EXAMINATION OF CONVENTIONAL STAGING AND PROGNOSTIC FACTORS IN COLORECTAL CANCER

Ph.D. Thesis Synopsis

Dr Rita Bori
Semmelweis University
School of Doctoral Studies of Pathology

Supervisor: Dr. Gábor Cserni, DSc

Critical examiners: Dr. Gábor Méhes, PhD,
Dr. Gábor István, PhD,

Members of the University Examination Committee:
Prof. Dr. Péter Nagy, DSc,
Dr. Zsolt Orosz, PhD,
Dr. László Vass, PhD,
Prof. Dr János Papp, PhD

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I. INTRODUCTION

Colorectal carcinomas (CRC) represent a significant public health problem all over the world, including Hungary. It is very important to recognize, diagnose and treat these cancers at the earliest stage possible.

The natural course of malignant tumours is influenced by a lot of circumstances (prognostic factors) which also determine their treatment. Well established and widely used prognostic factors (that can be defined by conventional methods) include the depth of invasion, the number of metastatic lymph nodes, the presence or absence of distant metastasis, vascular (mainly venous) invasion and the completeness of surgical resection.

One of the most important prognostic factors of CRCs is the status of the regional lymph nodes. Even a single metastatic lymph node causes a decrease in survival compared to lymph node negative tumours with similar features. Examining lymph nodes has become of greater importance since the introduction of adjuvant therapy as the status of lymph nodes may influence the systemic treatment of CRCs. The majority of patients having a positive lymph node status receive adjuvant chemotherapy.

The significance of identifying lymph node metastases when they are present is therefore understandable. The correct pN classification will obviously depend on the number of lymph nodes being examined.

Apart from distant and regional lymph node metastasis, the depth of invasion of the tumour is also among the major prognostic factors of CRCs. Both distant and regional nodal metastases, and even the presence of venous invasion depend on the depth of invasion. This latter is at least partly, time dependent, but on the other hand, may also be associated with the aggressiveness of the tumour. Aggressive tumours may infiltrate deeper faster. On the basis of the above considerations, tumours that have just infiltrated the external layer of muscularis propria are of less advanced stage, and seem to differ in their behaviour from tumours that deeply infiltrate the subserosal layer.

II. AIM

The aim of the present work was to examine the traditional prognostic factors, with special attention to details of the lymph node status and the depth of invasion. It seems, that not only the number of examined lymph nodes but also some of their qualitative features are also

important in identifying the metastatic ones. During the course of our work, we attempted to find such qualitative and quantitative characteristics that help differentiate among lymph nodes.

II.1. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

There have been a number of suggestions as to the minimum number of lymph nodes used for the precise determination of lymph node status in CRCs. We analyzed our data in retrospect to identify the minimum number of lymph nodes required for staging CRCs. Apart from this quantitative feature, we tried to search for some qualitative aspects which can help find metastatic lymph nodes.

As we have been gathering experience with the examination of sentinel lymph node in breast cancers for years, we would have liked to test whether this can be applied for CRCs. We therefore analysed the connection between sentinel lymph node metastasis and the status of the remainder of the regional lymph nodes.

II.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumour.

We supposed it to be very likely for the metastatic lymph nodes to be located near the tumour. To support our assumption, we examined the lymph nodes separately based on their distance from the tumour as measured along the bowel. We tried to define the minimum bowel length to be examined by which the metastatic lymph nodes and the stage of the tumour may be defined with the highest precision.

II.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

The number of metastatic lymph nodes is related to the prognosis of the tumour. The volume of tumour present in lymph nodes is usually reflected by the number of metastatic lymph nodes, and less attention is paid to the size of the metastasis, though there are some data referring to its relation to the prognosis. Our aim was to examine how the size of lymph node metastases related to further lymph nodes being affected.

II.4. Examining the heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

The main prognostic factors of CRCs include the presence of distant and/or regional lymph node metastasis as well as the depth of invasion. The presence of both distant and nodal

metastasis and also that of venous invasion is related to the invasion of tumour into the intestinal wall layers.

Our aim was to analyze whether the depth of tumour invasion influences nodal status, vascular invasion and distant metastasis within the pT3 group of colorectal carcinomas.

