 1, 39.5% (n=45) in Fong 2, 18.4% (n=21) in Fong 3 and 5.3% (n=6) in Fong 4.

Roughly half of patients were KRAS mutated (mKRAS) (46.5%; n=53) and, aside from a higher percentage of preoperative chemotherapy in the KRAS wild-type (wtKRAS) group (57.4% vs 37.7% p=0.036), there was no significant difference between wtKRAS and mKRAS groups in the studied parameters (Table 1).

Regarding perioperative treatment, 48.2% (n=55) of patients were treated with preoperative ("neoadjuvant" or conversion) chemotherapy. Target therapy was used on 30 patients, specifically 18 patients were treated with bevacizumab, 11 with cetuximab and one with panitumumab. To most patients (81.6%; n=93) postoperative chemotherapy was offered. As expected, the higher the Fong score, the more patients were treated with preoperative chemotherapy. Synchronous disease and wtKRAS status were significantly associated with preoperative chemotherapy (p=0.036 and $p<0.001$ respectively).

Regarding surgical treatment, 58.8% (n=67) of patients were submitted to non-anatomical resections and 41.2% (n=47) to anatomical resections, mainly

Table 1 – **Characteristics of the entire cohort stratified by KRAS status (CEA – carcinoembryonic antigen, mKRAS – mutated KRAS, Nº – number, wtRAS – wild-type KRAS)**

	wtKRAS (n=61)	mKRAS (n=53)	*p* value
Gender, n (%)			0.936
Female	20 (32.8%)	17 (32.1%)	
Male	41 (67.2%)	36 (67.9%)	
Age (years), n (%)			0.334
<61	32 (52.5%)	23 (43.4%)	
≥61	29 (47.5%)	30 (56.6%)	
Primary tumor location, n (%)			0.783
Right colon	10 (16.4%)	11 (20.8%)	
Left colon	26 (42.6%)	23 (43.4%)	
Rectum	25 (41%)	19 (35.8%)	
pT, n (%)			0.388
ypT0/pT1-2	9 (14.8%)	5 (9.4%)	
pT3-4	52 (85.3%)	48 (90.6%)	
pN, n (%)			0.482
pN0	17 (27.9%)	18 (34%)	
pN+	44 (72.1%)	35 (66%)	
Nº of metastases, n (%)			0.617
1	34 (55.7%)	32 (60.4%)	
>1	27 (44.3%)	21 (39.6%)	
Size of largest metastases (cm), n (%)			0.394
≤5	55 (90.2%)	45 (84.9%)	
>5	6 (9.8%)	8 (15.1%)	
CEA (n=103), (ng/ml), n (%)			0.103
<5	25 (43.9%)	13 (28.3%)	
≥5	32 (56.1%)	33 (71.7%)	
Timing of disease, n (%)			0.374
Synchronous	35 (57.4%)	26 (49.1%)	
Metachronous	26 (42.6%)	27 (50.9%)	
Bilateral disease, n (%)			0.092
No	41(67.2%)	43 (81.1%)	
Yes	20 (32.8%)	10 (18.9%)	
Preoperative chemotherapy, n (%)			**0.036**
No	26 (42.6%)	33 (62.3%)	
Yes	35 (57.4%)	20 (37.7%)	
Fong group, n (%)			0.356
0	7 (11.5%)	3 (8.8%)	
1	15 (24.6%)	17 (28.1%)	
2	21 (34.4%)	24 (39.5%)	
3	14 (23%)	7 (18.4%)	
4	4 (6.6%)	2 (5.3%)	
Type of surgery, n (%)			0.480
Anatomical	34 (55.7%)	33 (62.3%)	
Non-anatomical	27 (44.3%)	20 (37.7%)	
Margins, n (%)			0.767
R0	43 (70.5%)	36 (67.9%)	
R1	18 (29.5%)	17 (32.1%)	
Postoperative chemotherapy, n (%)			0.909
No	11 (18%)	10 (18.9%)	
Yes	50 (82%)	43 (81.1%)	
Recurrence, n (%)			0.569
No	7 (11.5%)	8 (15.1%)	
Yes	54 (88.5%)	45 (84.9%)	

bissegmentectomy. The surgery was considered R0 in 69.3% (n=79) of cases and, aside from a higher percentage of male patients, patients ≥61 years old and pT0-2 tumors in R1 group, there were no other significant differences between R0 and R1 patients, namely regarding KRAS status (Table 2).

