FROM SIN TO CIN AND BACK

The use of quantitative biomarkers in CIN diagnosis, grading, progression and regression

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SUMMARY

Treatment of cervical intraepithelial neoplasia (CIN) lesions heavily depends on CIN grade. However, intra- and inter-observer reproducibility of CIN-grading is not perfect and over- or under-treatment can be the result. Recent developments have shown that p16, MIB-1, p53 and Retinoblastoma protein, and, to a lesser degree cytokeratins-14 and 13, are useful criteria for diagnosis, grading and assessment of progression and regression, greatly exceeding the value of classical grade. As a result, both early CINs and CIN-3s that regress and progress can be accurately identified. For accurate results, it is essential that the biomarkers are determined by quantitative image analysis techniques, and separately in the superficial, middle and deep layers (excluding the basal layer) of the cervical epithelium. Quantitative biomarker analysis of CIN lesions is an important tool in daily surgical pathology practice.

INTRODUCTION

Treatment of cervical intraepithelial neoplasia (CIN) lesions heavily depends on CIN grade. However, for pathologists, the grading of CIN is problematic (1) as intra- and interobserver reproducibility of CIN-grading is not perfect (2-7). There are also difficulties in reliably distinguishing CIN from non-neoplastic lesions and over- or under-treatment can be the result (8-9). These points indicate the need for adjuvant methods for the distinction of CIN from non-neoplastic lesions, the identification of different CIN grades and the ability to predict the risk of progression of early CIN1 and 2 lesions ("early CIN").

As CIN involves progressive dysfunction of proliferation and differentiation activity of cervical epithelial cells, over the past decade we have concentrated on studying the value of proliferation and differentiation related features (10-18). Without doubt, p16 and Ki-67 (MIB-1) are the most widely available, robust, stable and strongest predictive biomarkers currently available for the handling of CIN lesions (19-22). This paper summarizes current knowledge and gives practical guidelines for the use of the most important biomarkers in daily surgical pathology practice.

CIN AND HPV

Persistent infection with high-risk human papilloma virus (*hr*HPV) is necessary in the evolution of cervical carcinomas and its precursors (23-32). Many believe that there is no CIN without HPV and that previous publication of HPV negative CINs is the result of poor methodology or material used. The viral load may be an important predictor of the development of a CIN lesion. Large, world-wide vaccination programs are in progress with promising results.

HPV is very infectious and has been found not only in the cervix, but also in the vagina, vulva, peri-anal region, urethra and even in tampons (33). HPV can enter only the (para)basal cells of the cervical epithelium, not the superficial cells. Thus, infection requires small trans-

epithelial micro-traumas of the cervical epithelium. HPVs enter the cells, resulting in slightly increased proliferation. However, only when HPV integrates in the host genome is the cellular metabolism of the epithelial cells dramatically changed. This causes increased proliferation and decreased differentiation. These morphologically abnormal cells migrate towards the surface (just as normal cells do) but without maturation. The dysplastic cells are estimated to arrive at the surface within 1-3 weeks and then desquamate.

Morphologically, the changes are visible as decreased upward maturation and proliferation (mitoses above the level of the parabasal cells). Depending on the height of the changes, CIN lesions are divided into CIN-1 (changes up to 1/3rd of the epithelial thickness), CIN-2 (up to 2/3rd) and CIN-3 (upper 1/3rd).

Ki-67 CELL CLUSTERS

TO DISTINGUISH CIN AND REACTIVE LESIONS

In agreement with Pirog et al (22), we have concluded that evaluation of MIB-1 positive cell clusters (=MIB-C) MIB-C is a strong diagnostic adjunct in distinguishing CIN from normal or benign reactive cervical squamo-epithelial lesions (16). However, to prevent overdiagnosis, MIB-1 positive tangentially cut parabasal cells, inflammatory cells and immature metaplasia must be carefully excluded.

