Histologic subtype of treatment failures after noninvasive therapy for superficial basal cell carcinoma: An observational study

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Background: There have been concerns that recurrences after noninvasive therapy for basal cell carcinoma (BCC) transform into a "more aggressive" histologic subtype.

Objective: We sought to evaluate the proportion of patients with a nonsuperficial treatment failure after noninvasive therapy for superficial BCC.

Methods: An observational study was performed using data from a single blind, noninferiority, randomized controlled trial (March 2008-August 2010) with 5-year follow-up in patients with primary superficial BCC treated with methylaminolevulinate–photodynamic therapy, 5-fluorouracil, or imiquimod. Data were used from 166 adults with a histologically confirmed treatment failure.

Results: A nonsuperficial subtype was found in 64 of 166 treatment failures (38.6%). Proportions with a more aggressive subtype than the primary tumor were 51.3% (38/74) for early and 28.3% (26/92) for later treatment failures (P = .003). The proportion of more aggressive early failures was significantly lower after imiquimod (26.3%) compared with methylaminolevulinate–photodynamic therapy (54.8%, P = .086) and 5-fluorouracil (66.7%, P = .011).

Limitations: There was limited information on the exact time of occurrence of treatment failures.

Conclusion: More aggressive treatment failure recurrences after noninvasive therapy for superficial BCC occur most often within the first 3 months posttreatment, probably indicating underdiagnosis of more aggressive components in the primary tumor rather than transformation. (J Am Acad Dermatol 2019;80:1022-8.)

Key words: 5-fluorouracil; basal cell carcinoma; histologic subtype; imiquimod; MAL-PDT; misclassification; noninvasive therapy; sampling error; superficial; transformation.

The criterion standard treatment for all basal cell carcinoma (BCC) subtypes is surgical excision, but superficial BCC is increasingly treated in a noninvasive manner.[1-3] Frequently used noninvasive alternatives for surgery are 5-fluorouracil (5FU) cream, imiquimod cream, and photodynamic therapy (PDT).[4,5]

Although cure rates of noninvasive modalities are lower compared with surgical excision, there are esthetic and practical advantages to noninvasive

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therapies.[2,6-8] A disadvantage is that before noninvasive treatment, final histologic diagnosis of BCC subtype has to be based solely on a punch biopsy specimen and cannot be based on the histologic examination of the complete tumor.

Recent literature describes a transformation of primary nonaggressive BCC into "more aggressive" recurrent subtypes (eg, primary superficial BCCs were of a nodular, infiltrative, or morpheaform BCC [mBCC] subtype on recurrence) after topical therapy or PDT.[9-12] This observation resulted in the hypothesis that there is a causal relationship between noninvasive therapy and transformation.[11]

However, other authors propose that discrepancies between histologic subtype of the primary tumor versus treatment failures might not be the result of transformation but of sampling error of punch biopsy specimens, resulting in underdiagnosis and consequently undertreatment.[2,13,14] Their hypothesis is that in the standard histologic examination of a punch biopsy specimen, part of the BCC that lies deeper is not always found and that these cells, because of their deeper localization, are insensitive to the effect of noninvasive therapy.[9] Some authors question the reliability of the standard histologic examination of a punch biopsy specimen.[13,15]

Until now, most studies conducted on this subject had small sample sizes, and a direct comparison between different noninvasive treatments; distinguishing between early treatment failures and later during follow-up has not been made.

This observational study used data from a multicenter randomized controlled trial (RCT) comparing methylaminolevulinate-PDT (MAL-PDT), imiquimod, and 5FU for the treatment of superficial BCC. We analyzed the histologic subtypes of the treatment failures (3 months posttreatment and later during follow-up) within the treatment groups. The primary objective was to compare the frequency of nonsuperficial subtypes between early and later occurring treatment failures. In addition, we evaluated whether the proportion of treatment failures with nonsuperficial subtype differs between treatment groups and is related to the location of the BCC.

CAPSULE SUMMARY

- Treatment failures after noninvasive therapy for superficial basal cell carcinoma are frequently of a nonsuperficial subtype.
- These nonsuperficial treatment failures probably result from underdiagnosed aggressive components in primary tumors, not from transformation.
- Nonsuperficial treatment failures were less frequently observed after imiquimod therapy than after treatment with methylaminolevulinate–photodynamic therapy or 5-fluorouracil and were more often located in the head and neck area. Close follow-up is necessary.