Table 2 – **Characteristics of the entire cohort stratified by margin status (CEA – carcinoembryonic antigen, mKRAS – mutated KRAS, Nº – number, wtRAS – wild-type KRAS).**

	R0 (n=79)	R1 (n=35)	*p* value
Gender, n (%)			**0.020**
Female	31 (39.2%)	6 (17.1%)	
Male	48 (60.8%)	29 (82.9%)	
Age (years), n (%)			**0.047**
<61	43 (54.4%)	12 (34.3%)	
≥61	36 (45.6%)	23 (65.7%)	
Primary tumor location, n (%)			0.172
Right colon	18 (22.8%)	3 (8.6%)	
Left colon	31 (39.2%)	18 (51.4%)	
Rectum	30 (38%)	14 (40%)	
pT, n (%)			**0.027**
pT0-2	6 (7.6%)	8 (22.9%)	
pT3-4	73 (92.4%)	27 (77.1%)	
pN, n (%)			0.581
pN0	23 (29.1%)	12 (34.3%)	
pN+	56 (70.9%)	23 (65.7%)	
Nº of metastases, n (%)			0.914
1	46 (58.2%)	20 (57.1%)	
>1	33 (41.8%)	15 (42.9%)	
Size of largest metastases (cm), n (%)			0.561
≤5	69 (87.3%)	31 (87.7%)	
>5	10 (12.7%)	4 (12.3%)	
CEA (n=103), (ng/ml), n (%)			0.718
<5	25 (35.7%)	13 (39.4%)	
≥5	45 (64.3%)	20 (60.6%)	
Timing of disease, n (%)			0.605
Synchronous	41 (51.9%)	20 (57.1%)	
Metachronous	38 (48.1%)	15 (42.9%)	
Bilateral disease, n (%)			0.716
No	59 (74.7%)	25 (71.4%)	
Yes	20 (25.3%)	10 (28.6%)	
Preoperative chemotherapy, n (%)			0.651
No	42 (53.2%)	17 (48.6%)	
Yes	37 (46.8%)	18 (51.4%)	
Fong group, n (%)			0.948
0	7 (8.9%)	3 (8.6%)	
1	24 (30.4%)	8 (22.9%)	
2	30 (38%)	15 (42.9%)	
3	14 (17.7%)	7 (20%)	
4	4 (5.1%)	2 (5.7%)	
Type of surgery, n (%)			0.859
Anatomical	46 (58.2%)	21 (60%)	
Non-anatomical	33 (41.8%)	14 (40%)	
KRAS status, n (%)			0.767
wtKRAS	43 (54.4%)	18 (51.4%)	
mKRAS	36 (45.6%)	17 (48.6%)	
Postoperative chemotherapy, n (%)			0.772
No	14 (17.7%)	7 (20%)	
Yes	65 (82.3%)	28 (80%)	
Recurrence, n (%)			0.260
No	12 (15.2%)	3 (8.6%)	
Yes	67 (84.8%)	32 (91.4%)	

With a median follow up of 43 months, there was recurrence in 86.8% (n=99) of patients: 37.4% (n=37) were isolated extra-hepatic, 32.3% (n=32) were isolated hepatic and 30.3% (n=30) were simultaneously hepatic and extra-hepatic recurrences. The median OS and RFS for the entire cohort were 53 and 11 months, respectively. The 1- and 3-year RFS for the entire cohort were 30% and 15%, respectively. In the wtKRAS group the median OS and RFS were 61 and 11 months, respectively. In the mKRAS group the median OS and FRS were 50 and 11 months, respectively. The difference in RFS between KRAS groups was not statistically significant (p=0.688), (Figure 1).