Ki-67 AND CIN GRADE

Ki67 immunoquantitation is important for grading support in CIN (17, 18). The 90th percentile of the stratification index (Si90) and the number of positive nuclei per 100 µm basal membrane is the best discriminating set of features to distinguish the three CIN grades at the same time. Some CIN-1 cases that were initially "misclassified", show, on re-cut of the paraffin blocks, a higher CIN grade while the other CIN 1 cases that were correctly classified with Ki67 quantitation remained CIN1 in the deeper cuts. In a subsequent prospective evaluation on 121 routine CIN cases (test set), agreement

between routine CIN grades (by 6 independent different pathologists) and quantitative Ki67 classification was 78%. However, when compared with the blind review of CIN grades by two expert pathologists, agreement was 97% and sensitivity, specificity, positive and negative predictive value were very high. Ki67 immunoquantitation is, therefore, a useful diagnostic adjunct to distinguish different CIN grades and may also be a sensitive biologic indicator of progression of seemingly low grade CIN. Ki67 immunoquantitative parameters are also correlated with the presence of hrHPV in CIN lesions. All cases with Ki67 Si90>O.60 are hrHPV positive; subjective impressions of Si90 are not as accurate or reproducible as quantitative image analysis results (17).

BIOMARKERS AND BIOLOGICAL AGGRESSION OF EARLY CIN LESIONS

Koilocytosis

Regression and progression depends on CIN grade, but the prognostic accuracy of CIN grade is not impressive (34). Koilocytosis is often regarded by pathologists as an additional sign of CIN aggression. However, our analysis of the course of the original early CIN lesion (as progression=increase of grade by at least 1, or not) showed that patients with koilocytosis had a significantly lower likelihood of progression. Moreover, in agreement with other studies, koilocytosis among observers was not well reproducible, indicating the need for alternative well reproducible and accurate prognostic features (14).

MIB-1

MIB-1 in small histological (marker) biopsies is the strongest single prognosticator in early CIN lesions to predict progression to CIN-3. Cox regression analysis showed that the percentage of Ki67 positive cells located in the middle third layer of the epithelium (MIDTHIRD) and Si90 is the best combination to predict progression. Furthermore, sensitivity (100%), specificity (56%), positive predictive value (23%), negative predictive value (100%) and overall percentage correctly classified cases (61%) of this Ki67 combination is

higher than that of subjective CIN grade or HPV status, either single or combined (both for routine and review CIN grades) (15). These results were promising. However, following Good Laboratory Practice standards (35, 36), a diagnostic, prognostic or predictive result in one group of patients must be validated in at least one independent test set of new patients. Application of the prognostic Ki67 combination obtained in the learning set, to a new test set of consecutive CINs gave comparable results. Ki67 immuno- quantitation of small histological CIN1 and CIN2 biopsies has strong independent prognostic value for progression.

The sets for learning and testing came from the same laboratory. The question was whether interlaboratory differences in tissue processing and staining of the sections influence the Ki67 features. In the development of a clinical laboratory test with potentially therapeutic consequences, it has been claimed that the laboratory test under development must be evaluated in another laboratory, without changing the decision thresholds of the relevant test features (35, 36). We therefore evaluated the Ki67 prognostic test in material from a laboratory in another country (Norway). Inevitably, differences in the processing and staining procedures exist between the Dutch and Norwegian laboratories. Each Norwegian patient in the new test set was classified as Ki67- model "Iow-risk" or "high-risk" (Si90 and MIDTHIRD), without changing the prognostically essential thresholds (see above) and then matched with the follow-up. Again the combination of MIDTHIRD and Si90 was highly significant ($p<0.001$, figure 1p) (15).

The next phase in the development of a prognostic laboratory test is ***prospective validation*** when the method is used routinely. This is probably the roughest phase for a laboratory test, as it is again inevitable that variations will be induced with routine use by technicians, even though the measurement and interpretation protocol is well defined. In spite of this, again, the Ki67 prognostic model was strongly predictive for CIN3 in the follow-up, whereas the

routine CIN grade was not (12).

