Sentinel Lymph Node and Occult Tumor Cells in Colon Cancer; the Good, the Bad, and the Ugly

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Abstract

Background: Node negative colon cancers pose a therapeutic dilemma, as there is a subset of patients who will have disease recurrence and who probably would benefit from chemotherapy. Sentinel lymph node biopsy (SLNB) and molecular diagnosis in search of occult tumor cells (OTC) in lymph nodes may aid in detecting these patients.

Methods: We performed a review of the literature in Medline and selected the most relevant articles which deal with SLNB and the molecular diagnosis of lymph nodes and discuss their relevance.

Findings: There is much heterogeneity among published studies (techniques and definitions). However, the presence of OTC in SLNB confer a negative impact on DFS and OS in Colon Cancer patients. OTC positive nodes is associated with disease recurrence of around 15-20% and 3 year DFS and OS of around 80%, much worse when compared with OTC negative patients.

Conclusions: The available literature suggests that the presence of OTC in Colon Cancer is an important prognostic variable. However, there is much heterogeneity among studies, and no prospective trials have been conducted to determine if these patients will benefit from chemotherapy.

Keywords: Sentinellymph node; Colno cancer; Occult tumor cells; Micrometastases; Isolated tumor cells

Discussion

Colon cancer (CC) is one of the most common cancers in the world. It is the third most commonly diagnosed cancer in males and the second most common in females. [1] The prognosis of patients with colon cancer is associated with the stage at the time of initial treatment. Patients with tumor confined to the colonic wall with no lymph node metastasis (LNMM) and no distant metastasis can expect a 5-year survival of 90%. [2] The most important prognostic indicator of survival is the presence or absence of LNM. The 5-year survival for patients who have LNM drops to 70.8%, [2] it is this group of patients who are chemotherapy (QT) candidates with the aim of improving systemic control. 5 year disease free survival (DFS) and overall survival (OS) with and without QT are as follows: DFS 45% surgery alone vs 63-64% for surgery + QT; OS 54% surgery alone vs 65-75% surgery + QT [3].

Approximately 30% of node negative patients (stage I and II) will develop loco-regional or distant disease recurrence [4]. However, it is not clear as to whether this group of patients benefit from QT. It has been argued that a high risk subset of node negative patients (T4, suboptimal lymph node harvest (<12 nodes), presence of lympho-vascular invasion, bowel obstruction, bowel perforation, and poorly differentiated histology) could benefit from QT [5]. Patients with high risk features have recurrence risks that approximate those of stage III disease [5]. When given QT (5-FU/Levamisol), this group will have a 31% decrease in tumor recurrence without a significant benefit in OS [6].

Patients without high risk factors classified as “average risk” have a 70-80% chance of cure with surgery alone, and apparently do not benefit from QT. The absolute risk reduction of administering QT (5y DFS and OS) is of 3% and 2% respectively when compared...
of micrometastatic disease, isolated tumor cells, occult tumor cells identified. Occult tumor cells (OTC) are defined as disease that is not detected by standard pathological techniques (either pN1mi or pN0i+). The number of lymph node sections needed to make this type of analysis is not clearly defined, being 2 the standard number performed currently in the H and E examination, and 3 or more for IHC. Moreover, not all studies analyse 12 lymph nodes as a minimum, which may lead to inconsistencies and possible down staging of disease.

An aspect that has drawn much attention is the type of analysis performed on the SLN and the prognostic implications that follow. The different types of analysis are IHC or RT-PCR. These techniques have been compared and the results are conflicting.

If we review results from older studies that look at the presence of pN1mi and prognostic significance, we can observe that only 3 of 8 studies performed with IHC show a positive result, as compared to 3 of 3 when performed with RT-PCR. A meta analysis of these data by Iddings et al. concluded that occult metastases identified by RT-PCR but not IHC are associated with a worse clinical outcome [31-42]. Iddings reports 3 year DFS and OS for patients pN0i- and with pN1mi to be 90 vs 78% and 97 vs 78% respectively [31].

