

Reply to Reviewers' Commentary

The authors are very appreciative for the critical appraisal and review of their manuscript and have answered each of the points raised in turn as set out below. Accordingly, the relevant changes have been made in the revised manuscript.

Reviewer 1

1. The title of the manuscript only refers to non-surgical factors and should be modified.

The title of the manuscript has been now been modified and reads as 'Factors influencing lymph node yield in colon cancer'.

2. In the conclusion, a summary of the sure, possible and unsure factors could be useful.

The following paragraph has been included in the Discussion section of the revised manuscript:

'It is difficult to make firm recommendations as concerns the quality of the 'evidence' presented here within. The authors think that the following is an accurate summary in terms of the strength of the data presented: that the tumour location and size are likely a strong influence on LN yield where as patient age and tumour differentiation seem to certainly have a strong association but possibly not to the same extent. The causal relationship reported between LN yield and ethnicity, gender, socioeconomic class, ASA grade, LVI and MSI/MMR appears to be far more uncertain and not nearly as compelling.'

Reviewer 2

1. Specific data MSI is useful data for assessing the risk of CRC development but probably not for LN invasion and for determining the presence of metastases. This aspect should be commented on in "Instability and Mismatch Repair".

The link between MSI and LN invasion and prognosis in terms of the presence of metastases has yet to be fully elucidated. However, there is a body of work demonstrating an association between MMR deficiency and a higher LN yield. The hypothesis for this is that MMR deficiency may result in a less aggressive colonic tumour due to the dense lymphocytic infiltration, amplified rate of apoptosis and decreased frequency of mutations (Guidoboni et al, Michael-Robinson et al, Nash et al.). These comments have now been included in the relevant section of the paper with the inclusion of the further references listed below.

1. Guidoboni M, Gafa R, Viel A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. Am J Pathol 2001;159:297-304. [PMID:11438476 DOI: [10.1016/S0002-9440\(10\)61695-1](https://doi.org/10.1016/S0002-9440(10)61695-1)]
2. Michael-Robinson JM, Biemer-Huttmann A, Purde DM, et al. Tumor infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut 2001;48:360-366. [PMID11171826 DOI: 10.1136/gut.48.3.360]
3. Michael-Robinson JM, Reid LE, Purdie DM, et al. Proliferation, apoptosis, and survival in high-level microsatellite instability sporadic colorectal cancer. Clin Cancer Res 2001;7:2347-2356. [PMID:11489812]
4. Nash GM, Gimbel M, Cohen AM, et al. KRAS mutation and microsatellite instability: two genetic markers of early tumor development that influence the prognosis of colorectal cancer. Ann

Surg Oncol 2010;17:416-424. [PMID: 19813061 DOI: [10.1245/s10434-009-0713-0](https://doi.org/10.1245/s10434-009-0713-0)]

2. The body mass index (BMI) should be included as a non-modifiable factor for LN harvest.

Thank you. This section has now been appropriately placed under the 'non-modifiable' heading.

3. The authors should provide some explanation about tattoo and related tumor LN identification because Indian ink is usually injected at a distance from the tumor and the tumor LN are probably in a different location that those related to the marking injection.

This is certainly an area that remains open to debate and really relates to the role, or not, of sentinel LN mapping in colorectal cancer (Cahill et al.). As the reviewer correctly points out, colonoscopic tattooing may not mark out the relevant tumour lymph node bed as it has been demonstrated that it may not discriminate between the first and second echelon nodes (Spatz et al.).

5. Cahill RA, Lindsey I, Cunningham C. Sentinel node mapping by colonic tattooing. Surg Endosc 2010;24:2365-6. [PMID: 20177923 DOI: [10.1007/s00464-010-0941-1](https://doi.org/10.1007/s00464-010-0941-1)]
6. Cahill RA, Lindsey I, Cunningham C. Sentinel node mapping by colonic tattooing. Surg Endosc 2010;24:2365-6. [PMID: 20177923 DOI: [10.1007/s00464-010-0941-1](https://doi.org/10.1007/s00464-010-0941-1)]