III. MATERIALS AND METHODS

The subjects of our analysis were patients with CRCs undergoing surgical resection of their tumours at the Department of Surgery in the Bács-Kiskun County Hospital or the Kiskunfélegyháza City Hospital between January 1996 and December 2007. All the specimens were assessed at our pathology department. In the beginning, the specimens were fixed in formalin, while later – in compliance with our agreement – we received them immediately after their surgical resection. The radial resection margin was marked with ink, the intestinal wall was cut parallel to the long axis of the bowel opposite the mesocolic margin, then these specimens were fixed first in 10%, later in 4% buffered formalin for 24-48 hours before being prepared. In the case of specimens removed by mesorectal excision, we examined the completeness of their excision. All lymph nodes recovered from the resection specimens were subjected to histopathology; this involved the investigation of haematoxylin and eosin-stained slides from the central cross-section of the nodes. There was a maximum of two lymph nodes embedded in one block.

III.1. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

We assessed the data of all resected primary CRC specimens removed between January 1996 and December 1998. We attempted to identify the minimum and optimal number of lymph nodes to be examined for the adequate staging of CRCs on the basis of the distribution of metastatic lymph nodes in relation to the total number of examined nodes.

Besides this mere quantitative model, we also set out to assess the nodal status from a more qualitative approach. Fom 1998, we attempted sentinel lymph node biopsy in a few CRCs. For this reason 2 ml of Patent blue dye was injected subserously immediately around the tumour during the course of the operation. The pathologist was responsible for identifying and removing the blue stained lymph nodes from the resection specimen. Then, these nodes

were examined separately from unstained lymph nodes and their metastatic status was also recorded separately.

Later the method of lymphatic mapping was somewhat modified. During the operation, the surgeon marked the first lymph nodes that stained blue (four nodes at most) with a suture. These nodes were examined more thoroughly when the other nodes were free of metastasis. In these cases step sections were made and cytokeratin immunohistochemistry was also used.

III.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumor.

Between December 1998 and December 1999, one hundred CRC specimens were assessed by means of dividing lymph nodes into 4 fractions. Before cutting out the blocks from the tumour, the specimens were divided into four pieces by cutting them perpendicular to the long axis of the bowel (Figure 1). Fraction A contained the mesocolic or perirectal tissues around the tumour and 1 cm away from it in both oral and aboral directions. Fraction B consisted of one or two 2 cm long segments distal and proximal to fraction A. Fraction C consisted of one or two 3 cm long segments from fraction B, and fraction D consisted of the remainder of the specimen.

Statistical comparisons between the lymph nodes taken from different fractions were made by means of Student's t-test.

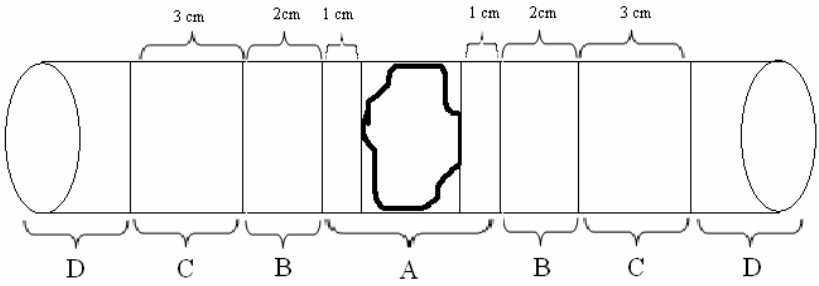

Figure 1. *Schematic drawing illustrating the different fractions separately analysed for their nodal status*

III.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

Between September 2000 and September 2005, CRC resection specimens with nodal metastasis were prospectively assessed with regards to the maximum size of nodal metastasis. Apart from the number of lymph nodes and the number of affected lymph nodes, the largest dimension of the largest lymph node metastasis was also recorded. For assessing the correlation of lymph node metastasis size and the number of affected lymph nodes Spearman's correlation was applied.

A statistical cluster analysis was used to establish different metastasis size determined prognostic groups, where prognosis was reflected by the number of involved lymph nodes or the lymph node ratio (metastatic lymph nodes/ examined lymph nodes) as surrogate markers of disease outcome.