Prognostic value of Ki-67 and other biomarkers in early CIN lesions

We have further analysed, in p16-positive early CINs with Ki67 cell clusters above the lower third epithelial layer (two features that are diagnostic for CIN, see above), the usefulness of quantitative Ki67 parameters, the cell cycle regulators Retinoblastoma protein pRb, p53, Cyclin-A, -E and -D, p16, p21, p27 and telomerase, and the cellular differentiation products involucrin, CK13, and CK14 (amongst other CKs). As the cervical epithelium is a dynamic structure, with cells being borne in the parabasal layer and then, in 1-3 weeks, maturing and migrating to the surface (where they are desquamated), all features were separately analyzed in the basal, deeper and upper half of the epithelium, using quantitative techniques. Progressors showed decreased Rb, CK13, CK14, and involucrin, but increased p21 and p27. Ki67-Si90 and Rb in the lower half of the epithelium (***RbDeep***) were the strongest multivariate independent progression predictors. Ki67-Si90>0.57 was unfavorable (progression risk=30%), but only if RbDeep<45% (progression risk=47%). All combined Si90>0.57 + RbDeep>45% or any Ki67-Si90 value below 0.57 were non-progressors (figure 2). In the high-risk progression subgroup (Ki67-Si90>O.57+ RbDeep<45%, 47% progression), CK13 and CK14 have additional prognostic value. All cases with combined CK14<50% and CK13<80% (both in the basal cell layer) (4% of all lesions) progressed. Thus, quantitation of combined Ki67, Rb, CK13 and CK14 gives accurate information about the progression risk of early CIN lesions (13). The results are summarized in the prognostic decision scheme shown in Figure 2.

On the basis of these findings, we have developed a model for the development and discourse of an early CIN lesion (Figure 3). It is hypothesized that hrHPV E7 expression reduces Rb, causing increased and upward proliferation (Si90>O.57). Increased RbDeep can reduce proliferation, and subsequently

reduce the upward spread of Ki67 positivity (decreased upward proliferation).

How to handle and interprete biomarker patterns in an early CIN lesion

On the basis of the above, we came to the following recommendations for the daily handling of a cervical biopsy in a surgical pathology laboratory, in the realm of the analysis of an early CIN lesion:

1. Analyse the diagnostic hematoxylin-eosin stained section, for routine evaluation.
2. Based on recent literature studies and our own routine use of p16 (unpublished results), scan the serial section stained for p16, to identify diffusely positive squamous areas. These are nearly always dysplastic (false positive p16 is very rare and easily recognized). The underlying cause of p16 positivity is very often hrHPV positivity of the p16 positive squamous cells.
3. Evaluate the next serial section further with Ki67. Ki67-positive cell clusters further indicate CIN.
4. Perform quantitative Ki67 analysis, for objective grading support and progression risk indication in case of CIN1 and CIN2. If Ki67 Si90 exceeds 0.57 and/or MIDTHIRD exceeds 30%, the likelihood of CIN3 in the follow- up is high (30%).
5. Then, in the subsequent section stained for Retinoblastoma protein (Rb), analyze the Rb positivity of nuclei in the lower half of the epithelium.
6. Interprete the results as follows. If the combination of Ki67-Si90>0.57 occurs together with Rb<40%, progression risk in CIN1 and CIN2 is very high (about 50%). Figure 2 illustrates this graphically. All other patients have a progression risk close to 0%. Moreover, in the high-risk subgroup, combined CK13<80% and CK14<50% identifies patients with an excessively high progression risk (Figure 3). In the other patients the cytokeratins are not informative.

CIN-3 (HSIL) AND BIOMARKERS

If untreated, the majority of cervical biopsies with high-grade squamous intraepithelial lesions (HSIL, CIN-3) will persist (as demonstrated by an HSIL in a follow-up biopsy), but approximately one-third will naturally regress (i.e., no HSIL detected on follow-up) (34). Consequently it is standard practice of care to ablate all HSILs. It would, therefore, be of clinical value if one could identify those HSILs that would regress.

We have recently analyzed a number of biomarkers to identify factors related to histological proven persistence or regression. Special attention was paid to p53 and pRb in biopsies as potential markers for *hr*HPV E6 and E7 function (35). Paraffin blocks taken from consecutive *small* cervical (diagnostic or marker) 4% buffered formaldehyde fixed biopsies for histological assessment (n=376) (routinely taken for abnormal cytological smears) and diagnosed as HSIL, were studied. Initial diagnostic marker biopsies that were large cone biopsies or Large Looped Excision of the Transformation Zone (=LLETZ) were not included in the study, as the CIN lesion is often widely excised with this procedure. The analysis was therefore limited to cases where the initial sample was a small diagnostic histological biopsy. The gynecologists who did the follow-up (colposcopy, biopsies) of the patients were unaware that the current study would be undertaken and hence were unaware of the results. Follow-up and treatment of the patients was done according to the national Norwegian treatment guidelines of patients with HSIL (see www.legeforeningen.no), as follows. When the diagnosis HSIL was made, all patients were offered a prompt follow-up LEEP or conisation under colposcopic visualization. Consequently, nearly all the follow-up biopsies (LEEPS or cones) were taken within a few weeks (median: 32 days) after the original HSIL diagnosis. This short-term intervention would exclude the possibility of studying the natural discourse of an HSIL lesion. However, for a variety of reasons the interval was much longer in the 28 patients analyzed. The

