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Actinic keratoses show variable histological basal growth patterns – a proposed classification adjustment

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ABSTRACT

Background Common histological classification schemes of actinic keratoses (AK) do not evaluate growth patterns at basal epidermal aspects of AK. Until now, the importance of basal epidermal growth patterns of AK has not been studied.

Objective To investigate the extent of atypical keratinocytes throughout the epidermis and variation in basal growth patterns of AK.

Methods AK lesions occurring on the head/face from patients seen in routine practice were assessed histologically. We determined histological grade (AK I-III), basal growth patterns of atypical keratinocytes (crowding, budding, papillary sprouting) and accompanying parameters.

Results Of the 246 lesions included, 28.0% were histologically classified as AK I, 46.7% as AK II, and 25.2% as AK III. 26.4% of the basal growth patterns were classified as crowding (pro I), 49.6% as budding (pro II), 17.9% as papillary sprouting (pro III) and 6.1% without basal directed growth. No significant correlation of the histological AK I-III grading and underlying growth patterns was observed (P= 0.4666). However, adnexal structure involvement (OR= 2.37; 95%CI 1.21-4.65), infiltration (OR= 2.53; 95%CI 1.31-4.90) and increased number of vessels (OR= 2.56; 95%CI 1.42-4.65) were independent positive predictive markers for pro II and pro III basal growth patterns.

Conclusions Basal growth patterns (pro I-III) in AK do not correlate with the established AK I-III histological grading system. Besides the degree of upward extension, varying degrees of downward extension exist. Histological classification should consider both, upwards and downward growth patterns when assessing AK.

INTRODUCTION

Actinic keratoses (AK) are regarded as early *in-situ* squamous cell carcinomas (SCC) and are frequently seen in dermatological practice[1]. Due to a change in leisure activities and demography in industrialized countries an increasing incidence of AK in people with fair skin types is observed. In chronically UV-exposed skin areas, AK present as erythematous macules, keratotic papules or plaques. They are diagnosed clinically and commonly classified based on the overall thickness of individual lesions[2]. However, recent studies have shown that the thickness of AK lesions neither correlates with its histological grading nor predicts its dysplastic severity[3,4]. To overcome the limitation of single lesion evaluation a new clinical classification, called the “actinic keratosis area and severity index” (AKASI), has

recently been proposed. This allows an objective assessment of field cancerization (AK severity) of the head/face based on parameters such as erythema, thickness and distribution[5].

Besides clinical grading, there are several histological classifications for grading AKs. Cockerell et al. introduced the term keratinocytic intraepidermal neoplasia (KIN) analogous to the cervical intra-epithelial neoplasia (CIN) and vulva intra-epithelial neoplasia (VIN)[6,7]. Three categories of KIN (KIN 1, KIN 2, and KIN 3) have been proposed, based on both clinical as well as histological features. However, the drawback of this classification is that the term KIN describes histopathological findings, which could also be used for other benign or epithelial neoplastic conditions such as seborrhoeic keratosis or Bowen's disease[8].

Another established histological classification was described by Röwert-Huber[8], which grades the extent of atypical keratinocytes throughout the epidermis. In grade AK I (mild), atypical keratinocytes are restricted to the basal and suprabasal layers, limited to the lower third of the epidermis. In grade AK II (moderate), atypical keratinocytes extend to the lower two thirds of the epidermis. In grade AK III (severe) full thickness atypia of the epidermis is found, and is equivalent to lesions called *in situ* SCC[4,8]. Despite this grading, every single AK lesion has the propensity to progress into invasive squamous cell carcinoma (iSCC) and subsequently metastasize[9-11]. The risk of progression of individual AK lesions into iSCC ranges from 0.025% to 16% per year.[12-16] Irrespective of this overall progression risk, it remains speculative whether a gradual proliferation of atypical keratinocytes throughout the epidermis leads to invasive tumour growth. It is therefore not possible to accurately predict the progression risk of individual AK lesions.

AKs have been recognized as part of a disease continuum described as the "classical pathway"[6]. But a recent study revealed that mild AKs with dysplasia restricted to the lower part of the epidermis are most frequently found adjacent to or overlying iSCC. Besides this "classical" pathway, another "differentiated" pathway has been reported, suggesting that penetration may possibly be independent of the extent of atypical keratinocytes throughout different layers of the epidermis[17,18]. Additionally, another study claims that the severity of AK dysplasia and expression of p53 is independent of clinical and histological thickness[3]. These results challenge the biological significance of the AK I-III grading system[3,17].