III.4. Examining the heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

Patients with CRCs undergoing surgical resection between April 2002 and December 2007 were subjects of the assessment.

In the final period of our study 117 tumours were routinely assessed by orcein elastic staining.

The pT3 category was divided into two subgroups labelled pT3a (up to 5 mm infiltration beyond the muscularis propria) and pT3b (more than 5 mm infiltration) on the basis of the depth of the invasion. We analysed the lymph node status, the presence of vascular invasion and the presence or lack of distant metastasis in relation to the depth of invasion. Colon and rectum carcinomas were studied separately.

Rectal cancer patients receiving neoadjuvant therapy were also included in the study. Their tumours were labelled according to the ypTNM categories and these latter tumours were analyzed separately.

The Pearson's chi-square test was used for comparing the data of tumours belonging to the different pT groups. The Fisher exact test was applied for low case numbers. The level of significance was $p<0.05$.

IV. RESULTS

IV.1. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

In the aforementioned period, 224 tumours were analyzed. In 40 (33%) of the 123 lymph node positive cases more than 3 lymph nodes were found to contain metastasis. When less than 6 lymph nodes were examined, the number of node positive cases was less than the number of node negative cases, although the opposite was true for the whole group and subgroups with greater number of lymph nodes examined. On the basis of a probability calculation, the examination of at least 16 lymph nodes is recommended for identifying tumours with nodal involvement (stage Dukes C) with at least 90 % precision, and this number of examined lymph nodes is also sufficient for the identification of pN2 group with similar precision.

We attempted to separate the sentinel lymph nodes from other lymph nodes merely on the basis of their blue staining in 28 cases. We found stained lymph nodes in 27 specimens.

Altogether 139 (average 5.15) lymph nodes stained blue and these were considered sentinel lymph nodes. There were 5 cases with metastasis only in the non-blue lymph nodes, these were considered false negatives as concerns the sentinel nodes being able to reflect the overall nodal status.. The sentinel lymph nodes were positive in 8 patients, and in 2 cases they were the only metastatic nodes.

The sensitivity of sentinel lymph node biopsy proved to be 62 %, whereas the negative predictive value was 74% (in relation to the overall nodal status). The false negative rate was 38%. By definition, the specificity and positive predictive values are 100%, since node negativity cannot occur in case of a positive blue node.

The modified method was applied in 30 patients. This method was unsuccessful in 5 patients. A total of 49 (an average of 2) lymph nodes were labelled as sentinel lymph nodes. The lymph node status was negative in 20 cases (pN0). There were 3 out of the 5 lymph node positive cases where metastasis was present only in non-sentinel lymph nodes. These cases were the false negative ones. There were only two cases with sentinel lymph node metastasis and only one where the sentinel lymph node was the exclusive site of nodal metastasis. The sensitivity and the negative predictive value were 40% and 87%, respectively. The false negative rate was quite high, 60%.

If isolated tumour cells (the recognition of which was made more likely with a more thorough examination) are also taken into consideration, 6 sentinel nodes were found to harbour isolated tumour cell type nodal involvement. Although these cases were not regarded as metastatic on the basis of TNM classification, their frequent occurrence in the blue lymph

nodes may suggest that the sentinel lymph node theory is correct in CRCs. With isolated tumour cells included in the group with lymph node involvement, the sensitivity, negative predictive value and false negative rate were 67%, 76% and 33%, respectively.

IV.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumour.

Significantly more lymph nodes were removed from fraction A than from fraction B, and more from fraction B than from fraction C.

The lymph node status could be determined in all but one tumour on the basis of examining the lymph nodes from fraction A. If we supplemented this with the examination of lymph nodes from fraction B, we could classify all tumours as affected by or free from lymph node metastasis. The correct identification of all but one patient with pN2 category (>3 metastatic lymph nodes) was also possible on the basis of examining only the lymph nodes from fractions A and B.

IV.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

During the course of our study, we assessed a total of 235 colorectal cancer specimens with lymph node involvement and we recorded data on the maximum size of metastasis.