Predictive survival factors were analyzed for the entire cohort as well as according to KRAS mutational status (Tables 3 and 4).

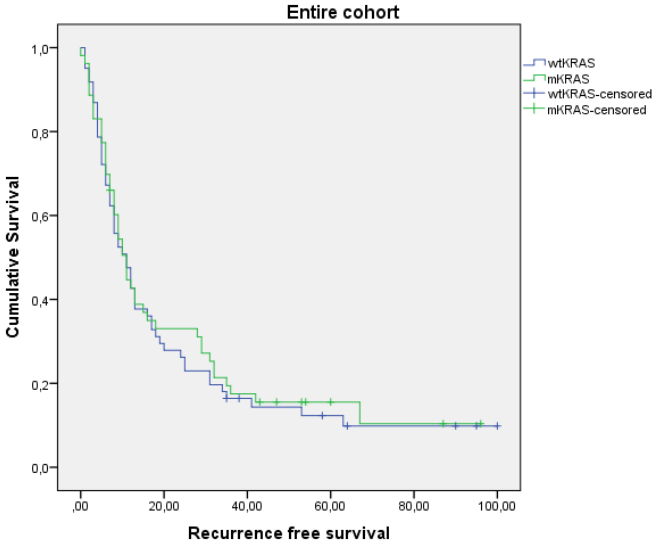

Figure 1 – **RFS in the entire cohort according to KRAS status: median RFS was 11 months in wtKRAS versus 11 months in mKRAS; log-rank test: p=0.688 (mKRAS – mutated KRAS, RFS – recurrence free survival, wtRAS – wild-type KRAS).**

Table 3 – **Predictive factors of recurrence free survival in the entire cohort (CI – confidence interval, HR – hazard ratio, mKRAS – mutated KRAS, Nº – number, RFS – recurrence free survival, wtRAS – wild-type KRAS)**

Predictive factors of RFS in the entire cohort				
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Gender (male)	1.24 (0.81-1.91)	p=0.327		
Age (≥61 years old)	1.30 (0.87-1.93)	p=0.200		
pT 3-4	1.08 (0.59-1.98)	p=0.800		
pN+	1.36 (0.87-2.11)	p=0.175		
>1 metastasis	1.26 (0.85-1.88)	p=0.255		
Size of largest metastasis >5cm	1.01 (0.55-1.85)	p=0.980		
CEA >5 ng/mL	0.95 (0.62-1.45)	p=0.808		
Metachronous disease	0.82 (0.55-1.23)	p=0.340		
Bilateral disease	1.05 (0.67-1.63)	p=0.844		
Preoperative chemotherapy	1.43 (0.96-2.12)	p=0.080	1.41 (0.95-2.10)	p=0.091
Anatomical resection	0.95 (0.64-1.43)	p=0.819		
R1 Margins	1.65 (1.08-2.53)	**p=0.020**	1.64 (1.07-2.50)	**p=0.023**
Postoperative chemotherapy	0.66 (0.40-1.09)	p=0.106		

Table 4 – **Predictive factors of recurrence free survival according to KRAS mutational status (CI – confidence interval, HR – hazard ratio, mKRAS – mutated KRAS, Nº – number, RFS – recurrence free survival, wtRAS – wild-type KRAS).**

