Cellular Oncology 28 (2006) 63–65
IOS Press

Letter to the Editor

Quantitative histopathological analysis of CIN sections

To the Editor,

Cervical histological and cone biopsies constitute a major portion of the work load in a surgical pathology department (estimated 3% of all histological specimens). Post-diagnostic treatment of patients with a histologically confirmed cervical intraepithelial neoplasia (CIN) largely depends on CIN grade, which is prognostic as to the risk of subsequent progression [14]. However, inter-observer reproducibility of normal, reactive, regenerative, metaplastic, and different CIN grades is notoriously low [8,17]. This explains the attempts, during the past three decades, to find quantitative histopathological sample classifiers for normal, koilocytotic and CIN grades.

In the past 15 years the Vancouver group has been in the forefront of this work [1,4,6,7,15,16,19,20]. The system they are developing allows surgical pathologists to mouse-select a certain diagnostic area in a section. Subsequent digital image-supported sample classification of this selected image is then performed in seconds, giving pathologists the adjunct information requested with a minimum of delay in the flow of routine work. This is an important factor in the acceptance of a new diagnostic or therapeutic laboratory test [3,18].

However, before arriving at the point of daily implementation of these methods, many hurdles have to be crossed. A recent work by Guillaud et al. [4] discusses methodological issues of *Quantitative histopathological analysis of cervical intra-epithelial neoplasia sections*. For this study, they used digitized images from 280 samples, classified by consensus expert gynaecological pathologists as normal ($n = 199$), koilocytosis (37), CIN 1 (18), CIN 2 (10) and CIN 3 (16). A cyto-technician delineated the basal membrane and superficial surface to select a particular epithelial (The intra- and inter-observer variation in number of cells selected is significant). This is the only interactive part of the analysis, the rest is automated. For densitometric features, normalization is performed by the system. The cells and samples are then classified using geometric (size, shape, others), densitometric (darkness, etc.) and texture (chromatin distribution) features of the nuclei. Using different feature combinations and groupings of the original 5 subclasses of cases, they arrive at a correct classification of roughly 85–95% of individual cells and 80% of the samples (65% for the CIN lesions). They concluded that (1) The use of intensity normalization from a subset of imaged non-overlapping intermediate layer cells works as well or better than any of the other methods tested and provides significant time saving; and (2) Although this result must be tested in a larger data set, the exclusive use of intermediate layer cells may be acceptable when using quantitative histopathology.

In spite of the significant amount of work done and information obtained, a number of questions remain.

1. They mention that 16% of all samples are technically inadequate with their technique [4], a figure comparable to the 21% we experienced with quantitative Ki67-based classification of CIN lesions [9–12]. Both techniques require a non-tangentially cut section of adequate size so that the conditions for the two different methods are probably very similar. It could be argued however, that other quantitative histopathological features than those they have used can still be informative also in sub-optimal and tangentially cut sections. Guillaud et al. [4] have used descriptors of nuclei only, not the orientation of the nuclei to each other. The latter is an important feature of upward maturation of cervical epithelium and therefore also in the classification of CIN lesions, as surgical pathologists know all too well. Interestingly, the orientation of nuclei in another study was strongly diagnostic for the presence of oncogenic Human Papilloma Virus = HPV, although less so for grade [8,13]. It would be interesting to investigate the SSA, nuclear geometric, density and texture features together with the quantitative Ki-67 features.
2. CIN is primarily a reaction of the epithelial cells on Human Papilloma Virus (HPV). It may well be that molecular markers are better discrimina-

tors of CIN grades, or better, predictors of the biological behaviour of CIN lesions than purely morphological descriptors they have used [8–13, 17].