The average number of examined lymph nodes was 18, the largest lymph node metastasis of each tumour was between 0.14 and 22.1 mm (median 6 mm) in size. 142 (60 %) of the tumours were classified as belonging to the pN1 and 93 (40%) as belonging to the pN2 category. There was significant correlation between the number of involved lymph nodes and the size of the largest metastasis ($p<0.0001$). There was also correlation between the lymph node ratio (LNR) and the largest metastasis size ($p<0.0001$). As expected, the lymph node ratio significantly correlated with the number of involved lymph nodes ($p<0.0001$).

The cluster analysis identified three relatively distinct groups, which logically ranged from low, through medium to more extensive nodal involvement.

Group 1 (low risk) was associated with a single metastatic lymph node, small metastasis size (average:4 mm) and very low LNR (average: 0,07)

Group 2 (medium risk) was associated with an average of 3 metastatic lymph nodes, medium metastasis size (average: 7 mm) and medium LNR (average: 0.21)

Group 3 (high risk) was associated with a large number of metastatic lymph nodes (average:13), large metastasis size (average:10 mm) and high LNR (average:0.55).

IV.4. Examining of heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

During the course of the studied period, the data of 701 tumours were analyzed. Among these, 108 (15%) patients with rectal carcinoma received radiotherapy or chemo-radiotherapy preceding their operation. The majority of these tumours belonged to the pT3 (429/593; 72%) or ypT3 category (66/108;61%).

In the pT3a subgroup, node positivity (44% vs 75%) or massive lymph node involvement (pN2) (9% vs 39%) was significantly lower than in the pT3b subgroup. The proportion of tumours with lymph node metastasis in the pT3a subgroup was significantly different from that of pT2 tumours.

In terms of frequency of venous invasion, there was also significant difference between the pT3a and pT3b groups (17% vs 30%), and that of the former group also differed from the rate of venous invasion in pT2 tumours. On the other hand, the frequency of vascular invasion in pT3b tumours was rather similar to that noted in pT4 cancers.

We only had data of metastatic status in 342 patients primarily treated surgically. As it was expected, the pT4 tumours had significantly higher rates of distant metastasis than the pT3 tumours or the pT3b subgroup. It was interesting to see that there was a significant difference between pT3a and pT3b tumours with regards to distant metastasis (11% vs 28%). When we assessed colon and rectal carcinomas separately, our results were similar for the two localisations.

The node negative patients were analyzed separately too. Obviously, the rates of vascular invasion and distant metastasis were smaller in this subset than in the whole series or in node-positive tumours. The difference in the rate of venous invasion was significantly different between pT3a and pT3b tumours, but there was no significant difference in the rates of distant metastasis between the two groups.

In the case of patients primarily treated with neoadjuvant therapy, there was a significant difference in the proportions of involved lymph nodes in the categories ypT2 and ypT3 or ypT3a, whereas the proportion of positive nodes was not significantly different between ypT3a (54%) and ypT3b (64%) tumours.

The overall venous invasion rate of ypT tumours was somewhat lower than that of the pT tumours. The highest rate of venous invasion was seen in the ypT3a subset. The distant metastasis rate of the tumours treated with neoadjuvant therapy was not suitable for analysis, as the rate of M1 cases was so low, due to selection bias.

V. DISCUSSION

V.l. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

There have been a lot of different suggestions as to the minimum number of lymph nodes to be examined in order to determine the correct stage of CRCs. Although all lymph nodes should be examined, provided that all lymph nodes are considered equivalent, our data suggest that nodal staging might often be unreliable when the minimum of 6 lymph nodes is not reached. The reliability of nodal staging seems acceptable when the optimum of 16 lymph nodes examined is reached..

We also brought up the possibility of a more qualitative approach and we attempted to identify the sentinel lymph nodes in a few cases of CRCs.

The sentinel lymph node theory states that lymph nodes which directly drain the tumours are the most likely to contain metastasis. Therefore, determining the lymph node status adequately may be based on the identification and correct histological examination of these lymph nodes.

The aim of identifying and evaluating sentinel lymph nodes is to define the stage of the tumours correctly, as the pathological stage of the disease influences the choice of adjuvant treatments. The other advantage of sentinel lymph node biopsy is that it can help discover aberrant lymphatic drainage.