reason for this was always patient driven: social reasons, work obligations, or planned holidays and so on. There were no medical indications to delay the biopsy as all patients with a histological diagnosis of HSIL were offered to be treated as soon as possible (i.e., with a wide (LEEP or cone) excision under colposcopic visualization). The follow-up treatment of the 28 study patients did not differ from that of the other HSIL patients. Thus, although the 28 study patients had a much longer follow-up biopsy interval than the other patients, their initial diagnostic marker biopsies were the same as in the other CIN-3 cases with a shorter interval. Likewise, they were also similarly treated with LEEP or cone biopsy, ensuring certainty whether the lesion persisted or regressed. Moreover, after the colposcopic follow-up biopsy, the patients were cytologically followed each 6 months. None of the regression cases after the follow-up biopsy developed cytological signs of occult persistent HSIL (median cytological follow-up time: 10.4 months, range: 5-23).

As mentioned, follow-up biopsies to determine progression, persistence or regression were included in the study only if they had been collected at least one hundred days post the diagnostic biopsy. This interval is important for the evaluation of the degree of dysplasia in the follow-up biopsy as the taking of the diagnostic biopsy procedure causes considerable damage in the cervix and consequently a local strong inflammatory and repair response. It is well known that this reaction without other superimposed infection is complete after approximately 3-6 weeks. Within this interval, there is a serious risk that dysplastic remnant lesions are over-diagnosed, due to the superimposed reactive changes in the epithelium. In order to minimize this risk, we excluded biopsies taken shortly after the initial biopsy, and only included those with an interval of at least one hundred days between the marker and follow-up biopsy. This was also practical as nearly all follow-up biopsies were taken either before 8 weeks or after 3 months. There were no detectable systematic reasons for the variation in the biopsy intervals.

This left 28 cases from the original sample for further analysis. In these, the presence and extent (in millimeters) of the HSIL and the resection margins were assessed. All lesions were high-risk (*hr*) HPV and p16 positive, 63% for HPV-16 or HPV-16 mixed with other *hr* genotypes, whilst 37% had other *hr*HPV types. The marker biopsies of the persistent HSILs had lower p53 and Retinoblastoma protein (pRb) detected in the deep half of the epithelium (P=0.001 and 0.02 respectively) than non-persistent HSILs. The degree of positivity of p16, Ki-67, CyclinD1, lesion extent, positivity of the resection margins and patient age were all unrelated to persistence or regression. Lesions with HPV-16 or mixed-16 genotypes had a significantly lower percentage of pRb (p=0.02), p53 (p=0.02) and Cyclin-D (p=0.04) positive nuclei in the deep epithelial layers. In agreement with this, type-16 positive HSIL lesions had a lower regression percentage than those with other HPV types, but the difference was not significant. We concluded that HSILs with combined negativity/low positivity for p53 and pRb protein in small histological biopsies are highly likely to persist, contrasting those in which one of these cell-cycle regulators is strongly positive (p53>15%, pRb>40%).

REFERENCES

1) Heatly MK. How should we grade CIN? Histopathology 2002;40:377-390.

2) Keenan SJ, Diamond J, McCluggage WG, Bharucha H, Thompson D, Bartels PH, Hamilton PW. An automated machine vision system for the histological grading cervical intraepithelial neoplasia (CIN). J PathoI 2000;192:351-362.

3) McCluggage WG, Bharucha H, Caughley LM et al. Interobserver variation in the reporting of cervical colposcopic biopsy