3. The percental distribution of different CIN grades in their material is unusual: most are "Normals", while the number of CIN 1, 2 and 3 is small and similar for the three CIN grades. Other large series of consecutive cases find few normal, mostly CIN 3 and few CIN 1 lesions. Why were there so "normal" biopsies, a fairly aggressive clinical procedure?
4. The very many "Normals" may give a too optimistic classification result. Depending on the *prior incidence rates* conditions set to the classification model in the linear discriminant analysis (?equal chances, ?chance set by incidence rates), the normal samples may be overemphasized in the computer classification. This would give well (i.e., too high) classifications for the Normal samples, but somewhat too low for the other diagnostic classes (in agreement with this, the classification results of the Normals is indeed very good but rather poor for the non-normals. *Linear* discriminant analysis method as they have used is especially sensitive to this classification bias result.
5. Important real-world diagnostic pitfalls to overdiagnose non-CIN lesions as CIN, are where reactive/inflammatory lesions and (atypical) squamous metaplasia are present. These diagnostic classes are supposedly much more difficult to classify with Quantitative Histopathology, but have not been investigated in the present study.
6. The use of intermediate-cells-only makes sense. Epithelial cells are "borne" in the parabasal cell layer and mature while they move to the surface. HPV penetrates, integrates and takes over cell metabolism in the (para)basal epithelial cells. HPV derived oncoproteins E6 and E7 then suppress p53 and retinoblastoma protein, resulting in changed cell morphology, with increased and upward proliferation. Analysis of whole thickness measurements therefore may give less sensitive classification results than targeted measurements in the lower half of the epithelium excluding the basal cells, as they propagate (in agreement with others [8–13]. However, the changes in the superficial cells are still strongly diagnostic as all pathologists know. Therefore, as an alternative they should separately measure superficial and deep-layer intermediate cells and compare their quantitative contribution in the discriminant analysis. Such an approach may better reflect the molecular processes underlying the CIN grade, or at least speak more to the imagination of the pathologists who in the end must use the methods.
7. Others [8] and we [2] have emphasized the need for prospective multicenter independent validation of methods. Therefore, in spite of the importance of the work done by Guillaud and his co-workers, such independent testing is probably more predictive of the error sources and usefulness of a method, than the fine technical details of a certain test. This is especially important as the standard deviations of features are so diagnostic, but, unfortunately, these features are not well reproducible. They should consider using median values. These may result in somewhat lower final classification results, but this would be compensated by their much better reproducibility.

References

[1] G. Anderson, C. Macaulay, J. Matisic, D. Garner and B. Palcic, The use of an automated image cytometer for screening and quantitative assessment of cervical lesions in the British Columbia Cervical Smear Screening Programme, *Cytopathology* **8**(5) (1997), 298–312.

[2] J.P. Baak, The framework of pathology: good laboratory practice by quantitative and molecular methods, *J. Pathol.* **198**(3) (2002), 277–283.

[3] J.P.A. Baak, A.-J. Kruse, E. Janssen and B. van Diermen, Predictive testing of early CIN behaviour by molecular biomarkers, *Cell. Oncol.* **27** (2005), 277–280.

[4] M. Guillaud, D. Cox, A. Malpica, G. Staerkel, J. Matisic, D. Van Niekirk, K. Adler-Storthz, N. Poulin, M. Follen and C. MacAulay, Quantitative histopathological analysis of cervical intra-epithelial neoplasia sections: Methodological issues, *Cell. Oncol.* **26** (2004), 31–43.

[5] P.A. Hall and J.J. Going, Predicting the future: a critical appraisal of cancer prognosis studies, *Histopathology* **35**(6) (1999), 489–494.

[6] A.G. Hanselaar, N. Poulin, M.M. Pahlplatz, D. Garner, C. MacAulay, J. Matisic, J. LeRiche and B. Palcic, DNA-cytometry of progressive and regressive cervical intraepithelial neoplasia, *Anal. Cell. Pathol.* **16**(1) (1998), 11–27.

[7] R.A. Kemp, C. MacAulay, D. Garner and B. Palcic, Detection of malignancy associated changes in cervical cell nuclei using feed-forward neural networks, *Anal. Cell. Pathol.* **14**(1) (1997), 31–40.

