Peer Review File
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Reviewer A

An interesting and well written manuscript about which I have very little things to criticize in current format. This gives good insights in a single center experience and helps in collecting more evidence.

The only substantial thing that is missing, and why I will suggest a revision (based upon if it is feasible to the authors), is the correlation of findings with clinical outcomes. As the patient information is collected from jan 2006 to dec 2017, 5 year follow up is complete in nearly all. Would it be possible to provide the difference in survival in micro metastatic disease findings yes/no?
Reply: Thank you for your comments. For the survival analysis, we have planned to conduct the analysis. However, we have observed that including such analysis would result in an excessive amount of information in this manuscript, including words, tables and various figures. Additionally, there is a set of follow-up data for some patients that has not yet been summarized.

Reviewer B

Siwachat and colleagues have submitted a manuscript that has as its stated goal to evaluate the incidence of lymph node micrometastases and nodal upstaging and to evaluate prognostic factors for lymph node micrometastases. I offer the following comments/criticisms and suggestions for revision.
1.The stated aims are not properly described. The first objective incorrectly references the term incidence. This is a statistical term that is used to describe the frequency of an event over time which is not the goal of this study. Rather, they should use the term frequency throughout the manuscript. The second objective incorrectly references the term prognostic factors. This is generally defined as a factor, measured before treatment, that has an impact on a patients outcome usually defined by survival, response to treatment etc. They should use the term predictive factors to describe their investigation of variables associated with the presence of lymph node micrometastases.
Reply: Thank you for your comments and statistical definitions. I've already changed the term "incidence" to "frequency" and the term "prognostic factor" to "predictive factor" throughout the manuscript as you can see with track change

2. For their multivariable analysis the authors have chosen to use the incidence rate ratio which is a relative difference measure that is used to compare incidence rates of events occurring at a particular point in time. As mentioned above, the study does not compare the incidence over time but rather the frequency of an event (a node positive for micrometastases) and thus they should reanalyze their data and report odds ratios.
Reply: Thank you for the suggestion. That's my big mistake. I have analyzed the data using both logistic regression and risk regression. In my assessment, logistic regression tends to

overestimate the results. I believe that risk regression is more suitable for this cohort, as the data collection encompasses all our patients rather than a sample.
I attached tables showing result of both risk and logistic regression (page 17,table 5)

3. In the discussion section the authors state “our study found a higher rate of NMM detection with three antibodies, 27 NMM from total of 1745 pN0 lymph nodes (1.55%) or 21 nodal upstaging from 98 patients (21.43%). This is misleading. The results in table 4 show that BerEp4 detected micrometastases in 12 of the patients but only 2 patients had this as the only IHC positive node. In the first of these patients an N1 node had been identified by BerEp4 in station 11, but that patient already had a micrometastasis detected in station 10 using cytokeratin and P53. As such, BerEp 4 results did not change the stage of the patient. In the second patient, BerEp4 detected a micrometastasis in a station 7 node in a patient who had a micrometastasis in station 8 detected by all 3 stains. Once again, it cannot be argued that the addition of BerEp4 resulted in better staging.
Reply: I understand the issue you commented on and have addressed it. I replaced the sentence “In contrast, our study found a higher rate of NMM detection with three antibodies, 27 NMM from total of 1745 pN0 lymph nodes (1.55%) or 21 nodal upstaging from 98 patients (21.43%)”to “In our study, utilizing three types of antibodies, we detected 27 NMM among a total of 1745 pN0 lymph nodes (1.55%) or 21 instances of nodal upstaging in 98 patients (21.43%). When compared to the prior studies, it was observed that the detection of NMM showed no significant differences” (see page 8, paragraph 2, line 24-27)