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Validity of the Histopathological Criteria used for Diagnosing Dysplastic Naevi

An interobserver study by the pathology subgroup of the EORTC Malignant Melanoma Cooperative Group

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Ten (dermato)pathologists studied 50 cutaneous melanocytic lesions including common naevocellular naevi, dysplastic naevi (DN), melanomas *in situ* and invasive primary melanomas, with emphasis on the histological criteria of DN. Using a standardised form, 20 defined histopathological features were scored (semi)quantitatively. Concordance of diagnosis, efficacy and reproducibility of features were investigated. DN were distinguished well from the other entities (mean P_o 0.87). Agreement on the degree of atypia of DN was low. The reproducibility of the scoring was best for the following features: irregular nests, lymphohistiocytic infiltrate, marked junctional proliferation and large nuclei. The overall values of these features to discriminate between DN and non-DN were better than for the other features studied. Using the presence of at least three of the four features as a condition for the diagnosis of DN, values for sensitivity, specificity and positive and negative predictive values were 0.86, 0.91, 0.96 and 0.73, respectively. On the basis of the results these features seem best suited as histological criteria for the diagnosis of DN.
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INTRODUCTION

THE DYSPLASTIC NAEVUS (DN) is considered a precursor to cutaneous melanoma. In addition, it may serve as a marker of an increased risk of developing melanoma. Clinical hallmarks often encountered are a maximum diameter of 5 mm or more, asymmetry, irregular borders and a variegated colour [1]. Histopathological examination of selected lesions is needed to confirm a clinical diagnosis of DN and to detect or exclude early melanoma. Elder *et al.* have first described the histopathological criteria for the diagnosis of DN [2, 3]. They consist of features of cytological atypia, architectural atypia and stromal changes. Later, these features were debated and others were added [4–19]. At present, it is not settled which of the histological criteria are obligatory for the diagnosis, and if so, to what degree and extent a feature should be present. Issues related to this problem are differences in the appreciation of the concept of DN as an entity and differences in the final interpretation of the observations. Basically, however, the selection and weight of a feature for a diagnostic purpose rests on its validity in terms of the discriminatory value (efficacy) and the reproducible recognition among observers. With regard to DN, research into the latter is sparse and concerns only some histological features [16]. As it is important to provide clinicians with a consistent histopathological diagnosis, the pathology subgroup of the EORTC Malignant Melanoma Cooperative Group, under the auspices of the E.C. Concerted Action on Melanoma, commenced the present study. The validity of the morphological features which have been propagated was investigated in relation to their interpretation in terms of concordance of diagnosis among observers. The results obtained are compared with other studies with respect to reproducibility [16] and efficacy [13] of features and concordance of diagnosis [19]. Although this work may be considered a pilot study, provisional guidelines are given on the histopathological classification of DN, which also may direct future research among (dermato)pathologists in a routine setting.

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MATERIALS AND METHODS

Histological slides

A set of 50 histological slides was used, taken from the files of the Pathology Panel of the Dutch Melanoma Working Party. Based on their individual diagnoses on all 50 slides, the set consisted of nine non-dysplastic benign melanocytic lesions, 25 DN, seven melanomas *in situ* and nine invasive melanomas. Thus, a wide spectrum of melanocytic lesions was included although enriched for certain categories. As the distinction between benign vs. malignant can serve as a quality check of the observers, rather a lot of premalignant and malignant lesions were included. DN were the lesions of principal interest in this

study. According to the Dutch Panel, following the criteria of Steijlen *et al.* [13], the set of DN consisted of approximately equal numbers of lesions with slight, moderate and marked atypia.

Observers

10 (dermato-)pathologists participated as observers. The same slides were sent to each observer and evaluated independently within a period of 3 weeks.

Morphological features and histopathological evaluation

A standardised form for histopathological evaluation was used, listing morphological features based on the literature and personal experience. In addition, criteria for melanoma *in situ* and invasive melanoma were listed (Appendix I). A lesion could be diagnosed as common naevocellular naevus, common naevocellular naevus with minor abnormal features, DN, melanoma *in situ*, superficial spreading melanoma or other lesion. In order to facilitate a consistent appreciation of the morphological features a list with detailed definitions of the criteria used was added (Appendix II). No set of criteria for the diagnosis DN or other diagnoses was proposed. The form was discussed and accepted by the observers before circulation of the histological slides. One slide per lesion was studied. In most cases several sections were mounted on the slide. No clinical data were provided.