METHODS

Patients and procedure

Data were used from an RCT including patients with a histologically proven superficial BCC who were treated with noninvasive treatment. All were diagnosed after obtaining a punch biopsy specimen. In this trial, 601 patients were randomly assigned to either treatment with MAL-PDT (2 sessions with a 1-week interval), imiquimod 5% cream (once daily, 5 times/week for 6 weeks), or 5FU 5% cream (twice daily for 4 weeks). For a more extensive description of the study design and execution, see Arits et al[7] and Jansen et al.[16] Follow-up with inspection of the primary lesion site took place at 3, 12, 36, and 60 months by an independent observer who was blinded to treatment allocation.[7,16,17] Patients were included in the present study if they had a histologically proven early (<3 months posttreatment) or later treatment failure (>3 months posttreatment). The institutional review board of the Maastricht University Medical Center+, in the Netherlands, approved the study protocol.

We collected pathology reports of punch biopsy specimens from patients' electronic files, and, if available, excision material of the treatment failures. Histologic diagnoses of early and later treatment failures were categorized into 2 subgroups: superficial or nonsuperficial subtype (Fig 1). **[F1-4/C]** Tumors were classified as nonsuperficial or more aggressive if either the biopsy specimen or the excision material contained a nodular, infiltrative, micronodular, basosquamous, or morpheaform element. In some cases, treatment failures were not surgically treated. In these cases, the punch biopsy specimen material was used to determine the subtype of the treatment failure.

Statistical analysis

For comparison of the distribution of baseline characteristics between treatment groups, the chi square test was used for categorical variables and analyses of variance and the Kruskal–Wallis test was used for continuous variables. The primary outcome measure was the proportion of treatment failures with a more aggressive subtype. Proportions were

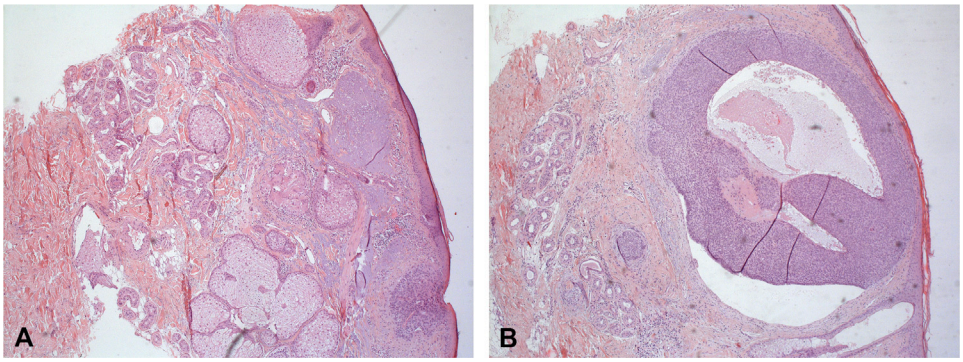

Fig 1. A, Punch biopsy specimen obtained from a primary superficial basal cell carcinoma before treatment with 5-fluorouracil cream. **B**, Punch biopsy specimen obtained from a patient with treatment failure (nodular subtype) of the noninvasively treated superficial basal cell carcinoma shown in *A*.

compared between early and later treatment failures and between treatment groups. Differences in proportions were tested for statistical significance using the Fisher exact or chi square tests. The assumption underlying this analysis was that a more aggressive subtype in early treatment failures was more likely to result from sampling error than in a tumor occurring later during follow-up. For the comparison between treatment groups, binary multivariable logistic regression analysis was used to adjust for differences in baseline characteristics between treatment groups. Odds ratios with 95% confidence intervals (CIs) were calculated to quantify the odds of an aggressive subtype after noninvasive treatment by MAL-PDT or 5FU compared with imiquimod (reference category).

$P \leq .05$ was considered statistically significant. Data analyses were performed with SPSS software (version 23.0; IBM Corp, Armonk, NY) and openepi.com.