[8] A.J. Kruse, J.P. Baak, T. Helliesen, K.H. Kjellevold, M.G. Bol and E.A. Janssen, Evaluation of MIB-1-positive cell clusters as a diagnostic marker for cervical intraepithelial neoplasia, *Am. J. Surg. Pathol.* **26**(11) (2002), 1501–1507.

[9] A.J. Kruse, J.P. Baak, E.A. Janssen, M.G. Bol, K.H. Kjellevold, B. Fianne, K. Lovslett and J. Bergh, Low- and high-risk CIN 1 and 2 lesions: prospective predictive value of grade, HPV, and Ki-67 immuno-quantitative variables, *J. Pathol.* **199**(4) (2003), 462–470.

[10] A.J. Kruse, J.P. Baak, E.A. Janssen, K.H. Kjellevold, B. Fiane, K. Lovslett, J. Bergh and S. Robboy, Ki67 predicts progression in early CIN: validation of a multivariate progression-risk model, *Cell. Oncol.* **26**(1–2) (2004), 13–20.

[11] A.J. Kruse, I. Skaland, E.A. Janssen, S. Buhr-Wildhagen, J. Klos, M.J. Arends and J.P. Baak, Quantitative molecular parameters to identify low-risk and high-risk early CIN lesions: role of markers of proliferative activity and differentiation and Rb availability, *Int. J. Gynecol. Pathol.* **23**(2) (2004), 100–109.

[12] A.J. Kruse, E. Gudlaugsson, T. Helliesen, E.A. Janssen, B. van Diermen, S. Sandvik and J.P. Baak, Evaluation of prospective, routine application of Ki-67 immunoquantitation in early CIN for assessment of short-term progression risk, *Anal. Quant. Cytol. Histol.* **26**(3) (2004), 134–140.

[13] A.J. Kruse, S. Buhr-Wildhagen, E.A. Janssen and J.P. Baak, The relationship between syntactic structure analysis features, histologicalgrade and high-risk HPV DNA in cervical intraepithelial neoplasia, *Cell. Oncol.* **26**(3) (2004), 135–141.

[14] A.G. Ostor, Natural history of cervical intraepithelial neoplasia: a critical review, *Int. J. Gynecol. Pathol.* **12**(2) (1993), 186–192.

[15] B. Palcic, C. MacAulay, S. Shlien, W. Treurniet, H. Tezcan and G. Anderson, Comparison of three different methods for automated classification of cervical cells, *Anal. Cell. Pathol.* **4**(6) (1992), 429–441.

[16] B. Palcic, Nuclear texture: can it be used as a surrogate endpoint biomarker?, *J. Cell. Biochem. Suppl.* **19** (1994), 40–46.

[17] E.C. Pirog, R.N. Baergen, R.A. Soslow, D. Tam, A.E. DeMattia, Y.T. Chen and C. Isacson, Diagnostic accuracy of cervical low-grade squamous intraepithelial lesions is improved with MIB-1 immunostaining, *Am. J. Surg. Pathol.* **26**(1) (2002), 70–75.

[18] R. Silvestrini, *Cell. Oncol.* **27** (2005), 355.

[19] F.S. Steven, M. Desai, J. Sin and B. Palcic, Fluorescent location of cells of cytological interest in cervical smears prestained with thionin, *Anticancer Res.* **16**(3A) (1996), 1193–1196.

[20] X.R. Sun, J. Wang, D. Garner and B. Palcic, Detection of cervical cancer and high grade neoplastic lesions by a combination of liquid-based sampling preparation and DNA measurements using automated image cytometry, *Cell. Oncol.* **27**(1) (2005), 33–41.

Jan P.A. Baak*

Departments of Pathology, Stavanger University Hospital, Stavanger, and Gades Institute, University of Bergen, Norway and Free University, Amsterdam, The Netherlands

*Address for correspondence: Prof. J. Baak, Department of Pathology, Stavanger University Hospital, Stavanger, Norway. Tel.: +47 51 519368; Fax: +47 51 519910; E-mail: baja@sir.no.