Author’s response to reviews

Title: Lymph nodal Fine Needle Cytology in the Staging and Follow-up of Cutaneous Lymphomas

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Author’s response to reviews:

Dear Editor, thank you for having reviewed the manuscript. We are glad for the flattering evaluation of the reviewer one, we read the observation and the remarks of reviewer 2 and we modified the manuscript accordingly. Namely the following modifications have been performed:

Major Revisions:

1. FNAC inherently bears the problem of generating false-negative results by poorly representative results (Papa IV et al: JCO 1996), as the samples differentiation depends on the localization within the lymph node, that might contain malignant cells, that are just not taken in the biopsy. This problem should be discussed in more detail and possible solutions/perspectives how to minimize that problem should be named.

R: We are aware of the problem of false negatives in lymph node FNC, This problem mainly concerns lymph nodes involved by specific neoplasm that determine partial and sub-capsular metastases, such as breast carcinoma and melanoma (Leenders MW et al: Ultrasound and fine needle aspiration cytology of axillary lymph nodes in breast cancer. To do or not to do? Breast 2012;21:578-583. Lam TK et al: False-negative sentinel node biopsy because of obstruction of lymphatics by metastatic melanoma: the value of ultrasound in conjunction with preoperative lymphoscintigraphy. Melanoma Res. 2009;19:94-99.) whereas, we have not been able to find the mentioned reference (Papa IV et al: JCO 1996). In these cases, the needle may not succeed in sampling the specific involved areas producing false negatives. Nonetheless, lymph node partial involvements is less frequently observed in haematological neoplasm (Jegalian AG et al. Follicular lymphoma in situ: clinical implications and comparisons with partial involvement by follicular lymphoma. Blood. 2011; 118:
2976-2984), including cutaneous lymphoma. Moreover, according to the standardized cytological technique of sampling, we moved the needle in different directions during the FNA in order to reach different areas of the lymph node and to increase the probabilities of a representative sampling. Finally small cell clones may be not detected by flow cytometry but, in much of cutaneous lymphoma, nuclear atypia are often quite evident to be identified at the microscopic examination of the smears and by immunocytochemistry, even though in small number of cells. Therefore all the ancillary techniques utilized in this study have been driven by the previous microscopic evaluation of the smears and these procedures have probably contributed to avoid false negatives in the small series studied. These consideration are now reported in the manuscript (see page11, line 16 and page17, line 1).

2. Both classification systems for the determination of the N state in the TNM system (Dutch and NCI-VA classifications) require a statement about the lymph node architecture, so the N state can only be defined by an excisional biopsy. The authors should address this point.

R: Both the Dutch and NCI-VA classifications require a statement about the lymph node architecture, hence histology. Nonetheless, the histology too is not always effective; in the Fraser-Andrews’ study (reference n. 14), six of 19 patients with uninvolved lymph nodes or limited histological involvement had a detectable T-cell clone at PCR investigation. Moreover lymph node biopsies are not always easily performed and may be complicated by sepsis in immunodepressed, especially erythrodermic patients. Therefore the more recent and widely accepted Cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). (Blood. 2007;110: 1713-1722) suggests FNC as a possible diagnostic procedure for lymph node assessment, possibly coupled with ancillary techniques. This has been reported in the new version of the manuscript (page 11, line 3).

3. Unfortunately, the ancillary methods, that represent one of the major novelties of this paper, cannot be compared by their quality, as they are differentially performed. So for example, no statement can be dared about the quality of flow cytometry, as it is only performed in 13/21 cases. Therefore the authors address to the sensitivity and specificity of performing their ancillary techniques.

R: We agree that ancillary techniques were not comparable in our experience either because the present series is too little either because the conditions of application were not homogeneous. As for flow cytometry, which is generally a powerful tool in lymph node FNC, it resulted in 8 not effective cases for different reasons and ICC and PCR were also used. Possible explanations for FC non effective cases have been considered and reported (see page 14, line 4).

4. The authors should express their statement of FNAC as a fist line method diagnostically equivalent to excisional biopsy more carefully. To really be able to state that in contradiction to the ISCL/EORTC recommendations, a bigger comparative study between these two methods would be compulsory. The results of FNAC and excisional biopsy would have to show high concordance in
order to establish FNAC as first line. This should be named in the discussion.

R: The present study does not aim to contradict the ISCL/EORTC recommendations. We only suggest that, notwithstanding histology is the gold standard in lymph node evaluation FNC might be considered as a possible first step procedure in PCL staging whereas larger comparative studies between the FNC and histology assessing their concordance are still compulsory. FNC coupled with ROSE and ancillary techniques, utilized according to the clinical context and the available material, might be utilized to reinforce the negative diagnoses based on clinical and or imaging alone and possibly to avoid difficult biopsies in cases unequivocally positive. This is now reported on page15, line 9.

5. Four new references have been added, according to the modifications requested.

Minor revisions:
1. page 16, line 6: must be "Papanicolaou" instead of "Papanicoplaou"
R: “Papanicolaou” has been corrected on line 6, page 16.

We hope that, with these changes, the manuscript may be accepted for publication in BMC Cancer.

Sincerely yours, Pio Zeppa.