CLINICAL RESEARCH OF PATIENTS RESECTED FOR COLORECTAL LIVER METASTASIS AFTER PREOPERATIVE CHEMOTHERAPY

PhD thesis

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Budapest, 2014

1 Introduction

The only potentially curative treatment of colorectal liver metastases (CRCLM) is surgical resection. There are many phases in the treatment algorithm where preoperative chemotherapy can be inroduced. There is the possibility to increase the number of resectable patients, or to increase the effect of the resection in patients with poor prognostic factors.

Patients with colorectal liver metastases can be divided into three groups. There are the easily resectable patients with low risk recurrens rate, where the recommendation is resection first. The so called „borderline" resectable patients, and patients with high risk of recurrens rate and poor prognostic factors usually candidates for a preoperative chemotherapy first, than resection, and adjuvant chemotherapy thereafter if necessary. The third group is the patients with irresectable colorectal liver metastases, who may became resectable after a very good response.

There are many types of chemotherapy and combinations of chemotherapy with other agents could be used as preoperative treatment. Nowadays, mostly the combination of a chemoherapy and targeted biological therapy is used as neoadjuvant treatment.

There are many new clinical and pathological findings and changes during surgical resection according to preoperative chemotherapy. The first very important question is the safety of hepatic resection after neoadjuvant chemotherapy. There are many new aspects in the evaluation of properative diagnostic modalities and also in the postoperative pathological examinations. The RECIST 1.0, which was used recent past, was frequently unsuitable and unaccurate in the evaluation of the effect of a preoperative treatment. The evaluation of diagnostic scans according to the new RECIST 1.1 is adopted recently in clinical

practice and it is particularly important after neoadjuvant chemotherapy in patients with colorectal liver metastases. There are many histomorfological changes detected in the tumor tissue after neoadjuvant chemotherapy, and these changes can predict survival. However, there is no widely expected and used criteria to define pathologic response for preoperative chemotherapy.

There are still many controversial factors should be clarified in the application of preoperative chemotherapy in the treatment of colorectal liver metastases.

2 Objectives

1. We analyed, first in Hungary, the effect of the preoperative chemotherapy on the results of liver resections of colorectal liver metastases in a singe tercier surgical department. We analysed the followings:

    a. The safety of liver resection after preoperative chemotherapy
    b. The changes of laboratory liver functional tests after liver resection with or without preoperative chemotherapy
    c. Survival after liver resection of colorectal liver metastases

2. We analysed the patholoical response after preoperative chemotherapy in colorectal liver metastases. The aims were the followings:

    a. To demonstrate the different histomorfological changes in colorectal liver metastases after preoperative chemotherapy
    b. To compare the preoperative imaging with the pathological findings
    c. To evaluate the correllation of the different pathological alterations with the survival

3 Patients and Methods

Patients undergoing liver resection with curative intent for colorectal cancer liver metastases at the Uzsoki Teaching Hospital in Budapest were analysed retrospectively from a prospectively collected database. There were 200 patients enrolled in the study between 01.09.2006 and 12.31.2013. (ethical license: ETT-TUKEB: 8-23/2009-1018EKU-ad.60/PI/09.) The following clinical data were collected: patients data (age, sex, comorbidities), preoperative chemotherapy (type of chemotherapy, duration of therapy), diagnostic examinations (type of diagnostic scan, the number-, size of metastases, the radiological response for treatment), perioperative data (type of resection, other surgical procedures, hospital days, operation time, ischaemic time, morbidity, mortality, laboratory tests), pathological examination (number and size of metastases, histomorfological changes after chemotherapy), survival (desease free-, and overall survival). Patients who underwent resection were divided into two groups: (1) patients who received preoperative chemotherapy and (2) patients resected without preoperative chemotherapy.

3.1 Preoperative diagnosis

Liver resection was performed after a CT and/or MRI scan. In the preoperative chemotherapy group, control CT scans were mandatory every 2-3 months to re-evaluate the treatment effect. To define the extent of the desease, the number and the size of the metastases were calculated. To compare the preoperative imaging with the pathologic findings, first we compared the number of metastases in the two different examinations. The size of the tumors were not compared, because these could be very different in the two diagnostic modalities, especially because of the shrinkage of the leasons during pathological processing

caused by fixation. The radiological response rate to chemotherapy was evaulated according to the RECIST 1.0 and this was compared to the pathological response in the chemotherapy group.