Scurry stated that the contiguous AK adjacent to and presumably antecedent to a nearby iSCC often has budding of the epithelium and marked atypia of the basal layer cells, while exhibiting less obvious nuclear atypia in the suprabasal layers.[19] There are no substantial data which demonstrate that Röwert-Huber's or Olsen's classifications are part of a disease continuum in AK. Therefore, an additional classification to evaluate basal growth patterns is required.

Thus, the aim of this retrospective analysis was to determine whether there is a correlation between the commonly used histological classification scheme for AK lesions proposed by Röwert-Huber and basal epidermal growth patterns of AK. Additionally, we investigated accompanying factors such as vascular density, inflammation and involvement of adnexal structures.

MATERIALS AND METHODS

Study population

This retrospective study was performed at the Skin Cancer Center of the Ruhr-University Bochum (Bochum, Germany). The database was searched for patients who had undergone oncological surgery (surgical and shave excisions) for those with histologically diagnosed AK located on the head/face. The study was restricted to these sites to ensure regional histological pattern assessment to improve comparison. Samples from immunosuppressed patients and samples with collision tumours were excluded from this study. The study was approved by the ethics review board of the Ruhr-University Bochum and conducted according to the Declaration of Helsinki.

Microscopic evaluation

All hematoxylin and eosin (H&E) stained sections (4 µm of thickness) were analysed at scanning magnification and at 20-fold magnification. The samples were classified as AK I, II or III according to Röwert-Huber et al. by two independent investigators (LS and TD).

The underlying basal growth pattern adjacent to the basement membrane zone was classified into three grades (**pro**truding I-III). In pro I (crowding) basal atypical keratinocytes were seen to be crowding, resulting in a more basophilic appearance of the basal epidermis. In pro II (budding) small hemispheric buds were seen at the basal epidermis starting to protrude slightly into the upper papillary dermis and forming round nests of atypical keratinocytes. They were clearly distinguishable from overlying parts of the epidermis by their basophilic appearance. However, the overall thickness of these buds did not exceed the thickness of the epidermis lying on top.

Pro III (papillary sprouting) was defined by spiky or filiform papillary elongation of atypical keratinocytes protruding into upper dermis. The thickness of the papillary elongation exceeded the thickness of the overlying epidermis (***Fig. 1***). If different grades of growth were demonstrated throughout a single lesion, the highest grade was chosen to define the lesion (AK I-III grading as well as pro I-III grading).

We defined upwards directed dysplasia (AK I-III), and downwards directed dysplasia (pro I-III) with a notional line drawn between the tips of upper dermal papillae and this was used to distinguish and evaluate both dysplastic entities, in a reproducible manner. Dysplasia criteria above (AK I-III) and below (pro I-III) this notional line have been evaluated (***Fig. 2***).

Moreover, underlying inflammation was classified semi-quantitatively into mild, moderate and intense infiltrate. Further accompanying parameters (involvement of adnexal structures, numerically increase in vessels) were also investigated. In the absence of consistency amongst the two investigators cases were discussed at a double-headed microscope until agreement was achieved.

Statistical analysis

Data analysis was performed using the statistical package MedCalc software version 15.2 (Ostend, Belgium). Distribution of data was assessed by the D`Agostino-Pearson test. Provided there was normal distribution, data were expressed as mean and standard deviation (SD), otherwise as median and range. Data were analyzed using the Chi^2 test (univariate) and logistic regression procedure (multivariate). P-values less than 0.05 were considered statistically significant.

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RESULTS

In total, 246 AK lesions were included in this study (***Table 1***). The median age of patients in the study population was 79 (56-94) years. The majority of patients were male (92.3%) and most of the lesions were located on the scalp (30.9%).

Overall, 69 lesions (28.0%) were histologically classified as AK I, 115 (46.7%) as AK II and 62 (25.2%) as AK III. With regards to basal growth pattern, 65 (26.4%) were classified as pro I (crowding), 122 (49.6%) as pro II (budding), 44 (17.9%) as pro III (papillary sprouting) and 15 (6.1%) were without signs of basal growth.

No significant correlation (P = 0.4666) between histological classification (AK I–III) and the underlying basal growth pattern (pro I-III) could be found (***Fig. 3***). In AK I, we observed 8 (11.6%) AK lesions without basal growth, 18 (26.1%) showed pro I, 31 (44.9%) pro II and 12 (17.4%) pro III features. In AK II, 5 (4.3%) AK lesions showed no basal growth, 32 (27.8%) pro I, 58 (50.4%) pro II and 20 (17.4%) had pro III features. In AK III, 2 (3.2%) AK lesions showed no basal growth, 15 (24.2%) pro I, 33 (53.2%) pro II and 12 (19.4%) had pro III features. Clinical examples of varying upwards and downwards directed growth patterns are shown in **Figure 4**.