Statistical methods

The interobserver agreement was studied by calculating the proportion of agreement (P_o) and the Kappa-coefficient (the proportion of agreement in excess of what is expected by chance, see Appendix III). The measures of agreement in diagnosis were also calculated when certain categories were combined. Both the mutual agreement in diagnosis of all observers and the agreement with the panel diagnosis were determined. The EORTC panel diagnosis was considered to be the diagnosis of a majority of the 10 observers. The reproducibility of the features was studied by calculating Pearson correlation coefficients and by applying Kappa statistics. The discriminating value of the features was described using discriminant analyses and Fisher exact tests. Sensitivity, specificity, positive and negative predictive value for the features with respect to the panel diagnoses were calculated.

RESULTS

Results reported here mainly concern the issues related to the diagnosis of DN.

Concordance of diagnosis

Before further analysis of the data, the observers' diagnoses were *a posteriori* classified in one of the six categories mentioned in Table 1. All observers agreed on 10 slides, while at least eight observers agreed on 23 slides, and at least five observers agreed on 46 slides. The distribution of the diagnoses per observer is given in Table 2. It illustrates the interobserver variation in the diagnoses made. All observers scored one or more lesions in category 2 (mean 6.5), but only in 1 case did a majority agree on this diagnosis.

As is shown in Table 3(a), the mean P_o and Kappa values for the comparison of benign vs. malignant lesions are high, i.e. 0.90 and 0.76, respectively. Also, mean values for the comparison II are relatively high, i.e. 0.79 and 0.58. These figures represent the means of 45 interobserver comparisons. When the diagnoses of one observer were compared to the diagnoses of a majority of the 10 observers ("panel diagnoses"), the values were higher [Table 3(b)]. Stratification of the diagnostic categories coincides with a decrease of the P_o and Kappa values, but for all diagnoses taken separately (analysis *V*) mean P_o and Kappa values are acceptable: 0.70 and 0.61, respectively. A low level of agreement (P_o mean = 0.28; S.D. = 0.16; 45 observer pairs) was found on the degree of histopathological atypia between pairs of

Table 1. A posteriori classification of the observers' diagnoses

Category 1
Benign proliferation of melanocytes, except Spitz naevus and freckle.
It includes:
- Common naevocellular naevus
- Congenital naevus
- Lentigo
- Blue naevus
- Combined naevus

Category 2
Common naevocellular naevus with minor abnormal features

Category 3
Dysplastic naevus

Category 4
Melanoma *in situ*.
It includes:
- *In situ* SSM
- *In situ* ALM

Category 5
Invasive melanoma.
It includes:
- SSM
- NM
- LMM
- ALM
- Unclassified melanoma
- Acral SSM
- Cutaneous melanoma metastasis

Category 6
Other diagnoses, including Spitz naevus (8×), freckle (7×), hyperpigmentation of basal keratinocytes (1×), functional hyperpigmentation (2×), pigmented Bowen's disease (1×), mastocytosis (1×), no diagnosis (4×)

SSM: Superficial spreading melanoma; NM: Nodular melanoma; LMM: Lentigo maligna melanoma; ALM: Acrolentiginous melanoma.

Table 2. Distribution of observers' diagnoses

Observer	Number of slides per diagnostic category					
	1	2	3	4	5	6
a	13	8	13	2	10	4
b	8	10	17	4	9	2
c	7	4	21	4	12	2
d	7	1	25	5	11	1
e	11	9	14	2	12	2
f	5	2	27	4	10	2
g	8	11	12	6	11	2
h	19	5	4	10	10	2
i	15	6	12	7	7	3
j	9	9	15	6	7	4
a–j mean	10.2	6.5	16	5	9.9	2.4
S.D.	4.3	3.4	6.8	2.4	1.8	0.8
EORTC panel*	9	1	23	6	9	2

*The diagnosis of a majority of the 10 observers.