specimens: comparison of grading systems. J Clin Pathol 1996;49:833-835.
4) Ismail SM, Colclough AB, Dinnen JS, Eakins D, Evans DMD, Gradwell E. Reporting cervical intra-epithelial neoplasia (CIN): intra and inter-pathologist variation and factors associated with disagreement. Histopathology 1990;16:371-376.
5) Ismail SM, Colclough AB, Dinnen JS et al. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. BMJ 1989;298:707-710.
6) Robertson AJ, Anderson JM, Swanson Beck J et al. Observer variability in histopathological reporting of cervical biopsy specimens. J Clin Pathol 1989;42:231-238.
7) Stoler MH, Schiffman M. Atypical Squamous Cells of Undetermined Significance- Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 2001 ;285:1500-1505.
8) AI Nafussi AI. Colquhoun MK. Mild cervical intraepithelial neoplasia (CIN1)- a histological overdiagnosis. Histopathology 1990; 17:557-561.
9) Creagh T, Bridger JE, Kupek E, Fish DE, Martinbates E, Wilkins MJ. Pathologist variation in reporting cervical borderline epithelial abnormalities and cervical intraepithelial neoplasia. J Clin Pathol. 1995;48:59-60.
10) Kruse AJ, Baak JP, Janssen EA, Kjellevold KH, Fiane B, Lovslett K, Bergh J, Robboy S. Ki67 predicts progression in early CIN: validation of a multivariate progression-risk model. Cell Oncol. 2004;26(1-2):13-20.
11) Kruse AJ, Buhr-Wildhagen S, Janssen EA, Baak JP. The relationship between syntactic structure analysis features, histological grade and high-risk HPV DNA in cervical intraepithelial neoplasia. Cell Oncol. 2004;26(3):135-41.
12) Kruse AJ, Gudlaugsson E, Helliesen T, Janssen EA, van Diermen B, Sandvik S, Baak JP. Evaluation of prospective, routine application of Ki-67 immunoquantitation in early CIN for assessment of short-term progression risk. Anal Quant

Cytol Histol. 2004 Jun;26(3):134-40.
13) Kruse AJ, Skaland I, Janssen EA, Buhr-Wildhagen S, Klos J, Arends MJ, Baak JP. 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. 2004 Apr;23(2):100-9.
14) Kruse AJ, Baak JP, Helliesen T, Kjellevold KH, Robboy SJ. Prognostic value and reproducibility of koilocytosis in cervical intraepithelial neoplasia. Int J Gynecol Pathol. 2003 Jul;22(3):236-9.
15) Kruse AJ, Baak JP, Janssen EA, Bol MG, Kjellevold KH, Fianne B, Lovslett K, Bergh J. Low- and high-risk CIN 1 and 2 lesions: prospective predictive value of grade, HPV, and Ki-67 immuno-quantitative variables. J Pathol. 2003 Apr;199(4):462-70.
16) Kruse AJ, Baak JP, Helliesen T, Kjellevold KH, Bol MG, Janssen EA. Evaluation of MIB-1-positive cell clusters as a diagnostic marker for cervical intraepithelial neoplasia. Am J Surg Pathol. 2002 Nov;26(11):1501-7.
17) Kruse AJ, Baak JP, de Bruin PC, van de Goot FR, Kurten N. Relationship between the presence of oncogenic HPV DNA assessed by polymerase chain reaction and Ki-67 immunoquantitative features in cervical intraepithelial neoplasia. J Pathol. 2001 Dec;195(5):557-62.
18) Kruse AJ, Baak JP, de Bruin PC, Jiwa M, Snijders WP, Boodt PJ, Fons G, Houben PW, The HS. Ki-67 immunoquantitation in cervical intraepithelial neoplasia (CIN): a sensitive marker for grading. J Pathol. 2001 Jan;193(1):48-54.
19) Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J PathoI 1998;153:1741-1748.
20) Klaes R, Friedrich T, Spitkovsky D, et al. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer 2002;92:276-284.
21) Keating JT, Cviko A, Riethdorf S, et al. Ki-67, cyclin E,

and p16INK4A are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Pathol 2001 ;25:884-891.
21) Nucci MR, Castrillon OH, Bai H, Quade BJ, Ince TA, Genest DR, Lee KR, Mutter GL, and Crum CP. Biomarkers in diagnostic obstetric and gynecologic pathology: a review. Adv Anat Pathol 2003;10:55-68.
21)
22) Pirog EC, Baergen RN, Soslow RA et al. Diagnostic accuracy of cervical low-grade squamous intraepithelial lesions is improved with MIB-1 immunostaining. Am J Surg PathoI 2002;26:70-75.
23) Kiviat N. Natural history of cervical neoplasia: Overview and update. Am J Obstet GynecoI 1996;175:1099-1104.
24) Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med 1992;327:1271-1278.
25) Woodman CBJ, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001 ;357:1831-1836.
26) Saito K, Saito A, Fu YS, Smotkin D, Gupta J, Shah K. Topographic study of cervical condyloma and intraepithelial neoplasia. Cancer 1987;59:2064-2070.
27) Schneider V. GIN prognotication: will molecular techniques do the trick? Acta CytoI 2003;47:115-116.
28) EI Hamidi A, Kocjan G and Du M-Q. Clonality analysis of archival cervical smears. Correlation of monoclonality with grade and clinical behavior of cervical intraepithelial neoplasia. Acta Cytol 2003;47: 117 -123.
29) Crum CP, Abbott DW, Quade BJ. Cervical cancer screening: from the papanicolaou smear to the vaccine era. J Clin Oncol 2003;21 :224-230.
30) Sun CA, Lai HC, Chang CC et al. The significance of human papillomavirus viral load in prediction of histologic severity and size of squamous intraepithelial lesions of uterine cervix. Gynecol Oncol 2001 ;83:95-99.