The high rate of false negative sentinel lymph nodes points out the limitations of determining sentinel lymph node in CRCs. There may be many causes for these unfavourable results. Some of these are of technical nature. The method to be followed is ambiguous in the literature and the definition of sentinel lymph node is also not consistent. The identification of real sentinel lymph nodes is endangered by extensive lymphatic invasion. Larger tumours may also lead to a higher possibility of misidentifying sentinel lymph nodes. Moreover, tumor invasion through the bowel wall may compress lymphatic vessels and the lymphatic drainage (and the blue dye) may deviate from real sentinel lymph nodes. In the future, sentinel lymph node biopsy should rather be tested in less advanced pT1 and pT2 tumours. In such lower stage tumours, the more thorough examination of sentinel lymph nodes (serial sections and immunohistochemistry) would help identifying patients who may benefit from adjuvant chemotherapy.

V.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumour.

Most studies on the subject conclude that as many as possible, but preferably all lymph nodes should be found and examined for the correct staging of CRCs. For the time being, the knowledge of lymph node status is important for the treatment of patients. At the same time, if we could separate lymph nodes containing metastasis with higher probability and frequency from those that are less likely to be affected, we could reduce the time spent for recovering nodes from pericolic or perirectal fat.

Our data suggest that the correct lymph node status can be determined by examining lymph nodes located in the fat tissues underneath the tumour and in the segments located 3 cm proximal and distal from it. Our results also suggest that if 3 lymph nodes are involved, further lymph nodes need to be examined in order to recognise the possible pN2 status.

With respect to the favourable results, we examined the previously separated fraction A and B together and the lymph nodes found in these were separated from the remainder (from the combined fractions C and D). The above mentioned suggestion was confirmed by the examination of 651 additional CRCs examined till the end of 2008. Among 322 lymph node positive patients we found 6 cases in which fraction CD contained metastatic lymph nodes while fraction AB was free from metastatic lymph nodes. Therefore, the false negative rate for the lymph nodes from fractions A and B to predict the overall nodal status was 1,9%. Based on the formulated suggestions, two cases would have been staged incorrectly as pN1 had there been further lymph nodes examined only when 3 lymph nodes in fraction AB were found affected. This would result in further 0.6% as false staging rate in determining the correct lymph node status. However, this latter error would not cause any difference in the treatment of the disease as the qualitative lymph node status (pN0 vs pN1 or pN2) outweighs in importance the quantitative lymph node status (pN1 versus pN2) in planning the treatment. These further cases analysed following our preliminary study, justified our conclusions.

V.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

Our data suggest that the largest metastasis size is in correlation with the number of lymph nodes involved in the metastatic process. That is, the larger the largest metastasis is, the higher the number of lymph nodes which are likely to be affected. This can be perceived as an indirect proof of the sentinel lymph node theory in CRCs. A cascade like mechanism was also proposed to explain the distribution of involved axillary lymph nodes in breast cancer: metastases spread from one (a few) lymph node – from the sentinel lymph node(s) in our

interpretation – to other lymph nodes (the second and then third echelon lymph node(s). The same seems to hold true for CRCs.

The number of metastatic lymph nodes was in strong correlation with the LNR In case of CRCs, the prognosis may be estimated on the basis of LNR just as well, or even better than using the pN categories of the TNM system. As the number of involved lymph nodes is a prognostic parameter in CRC, the largest metastasis size also reflects the prognosis indirectly, either by the shift from pN1 disease to pN2 disease or parallel with the number of involved lymph nodes or as being correlated with the LNR.

We found indirect evidence for a larger lymph node metastasis to indicate a higher probability for the disease to have spread to more lymph nodes and hence showing a worse prognosis.

V.4. Examining the heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

Our study also supports the previously described phenomenon that increasing invasion depth as reflected by the higher pT categories of the TNM classification is associated with other recognised prognostic markers: a higher rate of nodal involvement, a higher number of metastatic lymph nodes (more pN2 cases), a higher frequency of venous invasion and distant metastasis. Although we did not investigate survival rates, we looked at prognostic markers which are associated with survival.

The subgroups pT3a and pT3b distinguished on the basis of the depth of invasion beyond the muscular propria showed significant differences regarding the frequency of lymph node involvement, massive lymph node metastasis, vascular invasion and distant metastasis. As expected, the estimated prognostic markers of pT3a tumour fell closer to those of pT2 tumours, and those of pT3b tumours more closely resembled to the prognostic data of pT4 tumours.