*Table 3. Reproducibility of diagnosis expressed in P_o and Kappa values**

	P_o					Kappa				
	(a) Means and S.D. of 45 P_o and Kappa values of 45 comparisons of two observers (ab ....ij)									
	I	II	III	IV	V	I	II	III	IV	V
Mean	0.90	0.79	0.69	0.63	0.57	0.76	0.58	0.57	0.50	0.45
S.D.	0.06	0.09	0.10	0.09	0.09	0.13	0.17	0.12	0.10	0.10
	(b) P_o and Kappa of comparisons between observer and "panel" diagnosis for each of observers a–j									
a-panel	0.94	0.90	0.82	0.70	0.70	0.85	0.80	0.75	0.60	0.62
b-panel	0.96	0.86	0.86	0.76	0.70	0.90	0.72	0.79	0.67	0.61
c-panel	0.82	0.82	0.72	0.76	0.70	0.58	0.64	0.59	0.66	0.59
d-panel	0.98	0.92	0.82	0.82	0.80	0.95	0.84	0.73	0.74	0.71
e-panel	0.94	0.86	0.78	0.66	0.62	0.85	0.72	0.68	0.54	0.54
f-panel	0.94	0.90	0.82	0.84	0.80	0.85	0.80	0.73	0.76	0.71
g-panel	0.96	0.94	0.88	0.72	0.70	0.91	0.87	0.83	0.63	0.62
h-panel	0.92	0.70	0.62	0.54	0.54	0.82	0.38	0.52	0.43	0.45
i-panel	0.92	0.82	0.74	0.66	0.66	0.80	0.68	0.64	0.55	0.57
j-panel	0.96	0.98	0.92	0.78	0.78	0.90	0.96	0.88	0.70	0.71
Mean	0.93	0.87	0.80	0.72	0.70	0.84	0.74	0.71	0.63	0.61
S.D.	0.04	0.08	0.09	0.09	0.08	0.10	0.16	0.11	0.10	0.08

*Comparisons based on clustering of diagnoses (categories of Table 1):

I:	[1, 2, 3, 6] vs.[4, 5]	Benign/malignant
II:	[2, 3] vs. [1, 4, 5, 6]	
III:	[1] vs. [2, 3] vs. [4] vs. [5] vs. [6]	Categories two and three together
IV:	[1, 2] vs. [3] vs. [4] vs. [5] vs. [6]	Categories one and two together
V:	[1] vs. [2] vs. [3] vs. [4] vs. [5] vs. [6]	All diagnoses separately

observers. This comparison was made if both observers of a pair classified the lesion as either common naevocellular naevus with minor abnormal features or as a DN, thus constituting four levels of atypia, which could be scored (Appendix I, items 38 and 39).

Discriminative value

The sensitivity, specificity, and positive and negative predictive value of the features discriminating between a non-DN and a DN are given in Table 4. Highest values for sensitivity were found for the following features: percentage of melanocytic cells

Table 4. Sensitivity, specificity, and positive and negative predictive value of histopathological features in dysplastic vs. *non-dysplastic naevi**

Feature	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Large nuclei	0.87	0.67	0.87	0.68
Nuclear hyperchromasia	0.72	0.71	0.86	0.51
Nuclear polymorphism	0.87	0.67	0.87	0.68
Prominent nucleoli	0.67	0.80	0.89	0.50
Large cytoplasm	0.81	0.74	0.88	0.62
% melanocytic cells with at least one of the cytological atypia features	0.94	0.45	0.81	0.76
Irregular nests	0.89	0.84	0.93	0.76
Marked junctional proliferation	0.71	0.84	0.91	0.55
Dust-like melanin	0.62	0.75	0.86	0.45
Elongated rete ridges	0.87	0.17	0.71	0.35
Loss of preference for rete ridge tips	0.67	0.77	0.80	0.63
Shoulder phenomenon	0.70	0.80	0.89	0.53
Abnormal distribution of pigment	0.33	0.88	0.87	0.36
Lymphohistiocytic infiltrate	0.88	0.52	0.81	0.65
Lamellar fibroplasia	0.70	0.81	0.90	0.53
Neovascularisation	0.51	0.83	0.88	0.42

*Panel diagnoses were used (category one and two vs. three); for each feature the total of the data from eight observers was used to calculate sensitivity, specificity, and positive and negative predictive values; scoring of features was dichotomised (present vs. absent); data from two observers were incomplete.

with at least one feature of cytological atypia, irregular nests, lymphohistiocytic infiltrate, large nuclei, elongated rete ridges and nuclear pleomorphism. Highest values for specificity were found for the following features: abnormal distribution of melanin, marked junctional proliferation, irregular nests, neovascularisation, lamellar fibroplasia and shoulder phenomenon. Highest values for positive predictive value were found for: irregular nests, marked junctional proliferation, lamellar fibroplasia, nuclear polymorphism and shoulder phenomenon. Highest values for negative predictive value were found for: irregular nests, % melanocytic cells with at least one of the cytological atypia features, large nuclei, nuclear polymorphism, and lymphohistiocytic infiltrate. For these analyses panel diagnoses were used. Scoring of the features was dichotomised. Dichotomisation, other than into present vs. absent, resulted in lower figures. From two observers some data were missing, which prohibited a meaningful analysis.