31) Clavel C, Masure M, Levert M, et al. Human papillomavirus detection by the hybrid capture II assay: A reliable test to select women with normal cervical smears at risk for developing cervical lesions. Diagn Mol Pathol 2000;9:145-150.
32) Nobbenhuis MAE, Walboomers JMM, Helmerhorst ThJM, Rozendaal L, Remmink AJ, Risse EKJ, van der Linden JC, Voorhorst FJ, Kenemans P, Meijer CJLM. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a pospective study. Lancet 1999;354:20-25.
33) Fairley CK, Robinson PM, Chen S, Tabrizi SN, Garland SM. The detection of HPV DNA, the size of tampon specimens and the menstrual cycle. Genitourin Med. 1994 Jun;70(3):171-4.
34) Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol. 1993 Apr;12(2):186-92. Review.
35) Baak JP. The framework of pathology: good laboratory practice by quantitative and molecular methods. J Pathol 2002;198:277-283.
36) Hall PA, Going JJ. Predicting the future: a critical appraisal of cancer prognosis studies. Histopathology 1999;35:489-494.
37) Zielinsky GD, Snijders PJF, Rozendaal L, Fransen Daalmeijer N, Risse EKJ, Voorhorst FJ, et al. The presence of high-risk HPV combined with specific p53 and p16ink4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. J Pathol. 2003;201:535-543.
38) Baak JPA, Kruse A-J, Garland SM, Skaland I, Janssen EAM, Tabrizi S, Fagerheim S, Robboy S, and Nilsen ST. Combined p53 and retinoblastoma protein detection identify persistent and regressive cervical high-grade squamous intraepithelial