Additionally, we found a significant association between basal growth pattern (pro I-III) and involvement of adnexal structures (P = 0.0030), infiltration (>moderate) (P = 0.0012) and numerically increased underlying vessels (P = 0.0012) (***Fig. 5***). In logistic multivariate regression analysis we observed, as independent positive predictive markers for basal growth in pro II or pro III, involvement of adnexal structures (OR = 2.37; 95%CI 1.21-4.65), infiltration (>moderate) (OR = 2.53; 95%CI 1.31-4.90) and numerically increased underlying vessels (OR = 2.56; 95%CI 1.42-4.65).

DISCUSSION

Histological classification systems of AK should ideally assess lesion severity and its malignant potential to allow physicians to make appropriate treatment decisions[8]. Current classification systems are restricted to dysplasia in epidermal structures focussing on the extent of atypical keratinocytes throughout the epidermis. This mainly refers in analogy to classification schemes of AIN, VIN and CIN and regards AK progression in terms of a disease continuum. There are no data that substantiate Röwert-Huber's classification proposing a disease continuum from AK I to AK III. Furthermore, there is no evidence to show that a continuous progression of atypical keratinocytes throughout the epidermis necessarily leads to iSCC. We now understand that this disease continuum is not universal and Scurry reported that KIN 1 and KIN 2 lesions may progress directly to iSCC without progression to KIN 3 first. If the AKs from which AK-derived SCCs emerge are thus graded as KIN 1 or 2, the utility of the KIN 3 designation comes into doubt.[19] In this regard, it remains speculative whether Röwert-Hubert's classification contributes more than a morphological description of AK extent throughout the epidermis; predictive qualities with respect to tumour progression have not be demonstrated.

The present study is to our knowledge the first to systematically investigate different basal growth patterns in AK lesions. We observed three different growth patterns which are described as crowding, budding and papillary sprouting of atypical keratinocytes and were defined as protruding atypical keratinocytes grade I-III (pro I-III), accordingly. Our suggestion for a new classification of basal growth pattern does not show a correlation with the common histological classification scheme (AK I-III). An extended basal growth pattern (pro III) may be observed in mild actinic keratosis (AK I) and full thickness dysplasia (AK III) may only demonstrate mild proliferative

activity at the basement membrane zone (pro I). Thus, these classification schemes may complement each other. Furthermore, our classification system is easy to learn and easy to apply favouring its potential use in dermatopathology.

Previous studies have described features such as “buds of atypical keratinocytes”[8,19,20] which may exhibit a higher risk of progression, particularly in the context of Bowen´s disease (BD) and therefore downward growth in contrast to solely upwards directed growth may be of special interest in AK which progress to iSCC. Similar to AK III, BD is characterized by full thickness atypia throughout the epidermis; multinucleated cells containing clusters of nuclei are often present.[21] A striking difference in comparison with AK is the sharp border between epidermis and dermis and frequently observed pigmentation at the preserved basal layer (so called “eyeliner sign”).[22] Signs of basal growth like budding or protruding of cells towards the dermis are not characteristic in BD. This supports the assumption that growth pattern and growth direction may play an important role in terms of tumour progression, particularly as BD very rarely becomes invasive.[19]

Moreover, recent studies have shown that a classification scheme focussing on the extent of dysplasia throughout the epidermis does not correlate with the risk of invasive growth. On the contrary, AK lesions classified as AK I have been shown to be the most common lesions associated with iSCC of the skin[3,17]. Therefore, it might be particularly important to focus on dysplasia at the basement membrane where it is highly likely that the process of invasion starts.