3.2 Morbidity

Morbidity after liver resection was classified according to Clavien and Dindo. All patients were operated with the same technical conditions and the main steps of the surgical procedure were similar.

3.3 Laboratory tests

The following preoperative and postoperative laboratory tests were collected: serum bilirubin and INR for the evaluation of excretion and synthesis, and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) for the evaluation of cellular death.

3.4 Survival

Overall survival (OS) and desease free survival (DFS) were analysed. OS was defined as the time interval between liver resection and patient's death, while DFS was defined as the interval between liver resection and the recurrence of the desease. Data were collected from the medical computer system, on personal control examinations or with telephone consultations.

3.5 Pathological examination

5 μm hematoxilin-eozin slides were prepared from the tissue blocks. The tumor tissue and the normal liver parenchyma were both examined. More slides were examined according to the size of the tumor, and the morphological changes were calculated from the the average of the examined slides. Histopathological examination was performed by two gastrointestinal pathologist blinded for clinical data and outcome. Morfological changes were evaluated according to the literature:

- Tumor regression grade (TRG) scoring system, where TRG1 corresponded to absence of tumor cells replaced by fibrosis; TRG2 to rare scattered residual tumor cells and abundant fibrosis; TRG3 to a large amount of residual tumor cells with predominant fibrosis; TRG4 to tumor cells predominating over fibrosis; and TRG5 to almost exclusively tumor cells without fibrosis.

- Residual tumor cell ratio, where complete-, major-, and minor response were recorded (complete response: no residual tumor cell, major response: <50% residual tumor cell and minor response: >50% residual tumor cell)

- Tumor thickness at the tumor-normal interface (TNI). The focus in which the maximum contiguous tumor cell thickness was observed at the TNI was measured by a ruler. This focus was composed of uninterrupted layers of tumor cells without admixed fibrotic stroma, acellular mucin, or nonneoplastic liver parenchyma.

- Type of necrosis, where usual necrosis (UN) was defined as containing nuclear debris in a patchy distribution, with the necrosis admixed and bordered by viable cells, and infarct-like necrosis (ILN) was defined as being composed of large confluent areas of eosinophilic cytoplasmic remnants located centrally

within a lesion with absent or minimal admixed nuclear debris. ILN was considered a form of therapeutic treatment effect.

- The distribution of tumor cells where two models for the pattern was defined: in the first model viable tumor cells were more frequent in the periphery of metastases, in the second model, residual disease is randomly distributed throughout the original tumor volume.

Steatohepatitis (grade 0 ≤5%; grade 1 5-33%; grade 2 ≥33-66%; grade 3 ≥66%) and sinusoideal obstruction syndrome (grade 0 nincs; grade 1 <30%; grade 2 31-60%; grade 3 >60%) were analysed in the normal parenchyma. Hepatotoxicity was diagnosed when grade 2-3 of the above classification was defined.

3.6 Statistical analysis

The t-test and the Mann-Whitney test was performed to assess differences between continuous variables and the Chi-square test was applied to assess the association between categorical variables. Kruskal-Wallis test was used to compare liver function laboratory tests. Logistic regression analysis was used to compare complication rates. Survival probabilities were calculated by the Kaplan-Meier method and compared by the log rank test. A p value of less than 0,05 was considered statistically significant. All statistical analyses were performed using SPSS version 17 software (SPSS, Chicago, IL, USA).

4 Results

There were 102 CRCLM patients who were resected after a preoperative chemotherapy, and 98 patients were resected without preoperative chemotherapy. In the chemotherapy group there were 35 patients, who received only cytotoxic chemotherapy, 60 patients who received chemotherapy in combination with bevacizumab and 7 patients, who received chemotherapy with cetuximab before liver resection.

4.1 Baseline characteristics

Patients mean age was 63 years, there was no significant difference between the chemotherapy and non-chemotherapy groups (0,094). There was no difference in sex, in primary tumor stadium or in primary tumor localization between the groups (p=0,341; p=0,410; p=0,426). There was more solitary metastases in the non-chemotherapy group (p<0,001), but there was no significant difference between the size of the metastases (p=0,687). There was also no difference in the time interval from the primary tumor resection and the diagnosis of liver metastases (p=0,568), and R0 resection rate was the same as well (p=0,129). There was more major hepatic resections (≥3 liver segments resected) in the chemotherapy group, than in the non-chemotherapy group (p=0,015).