Discriminant analysis showed that using more than three to four features had no further discriminating value. Although the most discriminating features varied from observer to observer, certain features were more often among the top four. Table 5 shows the overall hierarchy of the best discriminating features. The average R^2 values for these features varied from 0.65 (for irregular nests) to 0.30 (for large cytoplasma). The hierarchical order obtained by discriminant analysis correlated well with the order obtained when applying a Fisher exact test, ranking the features according to significance (best P values). Features not listed, but with a relatively high discriminating value in a single observer were: bridges of melanocytes, lymphohistiocytic infiltrate, lamellar fibroplasia, dust-like melanin and elongated rete ridges.

Reproducibility of feature scoring

The concordance in the non-dichotomised scoring of the features among eight observers can be found in Table 6. From two observers some data were missing, which prohibited a meaningful analysis. The results of the analysis of all diagnoses (category one to six) did not differ from those of the analysis restricted to category one, two and three only. Highest mean Pearson correlation coefficients (≥ 0.65) with also the highest minimum values for a single observer (see range), were found for the following features: lymphohistiocytic infiltrate, irregular nests and marked junctional proliferation. Counting of the number of "bridges of melanocytes" was hardly reproducible (not shown).

*Table 5. Overall hierarchy in discriminating value of histopathological features for the discrimination between normal naevi and dysplastic naevi**

a	— Irregular nests
b	— Marked junctional proliferation
c	— Large nuclei
d	— Shoulder phenomenon
e	— Nuclear hyperchromasia
f	— Loss of preference for rete ridge tips
g	— Nuclear polymorphism
h	— Large cytoplasm

*Hierarchy based on discriminant analyses and Fisher exact tests. Some features not listed had a relatively high value in a single observer, e.g. bridges of melanocytes, lymphohistiocytic infiltrate, lamellar fibroplasia, dustlike melanin, elongated rete ridges.

*Table 6. Comparison of the scoring of histopathological features among eight observers using Pearson correlation coefficients**

	Correlation coefficient			
	A†		B	
Feature	Mean	Range	Mean	Range
Large nuclei	0.53	0.42–0.68	0.53	0.42–0.69
Nuclear hyperchromasia	0.45	0.10–0.59	0.46	0.10–0.59
Nuclear polymorphism	0.57	0.34–0.71	0.58	0.34–0.71
Prominent nucleoli	0.43	0.27–0.67	0.43	0.27–0.67
Large cytoplasm	0.53	0.26–0.79	0.53	0.26–0.79
% melanocytic cells with at least one cytological atypia feature	0.52	0.19–0.80	0.52	0.19–0.80
Irregular nests	0.72	0.56–0.81	0.72	0.56–0.81
Marked junctional proliferation	0.65	0.50–0.80	0.65	0.50–0.88
Dust-like melanin	0.41	0.00–0.77	0.41	0.00–0.77
Elongated rete ridges	0.53	0.31–0.78	0.53	0.31–0.78
Loss of preference for rete ridge tips	0.48	0.06–0.74	0.48	0.06–0.74
Shoulder phenomenon	0.60	0.22–0.74	0.60	0.22–0.74
Abnormal distribution of pigment	0.46	0.18–0.73	0.46	0.18–0.73
Lymphohistiocytic infiltrate	0.79	0.63–0.89	0.79	0.63–0.89
Lamellar fibroplasia	0.56	0.43–0.69	0.56	0.43–0.69
Neovascularisation	0.28	0.00–0.55	0.28	0.00–0.55

*Per observer the score (not dichotomised) of that observer was compared to the mean of the score of seven other observers; data from two observers were incomplete; per feature the mean and range of the results from eight observers is presented.
†A: All categories of Table 1 included; B: limited to categories one, two and three.

Based on the foregoing results, features were selected and Kappa values calculated for the agreement on their presence or absence in benign lesions (i.e. categories one, two and three). Best values were obtained for: irregular nests, lymphohistiocytic infiltrate, marked junctional proliferation and large nuclei. The results are shown in Table 7. The highest Kappa value was

*Table 7. Kappa values for the agreement on presence/absence of four features**

	Feature			
Observer	Irregular nests	Lympho-histiocytic infiltrate	Marked junctional proliferation	Large nuclei
a	0.60	0.38	0.36	0.40
b	0.65	0.49	0.40	0.44
c	0.52	0.40	0.46	0.28
d	0.60	0.56	0.28	0.46
e	0.38	0.50	0.47	0.37
g	0.67	0.45	0.55	0.48
h	0.54	0.56	0.31	0.19
j	0.68	0.38	0.47	0.33
mean a–j	0.58	0.47	0.41	0.37
S.D.	0.10	0.07	0.09	0.10

*Per observer the mean of Kappas of that observer compared with each of seven others is presented. Provided the own diagnosis of the two compared observers was one, two or three. Dichotomised: present vs. absent.