Our study has also demonstrated several parameters which positively correlate with basal growth (pro I-III). In logistic multivariate regression analysis we found as an independent positive predictive marker for pro II or pro III basal growth, the involvement of adnexal structures as atypical keratinocytes migrate down the

adnexal epithelium. In the literature, sparing of the epithelium of acrosyringia and acrotrichia is often described as a typical histological sign of AK.[6,8,23] Nevertheless, histological classification scheme by Cockerell states that in KIN II, involvement of upper acrotrichia and acrosyringia may be seen.[6] Furthermore, both Cockerell´s and Röwert-Huber´s classification scheme describe involvement of adnexal structures as part of their grade III KIN/AK.[6,8] Furthermore, it has been shown that patients with AK and follicular extension were more likely to progress towards invasive tumour.[24] The results of our study support this evaluation in terms of an increasing risk of progression with higher grades of basal growth pattern.[25] Our study showed more than a half (51.2%) of pro III graded AKs had adnexal involvement. This may have an impact on the choice of treatment as many lesion directed treatments target superficial disease and are not effective when there is deep follicular involvement, increasing the risk of recurrence.[26,27]

Additionally our study revealed that an underlying moderate to severe inflammatory infiltrate was a further positive independent predictor for pro II or pro III basal growth patterns. Berhane et al. proposed a classification system of AK lesions based on underlying inflammation. However, it has not been established in routine practice due to its inconsistency. Thus it cannot be predicted that the presence of an infiltrate will lead to progression or regression of AK lesions[28]. Our study showed that atypical epidermal structures protruding deeply into the papillary dermis positively correlate with inflammatory infiltration. Involvement of immune cells is a characteristic feature of the tumour microenvironment in invasive carcinoma.[29,30] Furthermore, our study showed numerically increased underlying vessels as an independent predictor for basal growth (> pro II).

We suggest that the histological classification scheme of AK should be amended by adding characteristics of basal lesion growth, which we have classified as pro I-III. Future studies are required to evaluate the relevance of basal proliferation in AK with regards to the risk of tumour progression. A histological review of AK adjacent to SCC could relate this histological grading adjustment to invasive tumour growth.

In conclusion, we have to broaden our view on the morphologic features leading to progression of AK lesions to invasive carcinoma. Although we are not able to conclude if, or which aspects of growth patterns may have an impact on progression, this study details the first classification scheme to evaluate basal growth patterns. Thus, it would be particularly interesting to apply our classification scheme to AKs adjacent to (early) iSCCs.

REFERENCES

1 Dirschka T, Gupta G, Micali G *et al.* Real-world approach to actinic keratosis management: practical treatment algorithm for office-based dermatology. *J Dermatolog Treat* 2016: 1-12.

2 Olsen EA, Abernethy ML, Kulp-Shorten C *et al.* A double-blind, vehicle-controlled study evaluating masoprocol cream in the treatment of actinic keratoses on the head and neck. *J Am Acad Dermatol* 1991; **24**: 738-43.

3 Heerfordt IM, Nissen CV, Poulsen T *et al.* Thickness of Actinic Keratosis Does Not Predict Dysplasia Severity or P53 Expression. *Sci Rep* 2016; **6**: 33952.

4 Schmitz L, Kahl P, Majores M *et al.* Actinic keratosis: correlation between clinical and histological classification systems. *J Eur Acad Dermatol Venereol* 2016; **30**: 1303-7.

5 Dirschka T, Pellacani G, Micali G *et al.* A Proposed Scoring System for Assessing the Severity of Actinic Keratosis on the Head: Actinic Keratosis Area and Severity Index (AKASI). *J Eur Acad Dermatol Venereol* 2017.

6 Cockerell CJ. Histopathology of incipient intraepidermal squamous cell carcinoma ("actinic keratosis"). *J Am Acad Dermatol* 2000; **42**: 11-7.

7 Fu W, Cockerell CJ. The actinic (solar) keratosis: a 21st-century perspective. *Arch Dermatol* 2003; **139**: 66-70.

8 Rowert-Huber J, Patel MJ, Forschner T *et al.* Actinic keratosis is an early in situ squamous cell carcinoma: a proposal for reclassification. *Br J Dermatol* 2007; **156 Suppl 3**: 8-12.

9 Ackerman AB, Mones JM. Solar (actinic) keratosis is squamous cell carcinoma. *Br J Dermatol* 2006; **155**: 9-22.

10 Quatresooz P, Pierard-Franchimont C, Paquet P *et al.* Crossroads between actinic keratosis and squamous cell carcinoma, and novel pharmacological issues. *Eur J Dermatol* 2008; **18**: 6-10.

11 Stockfleth E, Ferrandiz C, Grob JJ *et al.* Development of a treatment algorithm for actinic keratoses: a European Consensus. *Eur J Dermatol* 2008; **18**: 651-9.

12 Dodson JM, DeSpain J, Hewett JE *et al.* Malignant potential of actinic keratoses and the controversy over treatment. A patient-oriented perspective. *Arch Dermatol* 1991; **127**: 1029-31.