These findings differ from those by Sloothaak et al. They report on the prognostic value of pN1mi and pN0i+. In their analysis, they exclude studies that used RT-PCR technique and included only those studies that used IHC. They identify 8 studies that are useful for meta analysis, with a total number of 1359 (958 Colon cancer) patients. Cancer recurrence rates were reported only in 5 studies. Detection of pN1mi was associated with higher recurrence rates in patients with colon cancer (OR 7.25% CI 2.4-13.3), no difference was observed when analysing pN0i+ in colorectal cancer OR (1.00 95% CI 0.53-1.88) [43]. Some published literature suggest that the presence of pN1mi but not pN0i+ is clinically relevant, however, other studies have challenged this outcome. A large meta analysis performed by Rahbari et al. in 2011 that included 39 studies with more than 4000 patients concludes that positive molecular tumor-cell detection performed either with RT-PCR or IHC (or both) in regional lymph nodes (pN1mi or pN0i+) was associated with poor OS (HR, 2.20; 95% CI, 1.43 to 3.40), disease-specific survival (HR, 3.37; 95% CI, 2.31-4.93), and DFS (HR, 2.24; 95% CI, 1.57-3.20). In subgroup analyses their results confirm the findings reported previously for pN1mi with poor OS (HR 3.62, 95%CI 1.34-9.80), DFS (HR, 2.81; 95% CI 1.11-3.86). It is important to note that 13 of the 39 studies analysed fewer than 12 LN, and there is moderate heterogeneity among studies. Because of lack of studies they could not analyse the pN0i- subset independently, however because the pooled analyses concluded a worse prognosis, it hints that pN0i+ could also influence prognosis [44].
Bilchic et al. reports on the results obtained from two multicenter prospective trials that include 253 patients whose lymph nodes were examined with IHC techniques. Upstaging occurred in 20% of patients. Disease recurrence varied according to nodal status pN0i-: 6% (9/141); pN0i+: 7% (2/27); pN1mi: 22% (2/9); N1/2: 33% (25/76); p<0.001 [45]. Mescoli reports disease recurrence rates in pN0i- of 4.7% vs. 14% in pN0i+ patients (HR 3.00; 95% CI 1.23-7.32 P=0.013). Their IHC analysis was done with a highly accurate antibody. They report upstaging in 59% of patients with CC. They correlate the presence of ITC with other features previously discussed as high risk (tumor necrosis, perineural invasion) and with number of lymph nodes harvested. They perform an interesting multivariate analysis including variables as T stage, cancer grade, cancer necrosis, vascular invasion, perineural invasion and pN0i+ and found the latter to be the only variable associated with cancer recurrence (Cox model; hazard ratio 3.00; 95% CI 1.23-7.32, P=0.013) [46].

An interesting clinical trial performed recently by Protic et al aimed to explicitly assess the clinical significance of pN0i+ vs pN0i- patients treated solely by surgery in patients who had a lymph node harvest of 12 or more (as to minimise biases that could be introduced with suboptimal lymph node sampling). Pan cyto-kerating IHC technique was performed on all H&E negative LN. They compared disease recurrence in the pN0i- group vs pN0i+ group and found a statistically significant difference in disease recurrence of 2.6 vs. 16.7% respectively. DFS was 92.9 mo vs 71.8 mo for the pN0i- vs pN0i+ groups respectively (p=010001). They conclude that the group of pN0i- patients would not benefit from the addition of adjuvant chemotherapy [47]. In Table 1 we sumarry the impact of pN0i-, pN0i+, pN1mi according to the different authors exposed above.

The variations observed can be explained by the different sensitivities inherent to each technique, [6]. In as much as IHC a wide variety of antibodies have been used that could account for different sensitivities and specificities. Among them are AE1/AE3 (low specificity), Keratin 20, Keratin 19, Mucin apoprotein 2, Guanylyl cyclase C, Carcinoembryonic antigen, CEACAM6, CEACAM1-2, CEACAM1-L, CEACAM7-1, CEACAM7-2, c-Met, K-ras mutation, MNF 116 anticytokeratin antibody (high specificity and sensitivity) [46- 52]. The marker with the strongest theoretical value for occult CRC metastasis is the Cyto Keratin 20 gene (CK20) that is detected through RT-PCR. It’s expressed in almost all CRC (high sensitivity with low expression of CK20 in normal LN) [53].

Despite all of the research that has been performed on the implications of positive OTC in CC, it has not been demonstrated that the addition of systemic chemotherapy in these patients will prove beneficial, and this will only be settled with a prospective randomized trial. Before this is done, it’s imperative to define and standardize the mechanism by which OTC (pN1mi and pN0i+) are detected in patients with colon cancer.

Due to the implications that the presence of pN1mi have on prognosis, it is plausible that the presence of OTC will become another criteria defining high risk patients and as such, these will be candidates to receive QT. However it is important to keep in mind that the presence of OTC per se does not mandate recurrent disease, other factors which will influence this include clonogenic capacity, genotypic/phenotypic characteristics and micro-environment that may influence migration, survival and growth of these cells [6]. Again these are areas of future investigation that will ultimately lead to patient directed therapies, sparing those who will most likely not benefit from adjuvant treatments.