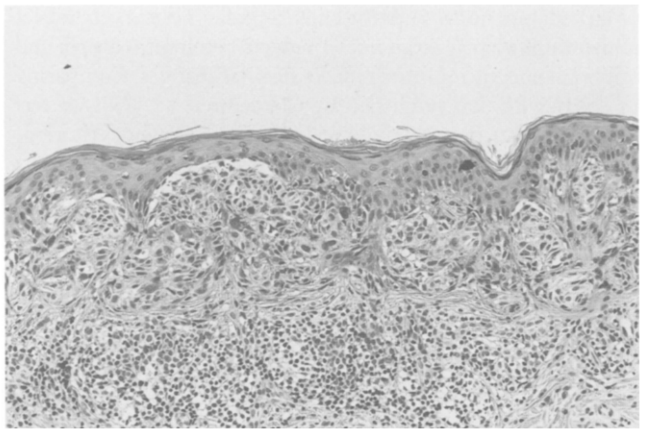

Fig. 1. Photomicrograph of a DN, showing marked junctional activity, irregular nests and lymphocytic infiltrate. At this magnification, large nuclei cannot be appreciated properly.

found for the first feature mentioned. Values were not higher when another dichotomisation then present vs. absent was used. The histopathological features are illustrated in Figs 1 and 2. Figures 3 and 4 show photomicrographs of lesions which were classified as common naevocellular naevus and melanoma *in situ*, respectively.

DISCUSSION

A first condition for a reliable diagnostic test is that the result is reproducible on a given specimen. As the scoring of multiple histopathological features in diagnosing melanocytic lesions is such a test, we investigated the reproducibility of the scoring. No clinical data were provided. The four best reproducible features were: irregular nests, lymphohistiocytic infiltrate, marked junctional proliferation and large nuclei. Although the Kappa values of these features are not very high, they are still better than those of the other features examined. The fact that, when another dichotomisation or further stratification was used, values became lower, indicates that reproducibility is best when a simple present vs. absent dichotomisation is used. Similarly, Ahmed *et al.* [16] found low Kappa values, using four levels that could be scored for junctional activity, irregular nests, dusty melanin and large melanocytic nuclei.

Given these basic values on the reproducibility of the scoring, examination of the validity of each of the four features to discriminate between DN and common naevi, showed that three of them scored the highest in the discriminant analysis. Also, their values for sensitivity, specificity, and positive and negative value were among the best. This is in agreement with the study by Steijlen *et al.* [13], who found the best efficacy for irregular

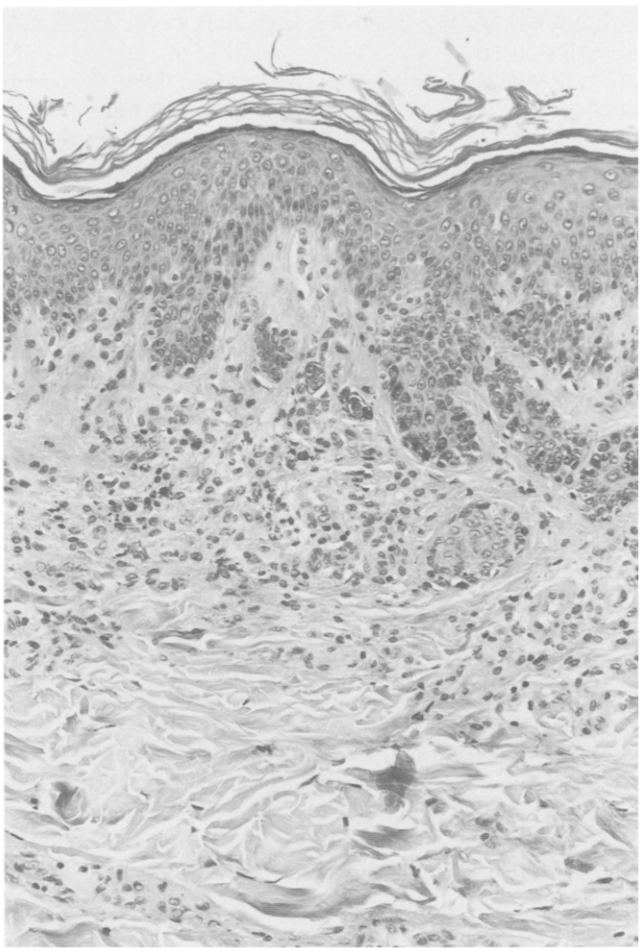

Fig. 3. Photomicrograph of a common compound naevocellular naevus, showing slight architectural abnormalities.