As nodal involvement is generally considered as a major prognostic disadvantage, the subset of node-negative cancers were also analyzed separately. The somewhat higher rate of distant metastasis in the pT3b category was not significantly different from that of pT3a tumours. However, there was a significantly higher proportion of cases showing venous invasion in more advanced pT3b carcinomas.

Although the number of cases analyzed after neoadjuvant therapy was smaller, and the related conclusion is therefore weaker, it seemed that the subdivision of ypT3 tumours into ypT3a and ypT3b is less useful here. There was no significant difference between the two subgroups in terms of nodal involvement or venous invasion. This phenomenon could be

related to the fact that neoadjuvant therapy destroys the tumour, but also destroys the bowel wall. Therefore it is problematic to determine the extension of invasion after neoaduvant treatments. In addition, venous invasion may be identified less easily in these cancers, and lymph node identification is also less fruitful.

VI. CONCLUSIONS

VI.1. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

We have demonstrated that the examination of 6 or less nodes may lead to the understaging of CRCs. Our series indicated the need of at least 16 lymph nodes to be assessed for recognising nodal involvement of most tumours, and this is seems sufficient for identifying extensive nodal involvement (pN2 stage) too. These numbers reflect the reliability of staging, for which all the surgically removed lymph nodes need to be examined histologically unless there is a method of selecting the nodes based on some qualitative factors.

One of these qualitative features could be the direct lymphatic drainage from the tumour site (sentinel lymph node).

Our series, however, points to the limitations of sentinel lymph node biopsy in CRC, as it did not seem useful for all the patients. By considering everything, we believe that sentinel lymph node identification in our patients (where tumours with deep infiltration were in abundance) did not fulfil our expectations neither with the original method, nor with the later applied modifications. The study was not continued as the surgeon involved changed hospitals.

We have to use traditional methods and cannot overcome the limitations of our technology until other methods are introduced for identifying sentinel lymph nodes (e.g. gamma probe guidance) or other qualitative features are found to help identifying metastatic lymph nodes correctly (e.g. size/localisation of lymph nodes in our previous studies). We must retain the standard procedure of recovering and microscopically examining all removed lymph nodes for the staging of CRCs, however, in compliance with what is said in part 2 of this work, the zone of identifying affected nodes could be limited to the immediate (3 cm distant) environs of the tumour.

VI.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumour.

Our method may reduce the optimal (but not the minimum) number of lymph nodes examined for reflecting reliable staging. On the basis of the analysis of over 700 patients, we recommend that resected CRC specimens should, if possible, be cut in both directions perpendicular to the longitudinal axis of the bowel, 3 cm from the edge of the tumour. Lymph nodes should be recovered only from this fraction of pericolic and/or perirectal tissues. Should the number of nodes found here be 6 or less (as this is the minimum number suggested by guidelines, and the risk of false negative lymph node staging may increase if less than 7 nodes are examined) and all of them be negative, or the number of involved nodes in this area be only 3, further nodes are to be sought for in the fat tissue of the remaining bowel specimen.

Several different studies have explored that the number of examined lymph nodes is a prognostic factor, mainly in pN0 colon carcinomas, and it would be logical to suggest that all removed lymph nodes need to be examined. However, the low number of Hungarian pathologists does not allow them to spend one hour or more time recovering all lymph nodes removed with the bowel. A considerable amount of time may be saved if the pathologist only needed to identify and remove lymph nodes from a shorter part of the bowel. Our results and the above mentioned suggestion may be a guide for that.

VI.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

In summary, the correlation of the largest metastasis size and the number of involved nodes may be considered as an indirect proof of sequential spread from first line lymph nodes (the sentinel lymph nodes) to further lymph nodes, though our data suggests a more complex process. As the size of the largest lymph node metastasis increases, the number of involved nodes increases more than might be expected, and also the variability of affected nodes become higher. This suggests a serial or cascade process, which becomes more unpredictable as the tumour volume increases.