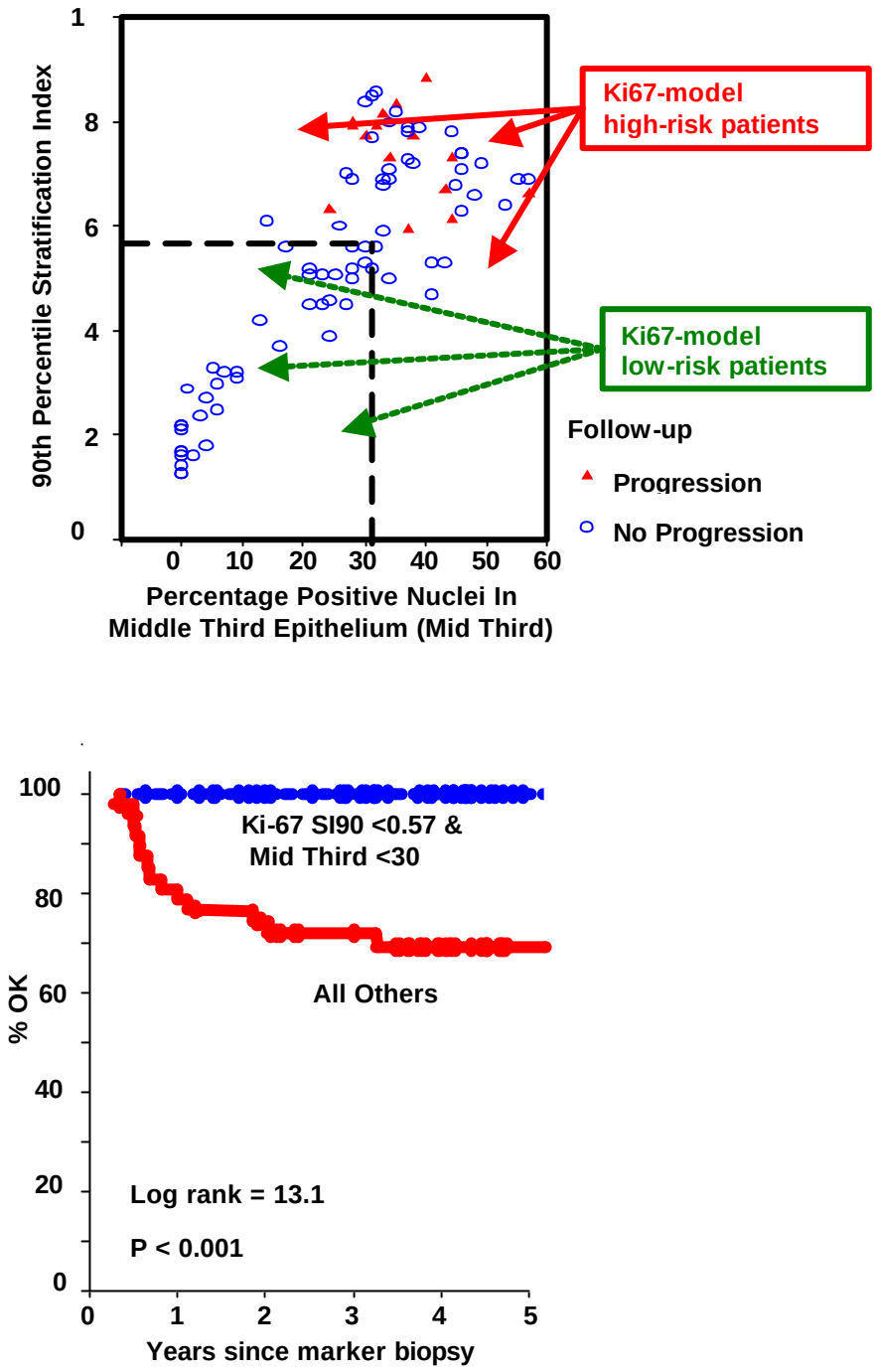

Figure 1. Top: Scatter plot and Bottom: Kaplan-Meier curve showing the percentage of patients with progression for the low-risk (blue circles and blue dotted line) and high-risk patients (red triangles and red continuous line) according to the Ki67 progression-risk model.

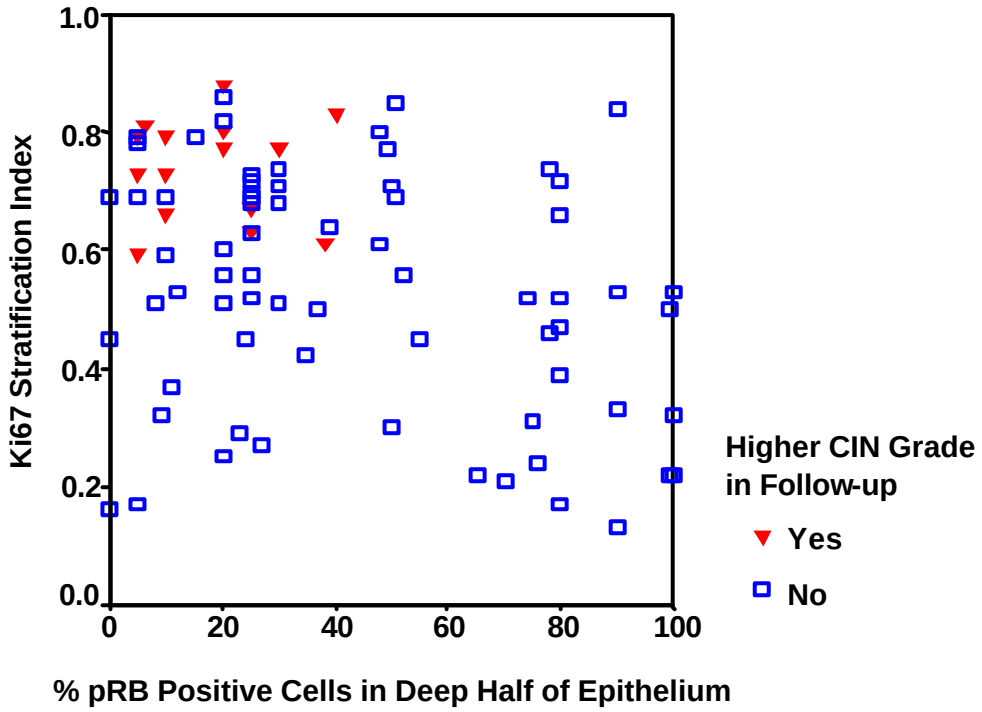

Figure 2. Scatterplot of the 90th percentile of the Stratification Index of the Ki67 positive nuclei and the % Rb positive nuclei in the deep half layer of the epithelium.