RESULTS

During the 5-year follow-up period, 171 of 601 (28.5%) patients developed an early or later treatment failure, of which 5 were not histologically confirmed.[16] Therefore, 166 patients were available for analysis. A total of 140 treatment failures were therapeutically excised after confirmation of treatment failure by examining the punch biopsy specimen. For 26 tumors, only a punch biopsy specimen was available; these were treated with either PDT, 5FU, imiquimod, or radiotherapy. The subtype of the tumor was defined as the most aggressive subtype found in either the punch biopsy specimen or the excision specimen.

For 166 patients with treatment failure, the distribution of patient and tumor characteristics according to treatment allocation are shown in Table I. For the overall 5-year tumor-free survival rate, we refer to the article by Jansen et al.[18]

Proportions with nonsuperficial subtype according to timing of treatment failure

Table II shows the proportions of patients with nonsuperficial subtype according to treatment group for early and later treatment failures. A more aggressive histologic subtype was found in 64 of 166 (38.6%) treatment failures, which is 10.6% (64/601) of the initially treated tumors. Nonsuperficial subtypes were less frequently diagnosed in later treatment failures when compared with early treatment failures; 38 of 74 (51.3%) early and 26 of 92 (28.3%) later treatment failures were of a nonsuperficial subtype ($P = .003$; Table II). This decrease was statistically significant for MAL-PDT ($P = .010$) and for 5FU ($P = .038$) but not for imiquimod ($P = .567$).

Proportions with nonsuperficial subtype according to treatment group

The proportion of early treatment failures with a nonsuperficial subtype was lowest in the imiquimod group (26.3%; Table II). Proportions were higher in the MAL-PDT (54.8%) and 5FU groups (66.7%). When compared with the imiquimod group as the reference category, differences were significant for 5FU ($P = .011$) but not for MAL-PDT ($P = .086$). The difference between MAL-PDT and 5FU was nonsignificant ($P = .39$). Proportions of later treatment failures with a more aggressive subtype were 24.4% (10/41) for MAL-PDT, 38.2% (13/34) for 5FU, and 17.6% (3/17) for imiquimod. Between-group differences were nonsignificant when compared with the imiquimod group as the reference category ($P = .610$ for MAL-PDT and $P = .150$ for 5FU).

Table I. Baseline patient and tumor characteristics in a population of 166 adults with early or later treatment failure of basal cell carcinoma

Characteristics	MAL-PDT, n = 72	Imiquimod, n = 36	5-fluorouracil, n = 58	Total, N = 166	*P* value
Men, n (%)	35 (48.6)	26 (72.2)	26 (44.8)	87 (52.4)	.024*
Women, n (%)	37 (51.4)	10 (27.8)	32 (55.2)	79 (47.6)	
Mean age, y (SD)	60.1 (12.9)	61.7 (11.3)	59.9 (11.6)	60.3 (12.1)	.762†
Tumor location, n (%)					.012*
Head/neck	12 (16.7)	4 (11.1)	11 (19.0)	27 (16.3)	
Trunk	48 (66.7)	15 (41.7)	33 (56.9)	96 (57.8)	
Upper extremities	8 (11.1)	5 (13.9)	5 (8.6)	18 (10.8)	
Lower extremities	4 (5.6)	12 (33.3)	9 (15.5)	25 (15.1)	
Median tumor size, mm^2 (range)	59.7 (4.71-1177.5)	50.2 (7.06-942)	62.8 (11.78-942)	56.5 (4.71-1177.5)	.189‡

MAL-PDT, Methylaminolevulinate—photodynamic therapy.
*Statistical significance ($\alpha \leq 0.05$) tested using group differences with the chi square test.
†Statistical significance ($\alpha \leq 0.05$) tested using group differences with the 1-way analysis of variance test.
‡Statistical significance ($\alpha \leq 0.05$) tested using group differences with the Kruskal—Wallis test.

Binary multivariable logistic regression analysis was used to correct for differences in baseline characteristics between treatment groups. The unadjusted odds ratio for a treatment failure with a more aggressive subtype, using imiquimod as the reference group, was 2.1 (95% CI 0.81-5.27) for MAL-PDT and 3.5 (95% CI 1.37-8.95 CI) for 5FU. The adjusted odds ratio from a multivariable regression model including treatment, age, sex, tumor size, and tumor location as independent variables was 1.5 (95% CI 0.53-4.30) for MAL-PDT and 2.9 (95% CI 1.03-8.46) for 5FU.