Predictive factors of RFS according to KRAS mutational status				
wtKRAS group			**mKRAS group**	
	Univariate analysis		**Univariate analysis**	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Gender (male)	1.48 (0.82-2.68)	p=0.192	0.97 (0.51-1.83)	p=0.928
Age (≥61 years old)	1.39 (0.81-2.39)	p=0.227	1.21 (0.67-2.19)	p=0.530
pT 3-4	1.50 (0.67-3.32)	p=0.322	0.57 (0.22-1.48)	p=0.249
pN+	1.38 (0.73-2.58)	p=0.321	1.28 (0.69-2.40)	p=0.433
>1 metastasis	1.50 (0.88-2.58)	p=0.139	1.02 (0.57-1.86)	p=0.937
Size of largest metastasis >5cm	1.39 (0.58-3.32)	p=0.456	0.81 (0.34-1.91)	p=0.621
CEA >5 ng/mL	1.03 (0.59-1.80)	p=0.921	0.80 (0.41-1.59)	p=0.529
Metachronous disease	0.87 (0.50-1.50)	p=0.618	0.80 (0.44-1.44)	p=0.456
Bilateral disease	1.19 (0.68-2.08)	p=0.542	0.82 (0.38-1.77)	p=0.617
Preoperative chemotherapy	1.29 (0.75-2.23)	p=0.357	1.53 (0.84-2.79)	p=0.168
Anatomical resection	1.11 (0.65-1.91)	p=0.701	0.78 (0.43-1.44)	p=0.428
R1 Margins	1.36 (0.75-2.44)	p=0.310	2.09 (1.11-3.91)	**p=0.022**
Postoperative chemotherapy	0.67 (0.34-1.32)	p=0.249	0.66 (0.30-1.43)	p=0.288

When evaluating the entire cohort, R1 margins were independently associated with worse RFS (27 versus 16 months, p=0.023). In the mKRAS group, R1 margins were the only predictor of worse RFS (31 versus 13 months, p=0.022; Figure 2), which was not verified in the wtKRAS group (24 versus 19 months, p=0.310; Figure 3).

Considering the recurrence pattern in the mKRAS group, the most common form of recurrence after R1 resections was extra-hepatic (12 cases), no isolated hepatic recurrence was recorded, and 4 patients had simultaneously hepatic and extra-hepatic recurrences. On the other hand, after R0 resections 11 patients had extra-hepatic recurrences, 8 isolated hepatic recurrences and 10 simultaneously hepatic and extra-hepatic recurrences. This difference in recurrence pattern was statistically significant (p=0.024). In the wtKRAS group, the most common form of recurrence after R1 resections was isolated hepatic recurrence (9 cases), with 4 patients with simultaneously hepatic and extra-hepatic recurrences and 3 patients with extra-hepatic recurrence. After R0 resections 15 patients had hepatic recurrences, 12 simultaneously hepatic and extra-hepatic recurrences and isolated hepatic 11 extra-hepatic recurrences.

Considering the impact of the type of hepatic surgery in the R1 mKRAS group, patients with anatomical resections had a better RFS than patients with non-anatomical resections, although this difference was not statistically significant (19 versus 7 months, p=290).

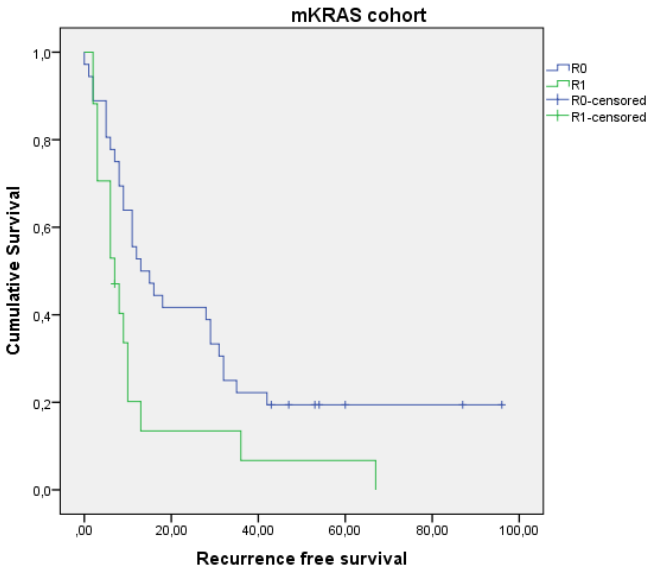

Figure 2 – RFS in the mKRAS cohort according to margin status: median RFS was 31 months in R0 versus 13 months in R1; log-rank test: p=0.022 (mKRAS – mutated KRAS, RFS – recurrence free survival).

