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Lymph node size as a simple prognostic factor in node negative colon cancer and an alternative thesis to stage migration

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Abstract

BACKGROUND: Stage migration is an accepted explanation for the association between lymph node (LN) yield and outcome in colon cancer. To investigate whether the alternative thesis of immune response is more likely, we performed a retrospective study.

METHODS: We enrolled 239 cases of node negative cancers, which were categorized according to the number of LNs with diameters larger than 5 mm (LN5) into the groups LN5-very low (0 to 1 LN5), LN5-low (2 to 5 LN5), and LN5-high ($\geq$6 LN5).

RESULTS: Significant differences were found in pT3/4 cancers with median survival times of 40, 57, and 71 months (P = .022) in the LN5-very low, LN5-low, and LN5-high groups, respectively. Multivariable analysis revealed that LN5 number and infiltration type were independent prognostic factors.

CONCLUSIONS: LN size is prognostic in node negative colon cancer. The correct explanation for outcome differences associated with LN harvest is probably the activation status of LNs.

Lymph node (LN) staging is still of crucial importance for the prognosis estimation and therapy stratification in colon cancer. It has been confirmed by many studies that the number of evaluated LNs correlates with patient outcomes in nodal negative colon cancers.[1–5] Missing LN metastases because of insufficient LN harvest could cause an incorrect stage classification, thereby confounding the survival analysis. A statistical stage migration effect, also known as Will Rogers phenomenon, is therefore the most frequently mentioned explanation for this association.[6] Recently, we questioned this hypothesis. We hypothesized that the reported superior survival data actually are caused by an immunological effect. Activation of the immune system could result in an enlargement of LNs, which are

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therefore easy to detect in high numbers. In the setting of conventional LN dissection, a high LN yield would not prevent understaging but indicates an activated state of the immune system. This could be a reasonable explanation of why advanced LN dissection techniques fail to improve the prognostic value of LN staging.

To determine whether this is also true using the conventional LN staging technique, we performed a retrospective study enrolling a larger number of nodal negative cases with long follow-up.

Patients and Methods

Patients and follow-up

Cases of nodal negative colon cancer were gathered from the files of the Institute of Pathology, Klinikum Augsburg between 2002 and 2005. Inclusion criteria were tumor-free resection margins, curative intention, and a follow-up of greater than or equal to 2 months. Follow-up data were provided by the Clinical and Population Based Cancer Registry Augsburg. The study was proven by the internal review board of the Klinikum Augsburg.

Histopathology

During the time period mentioned above, LNs were dissected using the conventional manual dissection technique. LNs larger than 5 mm in diameter were bisected. After paraffin embedding at least 2 step sections were cut from each block. Then the slides were stained with hematoxylin and eosin (H&E).

From each case, a representative slide of the tumor was re-evaluated by 2 independent investigators (B.M. and T.S.) blinded to the initial results. The evaluated features were pT-stage, histological type, invasion type according to Jass, tumor budding, lymphatic and venous invasion, lymphocytic infiltration, and Crohn-like inflammation. In discrepant cases, the slides were re-evaluated again, and a consensus decision was made. The invasion type was analyzed according to the description by Jass. Tumor budding was evaluated on H&E stained slides using the counting method. The cut-off value used was 30 buds/0.385 mm^2. The lymphocytic infiltration was graded qualitatively using a 3-tailed system (no or low, moderate, and severe). No discrimination between intra- and peritumoral infiltration was made. Crohn-like inflammation again was evaluated according to Jass' description.

In mucinous, poorly differentiated and medullary carcinomas, additional immunohistochemical investigations were performed with an MLH1 antibody to determine acquired microsatellite instability (MSI). In cases where at least one of the Bethesda criteria was fulfilled, full MSI investigation including immunohistochemistry MLH1, MSH2, MSH6, PMS2, and molecular MSI testing were performed. For immunohistochemistry, 3 to 5 μm thin sections were cut. All reactions were performed using a Ventana Benchmark Ultra system (Roche diagnostics, Mannheim, Germany). Prediluted mouse monoclonal antibodies including anti-MLH-1 (M1), anti-MSH2 (G219-1129), anti-MSH6 (44), and the rabbit monoclonal antibody PMS2 (EPR3947) were used. The Ventana Vision detection system (Roche diagnostics) was used for the development of the reactions.

Morphometry

For the LN morphometry, a simple caliber (Fig. 1) was used on the basis of H&E stained slides. The investigator (Y.K.) counted the number of LNs with diameters greater than 5 mm in each case. Because of the significantly lower LN harvest because of the conventional dissection technique, the cut-off value was adapted. A harvest of greater than or equal to 6 LNs with diameters greater than 5 mm qualified a case as LN5-high (LN5h). All cases with 2 to 5 such LNs were regarded as LN5-low (LN5l). The remaining cases with zero or one LN greater than 5 mm were classified as LN5-very low (LN5vl).

The study was approved by the internal review board of the Klinikum Augsburg.

Statistics

Depending on the results of normality testing, metric values were compared using either the Student *t* test or the Mann–Whitney rank-sum test. Dichotomous data were analyzed with the chi-square test or Fisher's exact test depending on the sample size. For the determination of the median follow-up time, the method of Schemper and Smith was used.[7] Kaplan–Meier curves were created and log-rank regression analyses were performed to compare overall and tumor-related survival of the different groups. Multivariable analyses were performed using the Cox regression

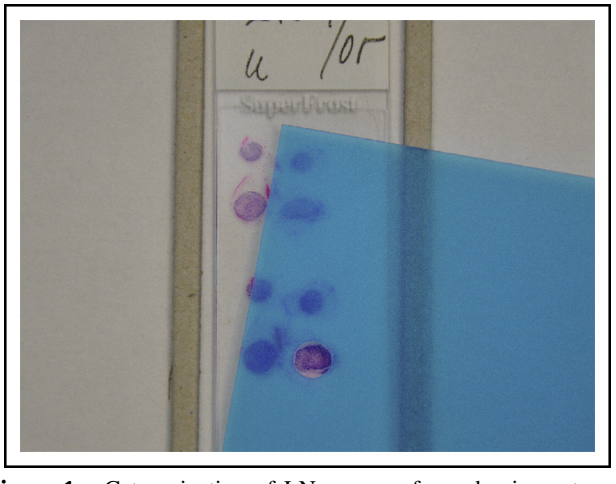

Figure 1 Categorization of LNs was performed using a transparent caliber with a 5-mm hole based on H&E stained slides. This proved to be a feasible, very simple, and fast method.