Proportions with nonsuperficial subtype according to tumor location

An additional analysis was performed for location—head or neck versus trunk or extremities. Nonsuperficial subtypes in both early and later treatment failures were more frequently observed in the head and neck area compared with the extremities and trunk. In the head and neck area, 12 of 12 (100%) early treatment failures were of a nonsuperficial subtype (1 was treated with imiquimod, 5 with 5FU, and 6 with MAL-PDT) compared with 26 of 62 (41.9%) tumors on the extremities and trunk ($P < .01$). For later treatment failures, more aggressive subtypes were observed in 9 of 15 (60%) tumors in the head and neck area versus 17 of 77 (22.1%, $P = .006$) on the extremities or trunk.

DISCUSSION

Our data show that 38.6% of treatment failures after noninvasive therapy for superficial BCC were of a nonsuperficial histologic subtype. The more aggressive subtypes are more frequently observed in early (51.3%) than in later treatment failures (28.3%). The risk of a more aggressive subtype is especially high for treatment failures in the head and neck area when compared with the extremities or trunk. Early treatment failures of a more aggressive subtype after imiquimod treatment are significantly less often observed than after other topical treatments.

A relationship between treatment with 5FU and the development of mBCC was suggested by Xiong et al,[11] who reported on 1131 veterans who had ≥2 previous keratinocyte carcinomas before study enrollment and who developed 50 mBCCs on the ears or face during a median follow-up of 3.6 years. Using logistic regression analysis, the most important risk factor for developing mBCC in comparison with a group that developed non-mBCC was a history of ever using 5FU (odds ratio 2.49; $P = .004$).[11] However, it is not clear whether 5FU was used at the same location of the new mBCC or at another location for other indications, such as actinic keratosis.

Other studies also found high proportions (62.5-87.5%) of more aggressive treatment failures in patients with initial nonaggressive BCCs in the head and neck area who were treated with PDT and imiquimod.[9,10,12]

These 4 studies that included only facial BCCs all suggested a transformation of nonaggressive to aggressive subtype BCC after treatment with 5FU, imiquimod, or PDT.[9-12] Our data show a similar high proportion of more aggressive treatment failures after noninvasive therapy in the head and neck area. However, we argue that the high proportion of nonsuperficial treatment failures is probably a result of misclassification and less likely caused by transformation.

This alternative explanation was proposed by other authors who argued that histologic subtype in the biopsy specimen of a primary lesion is often

Table II. Proportions of aggressive histologic subtypes of basal cell carcinoma, early and later treatment failures: Comparison between treatment groups

	Early treatment failure, % (N/total)	Later treatment failure, % (N/total)	*P* value
All, N = 166	51.3 (38/74)	28.3 (26/92)	.003
MAL-PDT, n = 72	54.8 (17/31)	24.4 (10/41)	.01
5-Fluorouracil, n = 58	66.7 (16/24)	38.2 (13/34)	.038
Imiquimod, n = 36	26.3 (5/19)	17.6 (3/17)	.567

Proportions of aggressive histologic subtype per treatment group for early (3 months follow-up) and later treatment failure of basal cell carcinoma (12, 36, and 60 months' follow-up) in % (N/total). Statistical significance was tested with the chi square test, with $\alpha \leq 0.05$.
MAL-PDT, Methylaminolevulinate–photodynamic therapy.

misclassified and underdiagnosed, leading to undertreatment with noninvasive therapy.[2,13,14] The risk of misclassification may be especially high in the head and neck area, because the prevalence of superficial BCC is lower in that location than in other areas and mixed subtypes occur more frequently.[19-21] One should therefore be aware that a BCC in the head and neck area that is treated noninvasively should be subjected to close follow-up.

