INVITED COMMENTARY

Towards developing a meaningful grading system for cervical squamous cell carcinoma[†]

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[†]Invited commentary for Jesinghaus *et al.* Introducing a novel highly prognostic grading scheme based on tumour budding and cell nest size for squamous cell carcinoma of the uterine cervix. *J Pathol Clin Res* 2018; **4**: 93–102.

Abstract

Cervical cancer is one of the commonest cancers worldwide, especially in developing countries. In early stage disease, a variety of pathological parameters are of prognostic value but currently this does not include tumour grade which, for a number of reasons, is of limited or no value in cervical squamous cell carcinomas. In a recent article in this journal, Jesinghaus and colleagues investigated a novel histopathological grading system based on tumour budding and cell nest size, which has been shown to outperform conventional grading systems for squamous cell carcinoma at several other sites such as lung, oral cavity, and oesophagus in terms of patient prognostication. They tested the prognostic value of this grading system in two independent cohorts of cervical squamous cell carcinoma. The grading system proved to be a highly effective, stage-independent prognosticator in both cohorts with small cell nest size and high tumour budding indicative of a poor prognosis. It is hoped that the results of this study will be validated in additional independent larger cohorts and will act as an impetus for the development of a meaningful and easy-to-implement grading system for cervical squamous cell carcinoma. As comparable tumour budding/cell nest size-based grading systems have been shown to be of prognostic value for squamous cell carcinomas at other sites, this shows the potential of these parameters to serve as the basis for a common grading system applicable to squamous cell carcinomas of different anatomic sites.

Keywords: cervix; squamous cell carcinoma; grading; tumour budding; cell nest size

Introduction

Cervical cancer is one of the commonest cancers worldwide. While many cases, especially in developing countries, are advanced stage at diagnosis, a significant number of cases are picked up at early stage; this is especially so in developed countries. In early stage disease, a variety of pathological parameters are of prognostic value, including tumour size, depth of invasion, presence or absence of lymphovascular space invasion, and lymph node status. However, especially with squamous cell carcinomas, tumour grade (a powerful prognostic factor in many other tumour types) is of limited prognostic value for a number of reasons (discussed below). In the recent International Collaboration on Cancer Reporting data set for reporting of cervical cancers, tumour grading is listed as a RECOMMENDED and not a REQUIRED element; the authors of the data set were not able to recommend any particular grading system for cervical squamous cell carcinomas [1]. In the recent recommendations of the European Society of Gynaecological Oncology, the European Society for Radiotherapy and Oncology, and the European Society of Pathology for management of patients with cervical cancer, tumour grade is not taken into account in patient management [2].

In a recent article in this journal, Jesinghaus and colleagues studied the prognostic value of a novel histopathological grading system based on tumour budding and cell nest size in cervical squamous cell carcinoma [3]. This system has been shown to outperform conventional grading systems in squamous cell carcinomas at several other sites such as lung, oral cavity, and oesophagus in terms of patient prognostication [4–6]. Herein, the limitations of conventional grading systems for cervical squamous cell carcinomas, the results of the study of Jesinghaus and colleagues and the potential impact of the newly proposed system are discussed.

Limitations of current grading systems for cervical squamous cell carcinomas

Tumour grade is regularly included in histopathology reports of cervical squamous cell carcinoma and adenocarcinoma. However, at present no particular grading system has achieved universal acceptance and grading of these tumours remains of uncertain clinical value. For example, grade is not amongst the factors considered in determining the Gynecology Oncology Group score which is used to assess the need for adjuvant therapy following surgery for low-stage cervical carcinomas [7]. Zaino et al investigated the prognostic value of classical morphological parameters (mitotic count, nuclear pleomorphism, keratinization) as well as several grading systems incorporating these parameters and found them to be highly limited [8]. Not uncommonly, studies that assess cervical carcinoma grade as a potential prognostic variable provide no details of the grading system employed.

As with grading of many tumours, grading of cervical squamous cell carcinomas has a considerable subjective component and this probably explains, at least in part, the variable proportion of well, moderately, and poorly differentiated tumours reported in different studies. Almost all cervical squamous cell carcinomas are human papillomavirus (HPV) associated and given that HPV-associated squamous cell carcinomas very commonly have a 'basaloid' morphology with minimal keratinization, they are almost always poorly differentiated when using grading systems which take into account the degree of keratinization of the neoplasm. Another factor which should be taken into account is that most clinically advanced cervical carcinomas are treated with primary chemoradiation and histological sampling is often limited to a small diagnostic biopsy which may not be fully representative with regards to grade due to tumour heterogeneity.