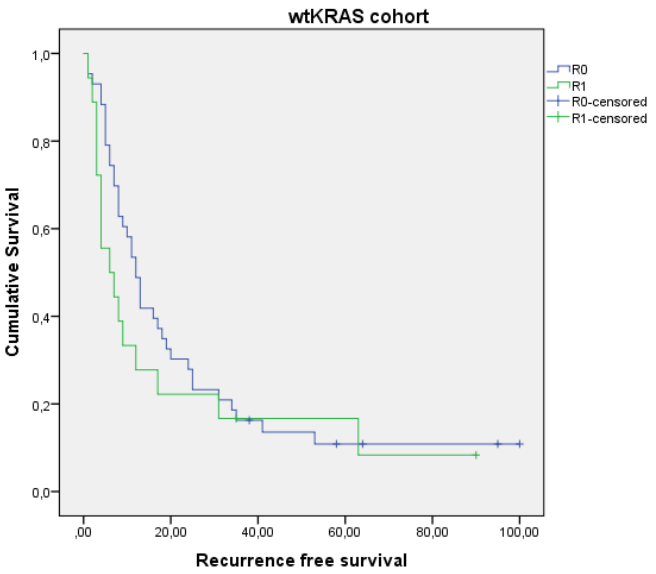

Figure 3 – RFS in the wtKRAS cohort according to margin status: median RFS was 24 months in R0 versus 19 months in R1; log-rank test: p=0.310 (wtKRAS – wild type KRAS, RFS – recurrence free survival).

DISCUSSION

Decisions regarding surgery selection for CLRM are complex and, besides the obvious questions of type of surgery (anatomical versus non anatomical) and margin status, there has been greater consideration towards other factors translating biologic behavior of the tumor that may have impact on outcome[18, 19]. Therefore, the goal of our study was to explore the influence of KRAS mutational status on the relative impact of the type of surgery and margin status on survival outcomes after surgery for colorectal liver metastasis. The type of surgery (anatomical versus non anatomical) had no impact on RFS, regardless of KRAS mutational status. On the other hand, R1 resection was not associated with worse RFS in the group of wtKRAS but was strongly associated with a worse RFS in patients with mKRAS tumors.

Regarding the type of surgery, previous studies have reported comparable survival outcomes between anatomical and non-anatomical liver resections for CRLM, although most of these studies didn't take into account the impact of tumor biology[20-22]. The rationale that an anatomical resection could confer a survival advantage in KRAS mutated tumors has been analyzed by Margonis et al.[14], and a survival advantage in these patients but not in KRAS wild-type tumors was found. In our study we did not share the same conclusion, as type of surgery (anatomical or non-anatomical) didn't have impact on RFS either in wtKRAS or mKRAS groups. This may be related to a different definition of anatomical resection, as well as the fact that the specific technical procedures applied in these types of surgery are not standardized among institutions.