4.2 Morbidity

The complication rate after hepatic resection was 39 % overall. There were more complications when major hepatic resection was performed (p<0,001). There was no significant difference in overall morbidity between the chemotherapy

and non-chemotherapy group, but the complications treated with surgical or radiological intervention were more common in the chemotherapy group (p=0,913; p=0,073). Comparing the subgroup of patients in the chemotherapy group, who received only cytotoxic chemotherapy, or chemotherapy with bevacizumab, we found no significant difference in morbidity or in complications treated with surgical or radiological intervention (p=0,929; p=0,097). In the chemotherapy group we found no correlation between morbidity and hepatotoxicity (p=0,413).

4.3 Laboratory liver function tests

Serum ALT and AST levels on the first postoperative day were significantly higher in the chemotherapy group than in the non-chemotherapy group (p<0,001; p<0,001), but on the later postoperative days there was no difference (p=0,099; p=0,436 és p=0,166; p=0,777). There was no significant difference in INR or serum bilirubin levels between the chemotherapy and non-chemotherapy group, not even on the first postoperative day (p=0,590; p=0,438 és p=0,777; p=0,915).

Comparing the subgroup of patients in the chemotherapy group, who received only cytotoxic chemotheray, or chemotherapy with bevacizumab, there was no significant difference in ALT, AST, INR or serum bilirubin levels (p=0,477; p=0,406; p=0,481; p=0,099).

4.4 Preoperative imaging

Comparing the number of laesions (≥ 1 cm) detected on preoperative imaging and on pathological examination, we found correspondence in 72 % of the cases. In 22 % of the patients the pathological examination found more laesions than it was expected on preoperative imaging.

There was no correlation between the RECIST and the pathologic response defined by the TRG (p=0,171).

4.5 Survival

After 20 months median follow up, the 5 years desease free survival (DFS) was 22%, the 5 years overall survival (OS) was 35%. The median OS was 41 months. We found a significantly worse desease free survival in the chemotherapy group than in the non-chemotherapy group (p=0,017), and overall survival showed numerous, but not significant difference between the two groups (p=0,065).

Focusing on the subgroup of patients in the chemotherapy group who received chemotherapy in combination with bevacizumab, the desease free survival of these patients was similar to the non-chemotherapy group (p=0,337). Comparing the subgroup of patients in the chemotherapy group, who received only cytotoxic chemotheray, or chemotherapy with bevacizumab, DFS was significantly better in the bevacizumab group (p=0,006), while OS showed no difference (p=0,262).

Analyzing the correllation between proeperative chemotherapy and survival according to the prognostic factors (tumor number, tumor size, time interval between primary and metastatic desease) we found that by good prognostic factors, survival was worst in the preoperative chemotherapy group than in the not treated group. In patients with poor prognostic factors, this difference disappeared.

4.6 Pathological examinations

Histomorfologic changes, which are likely to be associated to preoperative chemotherapy were analyzed in the chemotherapy and in the non-chemotherapy group, but none of these changes were observed only in the chemotherapy group.

The major response defined by the residual tumor cell ratio was a little more frequent in the chemotherapy group (65%), but in 41% of the patients in the non-chemotherapy group showed the same signs as a major response. There was no significant difference between the two groups (p=0,085). TRG scoring system showed significant difference between the groups (p=0,002), in the chemotherapy group 48% of the patients had TRG1, TRG2 or TRG3 stadium (response to chemotherapy), while in the non-chemotherapy group there were only 6 %. TNI was significantly shorter in the chemotherapy group than in the non-chemotherapy group (1,15 mm vs. 2,40 mm; p=0,021). Comparing the type of necrosis in the tumor, ILN was more frequent in the chemotherapy group than in the non-chemotherapy group (46% vs. 18%; p=0,031). Analyzing the pattern of tumor cells, tumor cells more more frequenty localized in the perifery of the metastases in the chemotherapy group than in the non-chemotherapy group (24% vs. 6%; p=0,009).

4.7 Morfological changes and survival

There were no correlation between any of the histomorfological changes and the desease free- or overall survival. Analyzing the survival plots, TRG showed a slight correlation with DFS and OS but these were not significant (p=0,089; p=0,169). Patients with a TNI <2mm had a better DFS and OS, than patients TNI >2mm, but this was not significant either (p=0,170; p=0,156).

