Letter to the Editor

Quantitative histopathological analysis of CIN sections

To the Editor,

We are very thankful to Dr. Jan Baak for his interest in our article "Quantitative histopathological analysis of cervical intra-epithelial neoplasia sections" [3] which give us the opportunity to discuss some of the salient points his comments raise and to clarify our thoughts. The aim of our paper was to investigate the different aspects of quantitative analysis that would be of importance in any potential implementation of these techniques in routine clinical use [1].

The issues raised by Dr. Baak are quite relevant to the intended thrust of our paper and warrant further discussion. The work described is part of a NIH NIC program project designed to evaluate emerging optical technologies for cervical cancer management. We have almost completed our planned large multi-centre screening (low disease incidence, only women with no history of an abnormal cervical smear were enrolled) and diagnostic (high disease incidence, only women with a history of an abnormal cervical smear were enrolled) trials and are now investigating the final results.

As addressed by Dr. Baak, we are now facing the challenge of the daily implementation of this technology in a routine clinical set-up [1,2]. The potential of quantitative analysis of cervical preneoplasia has been demonstrated by numerous authors [6,4].

Our work intended to investigate the possibilities of implementing this technology in an as automated a fashion as possible maintaining high throughput without losing any diagnostic or potentially prognostic information. Following this initial methodological paper, we reported on the "Exploratory analysis of quantitative histopathology of cervical intraepithelial neoplasia: objectivity, reproducibility, malignancy-associated changes, and human papillomavirus" [4]. In this work we evaluated the nuclear morphological features, architectural features and HPV status on 1200 biopsies and correlated these measures with the biopsy specimen histopathological interpretation. With the completion of the large multi-centres screening and diagnostic trials an extensive analysis will be being done on more the more than 4000 biopsies collected. We intend

to directly address the issues raised by Dr. Baak in our report on these final results.

At the present time, we can only address these issues with the currently available data from the ∼3000 biopsies so far collected (normal: 1853, Atypia/inflammation/metaplasia: 416, Koilocytosis: 258, CIN I: 150, CIN II: 119, CIN III: 154). All of these specimens were also tested for HPV using Hybrid Capture II and evaluation of p16 expression and Ki-67 has also been done on a subset of 300 of these specimens. As part of these studies for the women with no abnormal lesions under colposcopic evaluation, two normal regions were biopsied in order to study the intra- and inter-individual variability for the measures performed.

The approach we have taken for the analysis of these prenoplastic lesions is to create two data sets: a set of cells collected from histologically normal biopsies from subjects with no cervical abnormalities (in the biopsies, cytology and HPV tests) and a set of cells from CIS lesions. All of these cells are drawn from a single institution and a single device. Using the method described in detail elsewhere [5] for the quantitative assessment [5] of prenoplastic lesions of the lung a phenotypic descriptor score is to be calculated and will be evaluated on data from the same system, different systems and different institutions in the same fashion. This approach will allow us to investigate the equivalence between the two systems and the portability of our approach. A similar approach will be used to developed and validate an Architecture Score measuring the changes at the tissue organisation level. Following Dr. Baak's comment we will use the Voronoi Diagram to study the relative contribution of the different layers in the classification process between the different grades of dysplasia as well as the discrimination between the nondysplastic grades and CIN specimens.

One of the drawbacks of this approach concerns once again the compromise between loss of information and applicability of the technique. We believe that most of the information coming from the superficial layer would be significant to discriminate different degree of abnormality in High-Grade lesions, between

CIN II and CIN III. On the other hand, in a large number of specimens, the superficial layers are stripped off, increasing the number of inadequate specimens. The other shortcoming of our study is that while it is wide it is not currently long i.e. long term follow-up is not available which does not allow us to address the probability that molecular markers may be better predictors of the biological behaviour of CIN lesions than purely morphological descriptors [6–11].

Similarly, a deeper analysis of parabasal and basal layer do add significant information in the discrimination between normal, non-dysplasic and CIN I. But it does require a more subjective and time-consuming algorithm to manually select and cut the overlapping nuclei.

More specifically, the concerns of Pr. Baak concerning some pitfalls in the statistical analysis were justified. We have indeed imbalance set of normal and CIS used as the training sets. In this specific study, the a priori probability classification was set equal for the normal and the abnormal groups.

In our final analysis, as mentioned before, more than 400 non-dysplastic lesions (squamous metaplasia, inflammation, atypia) will be analysed, and the relevance of each the different features (morphometry, architecture, MIb and p16 positivity, HPV types) to discriminate these specimens from the low-grade dysplasia groups will be assessed.

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