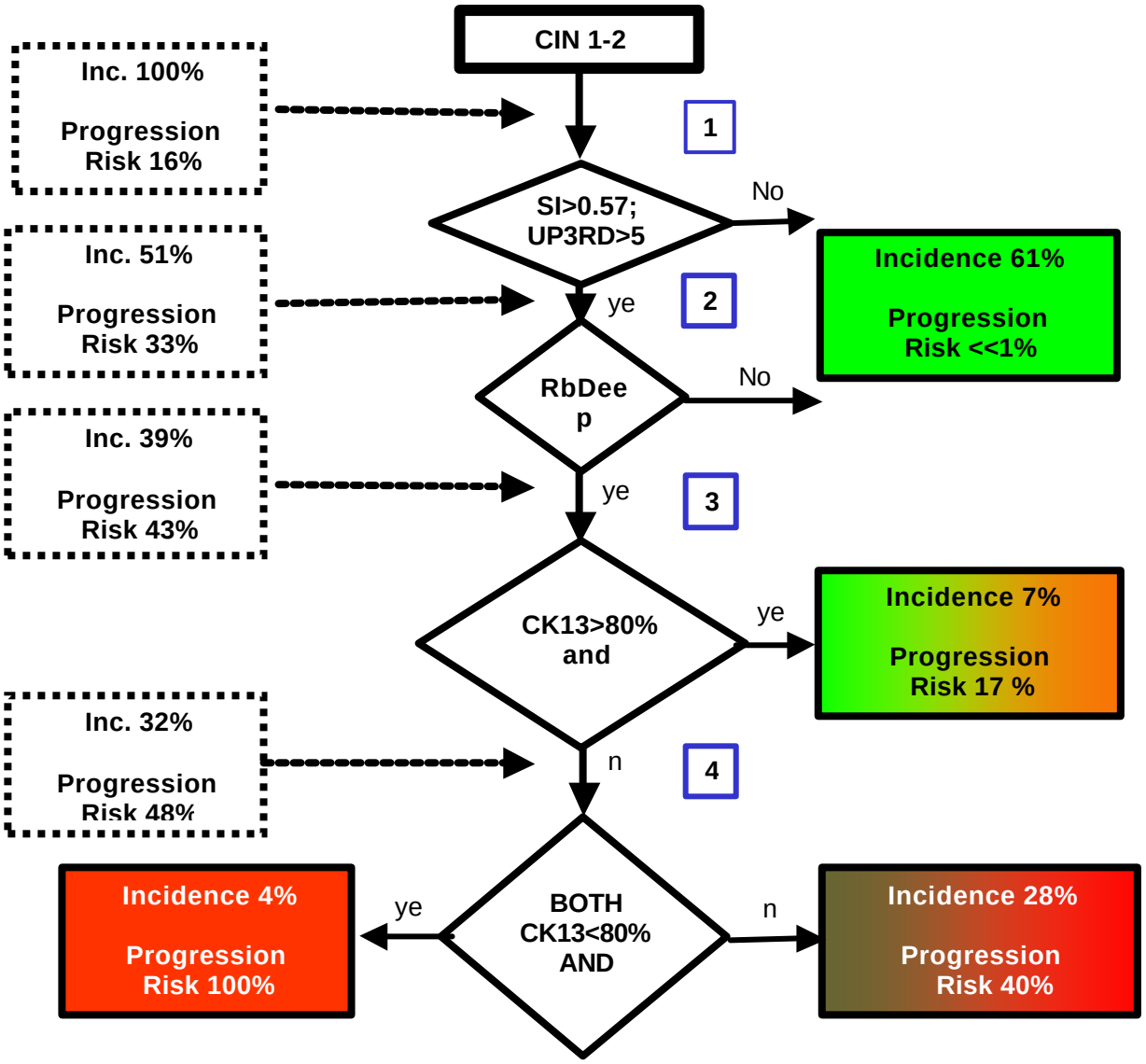

Figure 3. Progression Rates of different Ki67/RbDeep/CK13/CK14 combinations.