VI.4. Examination of heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

We demonstrated that CRCs invading 5 mm or less beyond the muscularis propia layer and infiltrate the subserosa or the mesorectal tissues (pT3a) are associated with a better prognostic profile in terms of nodal involvement, vascular invasion and distant metastasis than the tumours infiltrating deeper (pT3b). This observation was true for both carcinomas of the

colon and those of the rectum. However, it seemed that a similar subclassification was of no use following neoadjuvant treatment of rectal cancers.

The majority of CRCs fall into the category of pT3. Subcategorisation might be useful with respect to both predicting the prognosis and planning the treatment. It might be concluded that tumours which infiltrate the subserosa deeply should be treated with more aggressive chemotherapy than more superficial tumours, although, this issue needs further exploration.

VII. NEW FINDINGS

VII.1. Nodal staging of colorectal carcinomas. Quantitative and qualitative aspects – adapting the sentinel lymph node theory to colorectal carcinomas.

1. The examination of 6 or less nodes may lead to inaccurate pN0 staging of CRCs.

2. If we consider every lymph node equivalent, the examination of at least 16 lymph nodes seem to be necessary for identifying the pN categories of pTNM system accurately. These numbers reflect the reliabilty of staging rather than give a minimum number beyond which no efforts would be needed to recover further nodes.

3. The sentinel lymph node mapping of colon and proximal rectal cancers seems to be technically feasible.

4. This method might be considered experimental in CRCs because its false negative rate is high, and also because uniform technical methods of identification and a consistent definition of sentinel lymph nodes are lacking.

5. As the large volume of the tumours may be in the background of the high false negative rate, the examination of sentinel lymph nodes in pT1 and pT2 categories needs further studies.

VII.2. Nodal staging of colorectal carcinomas. Distance of lymph nodes from the primary tumour.

1. It is usually true that most lymph nodes are close to the tumour and there are fewer nodes further away from the tumour.

2. Metastatic lymph nodes are mainly located in the immediate vicinity of the tumour.

3. In compliance with the above said, appropriate staging may be obtained if lymph nodes are taken for examination from the bowel segment conatining the tumour and 3 cm distal and proximal to it.

4. In order to further decrease the number of cases classified incorrectly, it may be recommended that the lymph nodes of the remaining bowel fraction should be removed and examined if the pN0 category would be identified on the basis of few (less than 7) lymph nodes, or if the pN1 category would be identified on the basis of 3 metastatic lymph nodes..

VII.3. Nodal staging of colorectal carcinomas. Analysis of the largest metastasis size.

1. There is a correlation between the largest metastasis size and the number of involved lymph nodes, as well as the ratio of involved lymph nodes. Therefore, indirectly we can also assume that metastasis size is related to prognosis too.

2. The larger the largest lymph node metastasis, the higher the probability for a massive lymph node involvement. Our results also support the sentinel lymph node theory in CRCs.

3. On the basis of the cluster analysis, apart from a sequential process, a cascade-like, multiplicative metastatic process must also be assumed as an explanation to nodal involvement, since the level of massive nodal involvement may be less accurately forecast on the basis of the size of the largest metastasis.

VII/4. Examination of heterogeneity of pT3 colorectal carcinomas according to their depth of invasion.

1. Invasion depth of CRCs is an acknowledged prognostic factor, which is in connection with lymph node metastasis, venous invasion and distant metastasis. Two subcategories can be distinguished within the most frequent pT3 group on the basis of their invasion depth and these subcategories might be connected to different prognosis and therefore probably different treatment needs.

2. The significance of pT3a and pT3b subcategories might be important mainly in lymph node negative tumours.

3. Distinguishing between pT3a and pT3b subcategories may be relevant for both colon and rectal tumours.

4. Subcategorisation of the pT3 category on the basis of the depth of invasion seems to be less useful after neoadjuvant treatment.

VIII. OWN PUBLICATIONS

Cumulative impact factor (IF)**: 21,643**

Independent citations: **89**

Publications related to the topic of the thesis **(IF: 4,913)**

1. Cserni G, Vajda K, Tarján M, **Bori R**, Svébis M, Baltás B. (1999) Nodal staging of colorectal carcinomas from quantitative and qualitative aspects. Can lymphatic mapping help staging? *Pathol Oncol Res* 5:291-296. **IF(1999): -**

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