Alternatively, margin status had a significant impact on RFS in our population, namely in the mKRAS group. Although the role of KRAS mutation in determining worse prognosis after liver surgery for CLRM is well established[23, 24], its impact on the adequacy of surgical margins remains to be determined. The first study to provide a recommendation for margin width according to KRAS mutation status was the series by Brudvik et al.[25]. The authors reviewed 633

patients who underwent potentially curative resection of colorectal cancer liver metastases, 229 of which had RAS mutations. They found that among the patients with hepatic recurrence, the median tumor-free margins on pathological examination were much narrower in patients with RAS mutations (4 mm) than in wild-type RAS patients (7 mm) and that patients with mutant RAS had more than double the rate of microscopically positive margins than patients with wild-type RAS. Due to this finding that RAS mutation was associated with a higher rate of R1 resections and, since no specific recommendations for optimal margin width could be made based on the study findings, they recommended a 15mm margin for mutated RAS patients in order to diminish recurrence. Later, Margonis et al.[15] stratified margin width in 3 groups (1-4, 5-9 and ≥10mm) and reported that, specifically in mKRAS patients, none of these groups of additional margin clearance improved overall survival compared to margins <1mm. These findings contradicted the previous recommendation by Brudvik et al., but at the same time clearly reinforced the importance of KRAS mutational status when deciding on the optimal surgical margin in CLRM surgery.

In our series, R1 resection was associated with a decreased RFS but only in the mKRAS group, mainly due to distant recurrence. In this group, the most common form of recurrence after R1 resection was extra-hepatic, followed by simultaneously hepatic and extra-hepatic recurrences, while no isolated hepatic recurrence was recorded. The presence of KRAS mutation has been previously associated with an increased rate of vascular invasion and hematogenous metastases[26]. On the other hand, studies analyzing the liver parenchyma surrounding CLRM surgery after resection demonstrated that the presence of micrometastasis with KRAS mutant DNA is quite low[27, 28]. These findings suggest that margin status and tumor biology influence prognosis in more ways than merely local spread and as such we can hypothesize that R1 resection in mKRAS patients may induce distant metastasis. The pattern of recurrence in our patients, as previously described, supports this theory. Another potential factor to explain this finding is the presence of R1 vascular margins. As this was a retrospective series, this information was not always available, but R1 vascular margins have been demonstrated to have a different impact on survival and recurrence than parenchymal R1 margins[29]. In our series, patients with R1 resection were older, which may be explained by a less aggressive surgery in elderly patients, and had more pT0-2 tumors than in the R0 group, for which we could not find a clear justification, but that may be related with vascular R1 resection.

Some limitations of this study must be recognized regarding its retrospective nature, namely a possible selection bias. The adopted exclusion criteria allowed for a more homogenous and comparable groups, namely controlling for the type of surgery performed. However, during the study period, KRAS status was only determined if the patient was elective for systemic therapy. Therefore, this may result in a selection bias towards the wtKRAS patients with worse prognosis and the outcomes presented for this population may not be representative of all wtKRAS patients. Nevertheless, we believe this strengthens our results since no difference in R0/R1 rates were observed between KRAS groups, which were, in fact, comparable in most variables analyzed. Additionally, KRAS mutational status was determined either on primary tumor or in the metastasis, with the potential for a genetic discordance between the tumor sites. However, a high concordance of KRAS status between primary tumor and metastasis has been described previously[30], which may obviate this limitation. Nonetheless, despite alterations in RAS gene family have been found to be an important biomarker used clinically to determine the response to anti-epidermal growth factor receptor (EGFR) agents, multiple genetic subclones coexist and evolve simultaneously, with treatment acting as a selection pressure[11]. Therefore, the analysis of circulating tumor DNA allows the detection of genetic alterations as well as the sensitivity or resistance to targeting agents[31] and it should be studied in this context. Lastly

the technical aspects of liver transection differed according to surgeon's preference which may have impact on pathological margin analysis.

CONCLUSION

In mKRAS patients R1 resection was associated with a decreased recurrence free survival, mainly due to extra-hepatic recurrence. These findings were not replicated in the wtKRAS group suggesting that, in mKRAS tumors, aspects related to surgical treatment, namely R1 margins, may be the source of extra-hepatic disease spread. Therefore, KRAS mutational status should be considered while planning liver resection for colorectal liver metastases, namely in the decision of optimal margin width.

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