13 Glogau RG. The risk of progression to invasive disease. *J Am Acad Dermatol* 2000; **42**: 23-4.

14 Marks R, Rennie G, Selwood T. The relationship of basal cell carcinomas and squamous cell carcinomas to solar keratoses. *Arch Dermatol* 1988; **124**: 1039-42.

15 Marks R, Rennie G, Selwood TS. Malignant transformation of solar keratoses to squamous cell carcinoma. *Lancet* 1988; **1**: 795-7.

16 Nestor MS, Zarraga MB. The incidence of nonmelanoma skin cancers and actinic keratoses in South Florida. *J Clin Aesthet Dermatol* 2012; **5**: 20-4.

17 Fernandez-Figueras MT, Carrato C, Saenz X *et al.* Actinic keratosis with atypical basal cells (AK I) is the most common lesion associated with invasive squamous cell carcinoma of the skin. *J Eur Acad Dermatol Venereol* 2015; **29**: 991-7.

18 Fernandez Figueras MT. From actinic keratosis to squamous cell carcinoma: pathophysiology revisited. *J Eur Acad Dermatol Venereol* 2017; **31 Suppl 2**: 5-7.

19 Scurry J. Grading of actinic keratoses. *J Am Acad Dermatol* 2001; **44**: 1052-3.

20 Cockerell CJ. Pathology and pathobiology of the actinic (solar) keratosis. *Br J Dermatol* 2003; **149 Suppl 66**: 34-6.

21 Bagazgoitia L, Cuevas J, Juarranz A. Expression of p53 and p16 in actinic keratosis, bowenoid actinic keratosis and Bowen's disease. *J Eur Acad Dermatol Venereol* 2010; **24**: 228-30.

22 LeBoit PE. The thin brown line. *Am J Dermatopathol* 2004; **26**: 444-5.

23 Anwar J, Wrone DA, Kimyai-Asadi A *et al.* The development of actinic keratosis into invasive squamous cell carcinoma: evidence and evolving classification schemes. *Clin Dermatol* 2004; **22**: 189-96.

24 Pandey S, Mercer SE, Dallas K *et al.* Evaluation of the prognostic significance of follicular extension in actinic keratoses. *J Clin Aesthet Dermatol* 2012; **5**: 25-8.

25 Goldberg LH, Joseph AK, Tschen JA. Proliferative actinic keratosis. *Int J Dermatol* 1994; **33**: 341-5.

26 Simon JC, Dominicus R, Karl L *et al.* A prospective randomized exploratory study comparing the efficacy of once-daily topical 0.5% 5-fluorouracil in combination with 10.0% salicylic acid (5-FU/SA) vs. cryosurgery for the treatment of hyperkeratotic actinic keratosis. *J Eur Acad Dermatol Venereol* 2015; **29**: 881-9.

27 Stockfleth E. The importance of treating the field in actinic keratosis. *J Eur Acad Dermatol Venereol* 2017; **31 Suppl 2**: 8-11.

28 Berhane T, Halliday GM, Cooke B *et al.* Inflammation is associated with progression of actinic keratoses to squamous cell carcinomas in humans. *Br J Dermatol* 2002; **146**: 810-5.

29 Nissinen L, Farshchian M, Riihila P *et al.* New perspectives on role of tumor microenvironment in progression of cutaneous squamous cell carcinoma. *Cell Tissue Res* 2016; **365**: 691-702.

30 Pio R, Corrales L, Lambris JD. The role of complement in tumor growth. *Adv Exp Med Biol* 2014; **772**: 229-62.

Table1. Demographic and histological characteristics (N=246)

Characteristic	n (%)
Sex	
Male	227 (92.3)
Female	19 (7.7)
Age, years	79 (56-94)[a]
Location of lesion	
Balding scalp	76 (30.9)
Forehead	49 (19.9)
Left side of the face (+ear)	69 (28.0)
Right side of the face (+ear)	48 (19.5)
AK histological severity	
AK I	69 (28.0)
AK II	115 (46.7)
AK III	62 (25.2)
AK basal growth grading	
no basal growth	15 (6.1)
pro I	65 (26.4)
pro II	122 (49.6)
pro III	44 (17.9)
Accompanying parameters	

Infiltrate (>moderate)	95 (38.6)
Involvement of adnexal structures	86 (35.0)
numerically increased vessels	68 (27.6)

AK, actinic keratosis; pro, basal **pro**truding actinic keratosis

[a]Data are median (range).