Conventional grading systems for cervical squamous cell carcinoma

Historically, cervical squamous cell carcinomas were graded using Broder's system (or modifications of this), which is based on the degree of keratinization, cytological atypia, and mitotic activity [9–12]. In some schemes, the pattern of invasion (pushing versus infiltrative) has also been taken into account [13–15]. Traditionally, cervical squamous cell carcinomas have also been sub-classified into large cell keratinizing, large cell non-keratinizing, and small cell non-keratinizing categories, with these sometimes being regarded as approximately equivalent to well, moderately, and poorly differentiated, respectively. However, it should be noted that some studies have found that keratinizing variants of large cell squamous cell carcinoma actually have a poorer prognosis than non-keratinizing variants, an apparently paradoxical finding if keratinization is deemed evidence of better differentiation. This is similar to the situation in the vulva where better differentiated keratinizing squamous cell carcinomas (non-HPV-related) have a poorer prognosis than more poorly differentiated non-keratinizing neoplasms (HPV-related) [16]. The World Health Organization (WHO) blue book suggests that cervical squamous cell carcinomas be graded based on the degree of keratinization, nuclear pleomorphism, size of nucleoli, mitotic frequency, and necrosis but provides no details as to how this should be applied [17].

More complex multifactor grading systems that include both tumour and host/stromal parameters have also been assessed in cervical squamous cell carcinomas. For example, the system employed by Stendahl et al [18], based on that used in head and neck squamous cell carcinoma, comprised eight features which were both tumour-related and stroma-related (growth pattern, differentiation, pleomorphism, mitoses, pattern of invasion, depth of invasion, lymphovascular space invasion, and inflammatory reaction). Simplified modifications to this system have also been described, including systems that selectively focus upon the invasive tumour border or the patterns of tumour invasion [13–15]. These multifactor systems, as well as taking into account conventional grading parameters, also include factors such as depth of invasion and lymphovascular space invasion which are not traditionally part of tumour grading but which are reported separately in the pathology report.

In spite of these different grading systems, it is apparent that none has gained widespread acceptance or has been widely adopted in clinical practice and it is likely that in assessing tumour grade in cervical squamous cell carcinomas, most pathologists simply 'eyeball' the histological sections and provide an 'informal' grade.

Recently proposed grading system for cervical squamous cell carcinomas

Jesinghaus and colleagues studied two cohorts (test and validation cohorts; $n = 125$ and 122, respectively) of primary resection specimens of cervical squamous cell carcinomas from German patients [3].

Table 1. Proposed new grading system for squamous cell carcinoma of the cervix (Reproduced with permission from [3])

Tumour budding activity/10 HPF	
No budding	1
<15 budding foci	2
≥15 budding foci	3
Smallest cell nest size within the tumour	
>15 cells	1
5–15 cells	2
2–4 cells	3
Single cell invasion	4
Tumour grading	*Total score*
Well differentiated (G1)	2–3
Moderately differentiated (G2)	4–5
Poorly differentiated (G3)	6–7

All the carcinomas were graded (G1–G3) in accordance with the 2014 WHO classification and also divided into keratinizing and non-keratinizing neoplasms. In addition, the cases were graded using the novel grading system which, as discussed, has been shown to have prognostic value in squamous cell carcinomas arising at other anatomic sites. This grading system is based on tumour budding activity and the smallest identifiable cell nest size. Tumour budding was defined as dissociation of small nests of tumour cells (<5 cells) that 'bud' into the peritumoral stroma; budding was evaluated throughout the whole tumour and scored within the area showing highest budding. In evaluating cell nest size, nests consisting of 2–4 tumour cells were scored as small, 5–15 as intermediate, and >15 as large; the term single cell invasion was reserved for tumours harbouring single dyscohesive tumour cells; for every carcinoma, the smallest identifiable cell nests were used in scoring. Using this system, a score was attributed to both budding activity (1–3 points) and cell nest size (1–4 points). Briefly, tumours without budding activity are scored as 1, tumours with low budding activity [<15 buds per 10 high power fields (HPF)] as 2 and tumours with high budding frequency (≥15 buds per 10 HPF) as 3. Large cell nests are scored as 1 point, intermediate and small as 2 and 3 points, respectively, and single cells as 4 points. The sum of both scores results in a final grading score ranging from 2 to 7. Using this grading system, G1 tumours have a score ranging from 2 to 3, G2 from 4 to 5, and G3 from 6 to 7. Table 1 summarizes the scoring system.