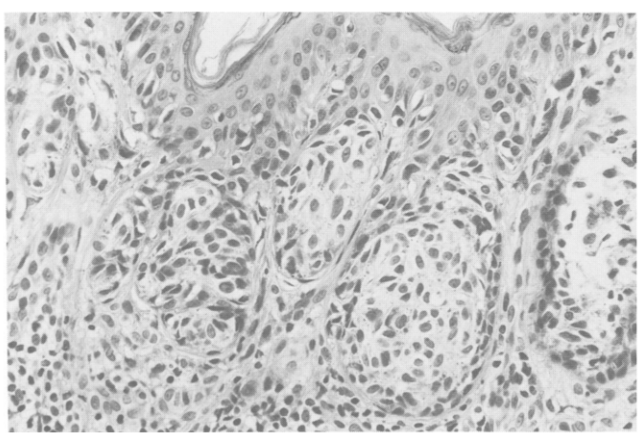

Fig. 2. Higher magnification of the lesion shown in Fig. 1. Note several melanocytic nuclei that are larger than those of adjacent keratinocytes.

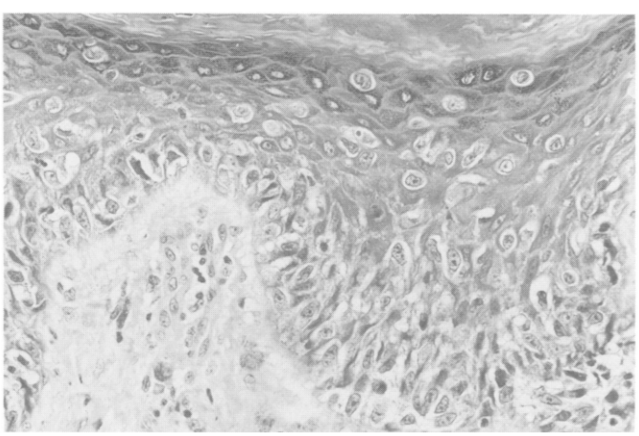

Fig. 4. Photomicrograph of a melanoma *in situ*, showing Pagetoid growth of atypical melanocytes.

nests, marked junctional activity, large melanocytic nuclei and dust-like melanin.

In 21 of the 23 lesions that in the present study were diagnosed as DN (panel diagnoses), all four best reproducible features were present, as determined by the score of the majority of the observers. However, features were missed in a number of cases by individual observers, illustrating the suboptimal reproducibility of the feature scoring. Based on these data and the fact that the discriminant analysis showed no further value in the discrimination between common naevi and DN when using more than four features, we examined the discriminative value of combinations of these features to distinguish DN from common normal naevi. Using the presence of all four features as a diagnostic prerequisite for DN, values for sensitivity, specificity, and positive and negative predictive value were 0.53, 0.93, 0.95 and 0.45, respectively. Thus, the missing of one feature by an observer would imply that DN would not be diagnosed as such. However, using the presence of at least three of the four features as a diagnostic prerequisite for DN, values for sensitivity, specificity, and positive and negative predictive values were 0.86, 0.91, 0.96 and 0.73, respectively. This implies that a very acceptable efficacy can be reached with a limited set of reproducible criteria, which has advantages in terms of learnability and applicability. For this practical reason we propose lesions be diagnosed as DN if at least three of the four features mentioned are present. In the lesions diagnosed as DN by the panel, nuclear atypia was nearly always scored as present by the individual observers. In our opinion lesions with one or two of the features should be classified as a common naevocellular naevus, while mentioning the minor abnormal features in the detailed microscopic report or note. They should not be diagnosed as a separate entity. For the 10 observers, the mean value for the correct classification with respect to the panel diagnosis in either category one and two or in category three was 88%, using the proposed approach summarised in Table 8. The morphological features mentioned are shown in Figs 1 and 2. In this approach grading of atypia in DN is not recommended since it is not reproducible. The features relevant for the differential diagnosis of DN, melanoma *in situ* and superficial spreading melanoma are also included in Table 8.

Table 8. Proposed diagnostic approach

Morphological features	Diagnosis
No or less than three of the features mentioned below for DN	Common naevocellular naevus
Three or more of the following features Marked junctional proliferation Irregular nests Large nuclei Lymphohistiocytic infiltrate	Dysplastic naevus
Pagetoid growth Continuous junctional proliferation	Melanoma *in situ*
Pagetoid growth Continuous junctional proliferation Invasion of markedly atypical melanocytes into the dermis	Superficial speading melanoma