**Conclusion**

In conclusion there is a very significant trend in patients with stage I or II Colon Cancer and the presence of OTC to have higher rates of disease recurrence. However there are studies that demonstrate that the SLNB in colon cancer has a high false negative rate, and a high percentage of skip metastasis; that along with the fact that there is much heterogeneity (methods, terminology, etc.,) among the different trials make it difficult to draw firm conclusions on the subject. For those reasons, more research is needed.

Future areas of investigation may include the use of *in vivo* SLNB with radio-tracer materials in early colon cancer treated by colonoscopic snare removal aimed at detecting OTC and upstaging disease (picking only positive sentinel nodes for pathological analysis through minimally invasive techniques). Another interesting area may be the use of SLNB to identify patients with colon cancer who have + LNM outside of the accepted areas of surgical resection and who would benefit from a directed or extended lymphadenectomy.

On a last note, is it possible that the T stage of the TNM system will ultimately disappear in Colon Cancer?, being relevant only if there is spread of the tumour outside of the colonic wall. Is the T stage (I, II and III) acting as surrogate marker for LNM that were not previously detected by conventional pathology? time will tell.
Table 1 NSS=Not Statistically Significant.

<table>
<thead>
<tr>
<th>Author name</th>
<th>Type of analysis</th>
<th>Outcomes</th>
<th>p value</th>
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<tbody>
<tr>
<td>Bilchik A (19)</td>
<td>IHC 4y recurrence Colorectal Ca</td>
<td>6.4% vs 7.4% vs 23% (pN0i- vs pN0i+ vs pN1mi)</td>
<td>p&lt;0.05</td>
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<tr>
<td>IHC 4y recurrence Colon Ca</td>
<td>5.9% vs 7.7% vs 22 % (pN0i- vs pN0i+ vs pN1mi)</td>
<td>p&lt;0.05</td>
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<td>Iddings D (meta analysis) (31)</td>
<td>IHC 3y DFS Colorectal Ca (4 studies)</td>
<td>80% vs 76% (pN0i- vs OTC)</td>
<td>NSS</td>
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<tr>
<td>IHC 3y OS Colorectal Ca (5 studies)</td>
<td>83% vs 81% (pN0i- vs OTC)</td>
<td>NSS</td>
<td></td>
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<tr>
<td>RT-PCR 3y DFS Colorectal Ca (4 studies)</td>
<td>97% vs 78% (pN0i- vs OTC+)</td>
<td>p&lt;0.05</td>
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<tr>
<td>Faerden AE (48)</td>
<td>IHC 5y DFS Colorectal Ca</td>
<td>93% vs 75% (pN0i- vs OTC+)</td>
<td>NSS</td>
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<tr>
<td>Mark B (49)</td>
<td>IHC 5y DFS Colorectal Ca</td>
<td>93% vs 75% (pN0i- vs OTC+)</td>
<td>NSS</td>
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<tr>
<td>Schaik PM (50)</td>
<td>IHC 5y DFS Colon Ca</td>
<td>72% vs 51% (pN0i- vs pN1mi)</td>
<td>p&lt;0.05</td>
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<tr>
<td>IHC 5y OS Colon Ca</td>
<td>79% vs 62% (pN0i- vs pN1mi)</td>
<td>p&lt;0.05</td>
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<tr>
<td>Protic M (47)</td>
<td>IHC DFS Colon Ca</td>
<td>92.9m vs 71.8m (pN0i- vs pN0i+)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>IHC Recurrence Colon Ca</td>
<td>2.6% vs 16.7% (pN0i- vs pN0i+)</td>
<td>p&lt;0.05</td>
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<tr>
<td>Mescoli C (46)</td>
<td>IHC Recurrence Colorectal Ca</td>
<td>4.7% vs 14% (pN0i- vs pN0i+)</td>
<td>p&lt;0.05</td>
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<tr>
<td>Sloatnaak DA (Meta analysis) (43)</td>
<td>IHC Recurrence Colorectal Ca (5 studies)</td>
<td>14% vs 48% (pN0i- vs pN1mi)</td>
<td>p&lt;0.05</td>
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<td>IHC Recurrence Colon Ca (3 studies)</td>
<td>9% vs 36% (pN0i- vs pN1mi)</td>
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<tr>
<td>IHC Recurrence Colorectal Ca (5 studies)</td>
<td>14% vs 22% (pN0i- vs pN0i+)</td>
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<td>IHC Recurrence Colon Ca (3 studies)</td>
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References