Figure 1 is a photomicrograph from the paper by Jesinghaus *et al* [1] illustrating the various parameters of the grading system (figure and legend reproduced with permission of the authors).

In the test cohort, the novel proposed grading system had a significant impact on overall, disease-specific, and disease-free survival (all $p < 0.001$).

Overall survival was 143.4, 124.2, and 86.1 months for G1, G2, and G3 squamous cell carcinomas, respectively. A significant impact of the new grading system on overall survival and disease-free survival was confirmed in the validation cohort ($p = 0.001$ and $p = 0.013$, respectively). Furthermore, lower tumour differentiation (G2/G3) using the proposed grading system was significantly associated with higher tumour stage, lymphovascular space invasion, and perineural spread in the test and validation cohorts. Lower tumour differentiation (G2/G3) was significantly associated with nodal metastases in the validation cohort. Multivariate survival analyses in the test cohort revealed a very strong significant stage and age-independent prognostic impact of the new grading system for overall, disease-specific, and disease-free survival. When this analysis was repeated in the validation cohort, again a stage- and age-independent significant impact on survival was observed for both overall and disease-free survival. Tumour budding activity and cell nest size were the most significant morphological factors in both cohorts with the other parameters evaluated, including WHO grading, failing to show prognostic significance. It should be noted that tumour budding on its own has been previously investigated as a prognostic factor in early stage cervical squamous cell carcinomas and high tumour budding shown to be a poor prognostic indicator [19].

Analysis of 20 cases in the study revealed a high interobserver reproducibility of the new grading system between two independent pathologists (Kappa-Cohens value: 0.857).

