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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 dilema, as there is a subset of patients who will have disease recurrence and who probably would benefit from chemotherapy. Sentinel lymph node biopsy (SNLB) 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 psrognostic 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: Sentinel lymph node; Colno cancer; Occult tumor cells; Micrometastases; Isolated tumor cells

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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 (LNM) 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 follow: 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

to surgery alone [7]. Still, 10% of patients with stage I disease and 15-30% patients with stage II have disease recurrence (loco-regional or distant) within 5 years. Possible reasons include aggressive tumor biology, disease outside of resection margin, or occult LNM (not detected by standard pathological techniques) [8]. This is where the sentinel lymph node biopsy [SNLB] may play an important role.

The development of the SNLB, first used in penile cancer, has become an accepted nodal staging procedure in certain types of malignant disease, particularly melanoma and breast cancer [9]. It offers node negative patients decreased morbidity that would result from an extensive lymphadenectomy; a second advantage is allowing a more thorough analysis of a small amount of tissue [8].

However, the technique is not perfect. False negative results for node positive patients has been reported anywhere from 3-50% of patients [10-12]. Moreover, lymphatic mapping techniques demonstrate that the purely anatomical concept of nodal spread is not true, and that sentinel lymph nodes (SLNs) may be located at unexpected sites [13]. In the colon, the presence of the SLN outside of the standard surgical resection may be seen in as many as 22% of the patients, [14] and skip metastasis (metastasis identified in other resected, nonsentinel nodes) have been reported in 10.4 - 53.8% [15-17].

In colon cancer SLNB is currently not aimed at modifying the surgical procedure, although it does permit a detailed analysis of a small amount of tissue with techniques that detect minimal disease. The application of immunohistochemistry (IHC) or reverse transcriptase polymerase chain reaction (RT-PCR) to the SLN in N0 (H&E staining) colon cancer leads to the discovery of occult tumor cells (OTC) in approximately 30% of node negative patients. This has spawned an interest in the detection and the implications of the OTC on prognosis and management of this disease.

As promising as the detection of OTC may seem (to further identify high risk patients), there are still many unresolved issues that stem primarily from lack of standardization. As such, the implications in the prognosis and management of the disease are yet unresolved. These issues, which will be discussed below, include problems with the technique, definitions, type of analysis performed, and lack of prospective randomized trials.

Different techniques, such as in vivo SLNB vs ex vivo pose different results. In the in vivo technique, 1 ml if isosulfan blue dye is injected into the subserosal plane around the tumor before ligating the mesenteric vessels, on the other hand in the ex vivo technique the specimen is removed and opened along the anti mesenteric border, then it is injected in the subserosal plane with isosulfan blue dye [11]. In both the blue staining lymph nodes are marked and removed. Accuracy and NPV are high, 90-95% and 93-97% respectively, with upstaging observed in 29-35%. Ex vivo accuracy is of 90-100%, NPV of 80-100%, upstaging of 19-57% [18-30].

Pathological analysis also poses inconsistencies. Definitions of micrometastatic disease, isolated tumor cells, occult tumor cells are not always specified in the studies. Briefly stated, when no search with IHC or PCR techniques is carried out, and the standard pathological techniques reveal node negative disease, the term pN0 is used. Conversely, when nodal ultra-staging is carried out, and in accordance with AJCC criteria one must define the following: micrometastases (pN1mi) as the presence of malignant cells between 0.2 and 2.0 mm in diameter; isolated tumor cells (pN0i+) as the presence of small clusters of malignant cells smaller than 0.2 mm; and pN0i- no micrometastatic disease or isolated 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 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 vs 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, perineurial 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, perineurial 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 sumarrize 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 standarize 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.

Author name	Type of analysis		Outcomes	p value
Bilchik A (19)	IHC	4y recurrence Colorectal Ca	6.4% vs 7.4% vs 23% (pN0i- vs pN0i+ vs pN1mi)	p<0.05
	IHC	4y recurrence Colon Ca	5.9% vs 7.7% vs 22 %(pN0i- vs pN0i+ vs pN1mi)	p<0.05
Iddings D (meta analysis) (31)	IHC	3y DFS Colorectal Ca (4 studies)	80% vs 76% (pN0i- vs OTC)	NSS
	IHC	3y OS Colorectal Ca (5 studies)	83% vs 81% (pN0i- vs OTC)	NSS
	RT-PCR	3y DFS Colorectal Ca (4 studies)	97% vs 78% (pN0i- vs OTC+)	p<0.05
Faerden AE (48)	IHC	5y DFS Colorectal Ca	93% vs 75% (pN0i- vs OTC+)	NSS
Mark B (49)	IHC	5y OS Colorectal Ca	64% vs 60% (pN0i- vs pN1mi)	NSS
Schaik PM (50)	IHC	5y DFS Colon Ca	72% vs 51% (pN0i- vs pN1mi)	p<0.05
	IHC	5y OS Colon Ca	79% vs 62% (pN0i- vs pN1mi)	p<0.05
Protic M (47)	IHC	DFS Colon Ca	92.9m vs 71.8m (pN0i- vs pN0i+)	p<0.05
	IHC	Recurrence Colon Ca	2.6% vs 16.7% (pN0i- vs pN0i+)	p<0.05
Mescoli C (46)	IHC	Recurrence Colorectal Ca	4.7% vs 14% (pN0i- vs pN0i+)	p<0.05
Sloothaak DA (Meta analysis) (43)	IHC	Recurrence Colorectal Ca (5 studies)	14% vs 48% (pN0i- vs pN1mi)	p<0.05
	IHC	Recurrence Colon Ca (3 studies)	9% vs 36% (pN0i- vs pN1mi)	p<0.05
	IHC	Recurrence Colorectal Ca (5 studies)	14% vs 22% (pN0i- vs pN0i+)	NSS
	IHC	Recurrence Colon Ca (3 studies)	6% vs 7% (pN0i- vs pN0i+)	NSS

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