5 Conclusions

Analyzing the perioperative results of patients with colorectal liver metastases resected after preoperative chemothery, we can conclude the followings:

a) Preoperative chemotherapy slightly increases the morbidity of liver resections, but there was no significant difference in the complications ratio between the preoperative chemotherapy- and non-chemotherapy group.
b) There was no increase in the morbidity of liver resections when preoperative cytotoxic chemotherapy was combined with bevacizumab.
c) Analyzing the postoperative serum bilirubin, INR, AST and ALT levels, there was no difference between the preoperative chemotherapy and non-chemotherapy groups.

We conclude, that after preoperative chemotherapy it is safe to perform hepatic resection. According to the literature, liver resection should be performed 4-5 weeks after the last dose of preoperative chemotherapy, which should not be longer than 3-6 cycles.

d) Analyzing the preoperative imaging and the pathological reports, the number of metastases were the same in the two modalities in 72% of the patients. There were 22% of the patients were more metastases were verified on the pathological examination than it was expected preoperatively.

e) Survival data after liver resection of CRCLM patients were similar to the international results. Desease free survival was worst in patients resected after preoperative chemotherapy than in patients resected without chemotherapy, which could be explained by the fact, that there were more patients with poor prognostic factors in the preoperative chemotherapy group.

f) Patients with good prognostic factors must be resected firstly. According to our data, patients with good prognostic factors resected after preoperative chemotherapy had worst survival than patients with good prognostic factors resected firstly.

g) Patients receiving chemotherapy in combination with targeted therapies had better survival than patients receiving only cytotoxic chemotherapy. Survival data of patients receiving chemotherapy in combination with targeted therapies had similar survival than patients resected without chemotherapy.

In conclusion, resectable patients, especially with good prognostic factors must be resected firstly. Borderline resectable patients should be treated with neoadjuvant chemotherapy preferably in combination with targeted therapies. In the group of resectable patients with poor prognostic factors, a short neoadjuvant chemotherapy should be considered before resection.

We analyzed firstly in Hungary the so far described different patho-morphological changes in colorectal liver metastasis in a single institution. According to the pathological examinations we can conclude the followings:

a) Steatohepatitis was observed in the preoperative chemotherapy and non-chemotherapy group as well, but severe (grade 3) steatohepatitis was present only in patients receiving preoperative irinotecan.
b) Patho-morfological changes described after preoperative chemotherapy could be observed in the resected colorectal liver metastases and the extension of these changes showed correlation with preoperative chemotherapy.
c) From the different patho-morfological changes, TRG (Tumor Regression Grade) and TNI (Tumor Thickness at the Tumor-Normal Interface) had correlation, but not significant correlation with desease free-, and overall survival. Fibrosis and residual tumor cell ratio together seems to predict the effect of a preoperative chemotherapy in CRCLM patients.

Our conclusion is that special patho-morfological changes in the resected colorectal liver metastases should be investigated, especially TRG status and TNI measurement is recommended. Analyzing these morphological changes would help to predict the effect of a chemotherapy and could help to specially target the adjuvant or later oncologic treatment after liver resection.

6 Publications

Publications connected to the dissertation

1. **Dede K,** Salamon F, Landherr L, Jakab F, Bursics A. (2015) Pathologic Assessment of Response to Chemotherapy in Colorectal Cancer Liver Metastases after Hepatic Resection: Which Method to Use? Pathol Oncol Res 21:173-9.
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7 Acknowledgements

I would like to thank to Professor **Ferenc Jakab** and to **Attila Bursics** that I could became familiar with liver surgery and to be interested in research work.

I would like to thank the everyday work to my collegues at the **Department of Oncosurgery** in the **Uzsoki Teaching Hospital.**

The research wouldn't be finished without the help of **Tamás Mersich, Zsolt Baranyai, István Besznyák** and **Attila Zaránd**.

Thank for the pathologic examinations and research planning to **Ferenc Salamon** and **Andrea Farkas**, and for the administrative work to **Andrea Katona**.

I would like to thank to **Marcell Szász** and **István Kenessey** for the help in the statistical analysis.

The research has many oncological and radiological attachments, I would like to thank to **László Landherr**, **Zsófia Egyed, Beáta Blans** and **Klára Hajnal** for their support.

Finally, I would like to thank to my **wife,** my **doughter,** my **parents** and my **sister** for the patience and support in the last couple of years.