The finding in our study that proportions of treatment failures with a nonsuperficial subtype were significantly higher in early than in later treatment failures (51.3% vs 28.3%) also supports the hypothesis of misclassification. We speculate that if a superficial BCC transforms into a more aggressive subtype, this would occur after a long period of time because BCCs grow slowly and would therefore transform slowly. Consequently, the proportion of more aggressive subtypes would increase over time. In our study, an opposite trend was observed. Nevertheless, the hypothesis of Xiong et al[11] that there was a causal relationship between treatment with 5FU and the development of mBCC cannot be completely refuted by our study. After 5FU treatment, more aggressive subtypes were still observed in 38.2% of later treatment failures. However, it is also possible that nonsuperficial treatment failures reappearing later during follow-up represent slow-growing, more aggressive components that were already present but underdiagnosed before treatment.[22] Recurrences with a more aggressive subtype than the primary tumor were also observed after surgical treatment for any subtype BCC; proportions of these nonsuperficial treatment failures ranged from 16.7% to 51%.[23-25] Treatment failures with a more aggressive subtype are therefore not only observed after noninvasive treatment. More aggressive recurrences are also described by van Loo et al,[26] who reported the occurrence of more aggressive treatment failures >5 years after surgical excision or Mohs' micrographic surgery, which supports the notion of slow-growing aggressive components.[26]

In our study, we also found that after imiquimod treatment, nonsuperficial treatment failures were found less often than after treatment with MAL-PDT or 5FU. There was also a smaller difference between early and later treatment failures in proportions with nonsuperficial subtypes in the imiquimod group. The effectiveness of imiquimod in targeting more aggressive components in BCCs that have been inaccurately diagnosed as superficial BCCs may explain why histologic subtype changes were found less often. Imiquimod has shown promising therapeutic effects on nodular and infiltrative BCCs, even though imiquimod is solely approved by the US Food and Drug Administration for the treatment of superficial BCC.[27-31]

Punch biopsy specimens have the advantage of representing the depth of the specimen compared with an increased width in the case of a shave biopsy specimen. One should, however, bear in mind that, by definition, a punch biopsy specimen represents a small part of a lesion.[32,33] Therefore, the misclassification of BCCs leading to underdiagnosis and undertreatment might be a result of sampling error of the biopsy specimen.[33-35] Several studies have analyzed the discordance rate between subtype based on punch biopsy specimen versus subtype based on excision material of the same BCC. These studies concluded that in 11.2% to 18.4% of the cases, a more aggressive subtype was missed if subtyping was based solely on biopsy material.[32,33,35,36] In our study, 64 treatment failures were of a nonsuperficial subtype, constituting 10.6% (64/601) of the entire population of the initial RCT. This proportion does not exceed the proportion of misclassification that can occur because of sampling error. This finding supports the idea that misclassification rather than transformation explains the occurrence of treatment failures with a more aggressive subtype.

For the optimization of BCC subtyping using material from a punch biopsy specimen, deeper

biopsy specimens or mapping (ie, obtaining multiple biopsy specimens) could be used.[13,37] Furthermore, promising new noninvasive diagnostic modalities, such as optical coherence tomography and reflectance confocal microscopy, may prevent sampling error in the future and could possibly be used to study the transformation of BCCs.[38,39]

Limitations

We reviewed pathology reports and not the original slides. It may be possible that pathologists were more alert to the presence of aggressive components because of the pathology request specifying the lesion as possible treatment failure. Such information bias could be responsible for improved identification of aggressive subtypes that remained unnoticed during diagnosis of the primary BCC.

For the distinction between early and later treatment failures, an arbitrary cut-off point of 3 months was chosen. It cannot be ruled out that possible aggressive components in primary BCCs take >3 months to become visible. Follow-up visits in the initial RCT took place at 3, 12, 36, and 60 months. Therefore, we cannot provide additional information on the exact timing of treatment failures with a nonsuperficial subtype during follow-up.

In conclusion, the highest frequency of treatment failures with a more aggressive histologic subtype was observed ≤3 months posttreatment, and therefore it seems likely that these tumors have been underdiagnosed before treatment, either because of a sampling error of the punch biopsy specimen or slow-growing aggressive components that become manifest during follow-up. The risk of misclassification of subtype may be especially high for BCCs in the head and neck area when compared with the extremities or trunk. More aggressive early and later treatment failures were observed less frequently after imiquimod treatment compared with 5FU and MAL-PDT, suggesting that part of the (unrecognized) nodular or infiltrative BCCs may respond to imiquimod.

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The initial trial was registered as an International Standard Randomized Controlled Trial (ISRCTN 79701845). Drs van Delft and Nelemans had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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