Marked junctional activity and irregular nests both reflect architectural atypia, large nuclei reflects cytological atypia and lymphohistiocytic infiltrate reflects stromal changes. Comparing our study with that published by Clemente *et al.* [19], we feel that marked junctional activity is similar to their major criterion, basilar proliferation of atypical melanocytes (extending at least three rete ridges or "pegs" beyond any dermal naevocellular component). In addition, our "irregular nests" is comparable to their other major criterion, "organisation of the melanocytic proliferation in a lentiginous or epitheloid-cell pattern". Our feature "large melanocytic nuclei" is included in their first mentioned criterion, as the term "atypical melanocytes" is used. Of their minor criteria, i.e. "lamellar fibrosis or concentric eosinophilic fibrosis", "neovascularisation", "inflammatory response", and "fusion of rete ridges", the feature "lymphohistiocytic infiltrate" was most reproducible in our study. Clemente *et al.* [19] found good concordance of diagnosis, using a previously agreed upon set of criteria. In the present study, no set of criteria was suggested by and to the observers. Emphasis was put on the definition of the features, which were scored. The interpretation was left to the observers. Doing so, we also found a very acceptable concordance of the diagnosis for most observers.

In the present study the matter of the preferred nomenclature of DN was not addressed. The most important issue to our opinion is that dermatologists, pathologists and dermatopathologists formulate a reproducible scheme for diagnosing and reporting these naevi. We feel that studies on the efficacy and reproducibility of histopathological features for diagnosing DN are more instrumental to solve this issue than semantic discussions on the nomenclature of the lesion.

In conclusion, our study indicates that it is possible to reliably diagnose DN by histopathological examination. A reproducible set of criteria with, in theory, good discriminating value can be compiled. Our study was set up as a first step to standardise the histopathological diagnosis of DN among European centres. Further studies should reveal whether our proposed diagnostic approach can add to a consistent diagnosis of DN, among pathologists and dermatopathologist in centres, who may coordinate continuing education and quality control, and, more importantly, among those in a routine setting.

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APPENDIX I

EORTC-MMCG PATHOLOGY FORM ON THE DIAGNOSIS OF DYSPLASTIC NAEVUS

SLIDE NUMBER	:	1–2
Date (day, month, year)	:	3–8
Reader (write or stamp)	:	9–10

HISTOPATHOLOGICAL EVALUATION

General remarks:

(1) The criteria for cellular and architectural atypia ad A concern the intraepidermal (including the junctional) component only.
(2) If asked for, the proportion of atypical structures (e.g. melanocytic cells, nests) as part of the total in the lesion, is given in a percentage estimated in one of the following classes:
0% (code: 0), 1–10% (code: 1), 11–50% (code 2), 51–100% (code 3).
(3) Further definition of the criteria: see addendum.

(A) MAJOR CRITERIA

(1) Cytological atypia	
Large nuclei (percentage code)	11
Nuclear hyperchromasia (percentage code)	12
Nuclear polymorphism (percentage code)	13
Prominent nucleoli (percentage code)	14
Large cytoplasma	15
What is the estimated percentage of melanocytic cells with at least one of the cytological atypia features? (percentage code)	16
(2) Architectural atypia	
Irregular nests (percentage code)	17
Marked junctional proliferation (yes=1, no=0)	18

(B) MINOR CRITERIA

(3) Cytological change	
Dust-like melanin (percentage code)	19
(4) Architectural changes	
Elongated rete ridges (yes=1, no=0)	20
Bridges of melanocytes (maximal no. per section)	21–22
Loss of preference for rete ridge tips (yes=1, no=0)	23
Shoulder phenomenon (yes=1, no=0)	24
Abnormal distribution of pigment (yes=1, no=0)	25
Lymphohistiocytic infiltrate (see addendum)	26
Lamellar fibroplasia (yes=1, no=0)	27

Neovascularisation (yes=1, no=0) 28

Slide number : 29–30
Reader (write or stamp) : 31–32

(C) CRITERIA FOR MELANOMA (*IN SITU*)

Continuous atypical melanocytic proliferation (yes=1,no=0) 33
Pagetoid cells present? (see adendum, yes=1, no=0) 34
Ascension of atypical melanocytes reaching granular layer (mention maximal number found in one section) 35–36
Lack of maturation (yes=1, no=0) (no decrease in nuclear size of dermal melanocytic cells as compared to junctional component) 37

DIAGNOSIS (according to your own criteria) 38

Common naevocellular naevus =1
Common naevocellular naevus with minor abnormal features =2
Dysplastic naevus =3
Melanoma *in situ* =4
SSM =5
Other, please specify ..... =6

If a dysplastic naevus please indicate degree of atypia: (according to your own interpretation) 39