Rationale for proposed grading system and value in other organs

As already discussed, the tumour budding/cell nest size grading system for cervical squamous cell carcinoma can be regarded as another cross-organ validation of this grading system and suggests that these parameters have the potential to constitute the pillars of a highly prognostic grading system that is applicable to squamous cell carcinomas at different anatomic sites. It is also worth noting that tumour budding has been shown to be of prognostic value in other tumour types, for example, colorectal adenocarcinomas where tumour budding is strongly predictive of lymph node metastasis and tumour recurrence [20]. These parameters are likely indicative of a carcinomas ability for cellular dissociation which is indicative of aggressive behaviour.
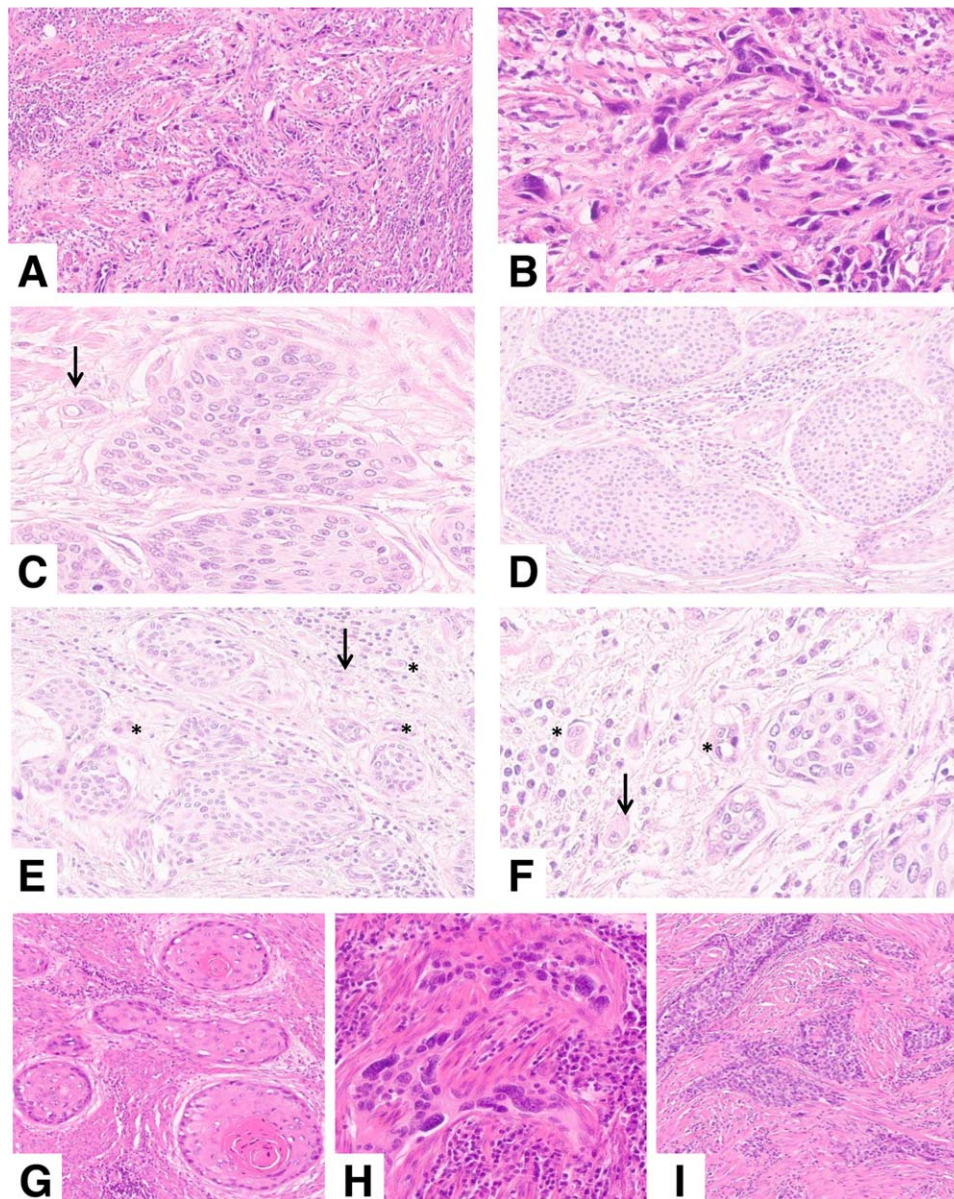
Figure 1. Histomorphological characteristics of squamous cell carcinoma of the uterine cervix. Tumour budding: Overview (A) and details (B) of a cervical squamous cell carcinoma with high tumour budding activity, indicated by the branching of numerous small tumour clusters of <5 cells into the surrounding tissue; (C) details of low tumour budding activity with a single tumour cluster <5 cells (arrow) budding into the tumour stroma. Cell nest size: intermediate magnification (D) of large and medium sized cell nests. Details (E, F) of small cell nest size (stars) and single cell invasion (arrows). Histological subtype, nuclear size, and stromal content: representative images of a keratinizing cervical squamous cell carcinoma (G), large tumour cell nuclei (H), and high stromal content (I). Reproduced with permission from [3].

Conclusions and future directions

The proposed grading system for cervical squamous cell carcinomas has been shown to be of prognostic value for this tumour type at a variety of other sites, such as lung, oral cavity, and oesophagus. This suggests the potential of the parameters (tumour budding and cell nest size) to serve as the basis for a common grading system applicable to squamous cell carcinomas of different anatomic sites. Given the lack of prognostic value provided by current grading systems for cervical squamous cell carcinomas and the absence of a universally used grading system, it is hoped that the results of this study will be validated

in additional independent larger cohorts. Future studies should also address the use of this system in biopsy material (the study of Jesinghaus *et al* only included resection specimens [3]) and the reproducibility of this scoring system, the reproducibility only having been addressed between two pathologists in a small cohort of 20 cases. It is likely that high tumour budding and small cell nest size are associated with specific tumour-host interactions and molecular events and this should also be addressed by future studies. For example, in colorectal adenocarcinomas, tumour budding has been linked to epithelial-mesenchymal transition [21]. Similarly, it has also been shown that small foci of invasion in stage IA cervical squamous cell carcinomas are associated with immunohistochemical alterations associated with epithelial-mesenchymal transition such as increased cyclin D1 expression and a reduction in E-cadherin and beta-catenin staining [22].

Author contributions statement

WGM is solely responsible for the writing of this article.

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