Not applicable =0
Slight =1
Moderate =2
Marked =3

CONTEXT OF LESION 40

Not applicable =0
Dysplastic naevus associated with common naevocellular naevus =1
Melanoma *in situ* associated with common naevocellular naevus =2
Melanoma *in situ* associated with dysplastic naevus =3
SSM associated with common naevocellular naevus =4
SSM associated with dysplastic naevus =5

If the lesion would not have been completely resected, would you consider re-excision? (yes=1, no=0) 41

COMMENTS

APPENDIX II

ADDENDUM TO THE EORTC-MMCG PATHOLOGY FORM ON THE DIAGNOSIS OF DYSPLASTIC NAEVUS

Further definition of the criteria

Large nuclei:	nuclear size ≥ nuclei of basal keratinocytes
Nuclear hyperchromasia:	nuclear density ≥ nuclei of lymphocytes
Nuclear polymorphism:	irregular size/shape/orientation
Prominent nucleoli:	nucleolar size ≥ one-third of erythrocytes
Large cytoplasma:	as compared to melanocytes in perilesional skin
Irregular nests:	irregular size/shape
Marked junctional proliferation:	number of melanocytes (either as single cells or loose aggregates) ≥ number of basal keratinocytes, along at least three adjacent rete ridges (nestlike or lentiginous)
Dust-like melanin:	finely and equally dispersed grey-brown pigment
Elongated rete ridges:	as compared to those in perilesional epidermis
Bridges of melanocytes:	rete ridges connected by bridging nests of melanocytes
Loss of preference for rete ridge tips:	arrangement of solitary units and/or nests of melanocytes at the lateral side of the rete ridges and/or over the top of dermal papillae

Shoulder phenomenon:	extension of junctional component over at least three rete ridges beyond the dermal naevocellular component
Abnormal pigment distribution:	retention of melanin throughout dermal naevocellular component
Lymphohistiocytic infiltrate:	0=no; 1=slight, perivascular; 2=moderate, aggregate-like; 3=marked, band-like
Lamellar fibroplasia:	as compared to the papillary dermis in perilesional skin
Neovascularisation:	as compared to the papillary dermis in perilesional skin
Pagetoid cells:	abnormal melanocytes with abundant pale cytoplasma containing dusty melanin

APPENDIX III

The observer agreement for categorical data*

P_o represents the observed proportion of agreement between two observers (the observed proportion of subjects with equal diagnosis for the two observers).

K represents the proportional excess of agreement beyond what is to be expected under independence. The coefficient Kappa is defined by

$$K = \frac{o - e}{1 - e}. \quad (o = \text{observed proportion of agreement}, e = \text{expected proportion of agreement})$$

*Cohen, J. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement* 1960, **20**, 37–46.

Quality of Kappa†

≤ 0.20 Poor/slight
0.21 – 0.40 Fair
0.41 – 0.60 Moderate
0.61 – 0.80 Substantial
0.81 – 0.99 Almost perfect
1.00 Perfect

†Landis J.R. and Koch G.G. The measurement of observer agreement for categorial data. *Biometrics* 1977, **33**, 159–174.

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Lipid-bound Sialic Acid, Prostaglandin E and Histamine in Head and Neck Cancer

Ivica Klapan, Vladimir Katić, Filip Čulo, Domagoj Sabolović, Višeslav Ćuk, Ksenija Fumić and Stjepan Simović

Blood concentration of lipid-bound sialic acid (LBSA), prostaglandin E (PGE) and histamine were determined in 37 patients with carcinoma of hypopharynx and larynx (supraglottic and glottic), in 12 non-cancer patients and in 10 healthy subjects. The concentration of LBSA was significantly increased in 94.4% cancer patients preoperatively and fell to somewhat lower levels within 1 month after tumour resection. In patients with complete tumour resection and no tumour recurrences within 2 years, it steadily decreased thereafter, reaching normal levels within 6–24 months after surgery, whereas in patients with tumour recurrences or incomplete tumour resection it rose again within 6 months after tumour resection. Similarly, the concentration of PGE was significantly increased in about two thirds of cancer patients (67.6%) preoperatively, dropped significantly within 1 month after tumour resection and rose again in patients with tumour recurrences. Preoperative histamine concentration was decreased in 24.3% of cancer patients and postoperatively it rose both in patients with or without tumour recurrences.
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INTRODUCTION

MANY PROGNOSTIC factors have been identified in cancer [1, 2]. Recent investigations have shown that concentrations of serum lipid-bound sialic acid (LBSA) [3–5], blood histamine [6] and serum level or tissue content of prostaglandin E (PGE) [7, 8] can serve as markers for the detection of tumour spread and progression.

Increased serum LBSA [5, 9] and PGE plasma [10] levels have been observed in head and neck carcinoma and both have been reported to correlate with the presence of the disease. Blood