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Morphometric Image Analysis as a Tool in the Diagnosis of Transected Squamous Neoplasms

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Introduction

Superficial skin biopsies are common in dermatopathology specimens and can pose a diagnostic challenge especially in cosmetic sensitive areas such as the face. When broadly transected so that the base of the lesion is not visualized, well-differentiated squamous cell carcinomas (SCC), hypertrophic actinic keratoses (HAK), irritated seborrheic keratoses (ISK), and verruca vulgaris (VV) can look quite similar on light microscopic examination. When we encounter such a biopsy in which atypia is not visualized in the upper half and thus a benign lesion is favored but a premalignant or malignant squamous neoplasms cannot be excluded, we sign out these cases as squamous acanthomas (SA) transected cannot rule out malignancy with a note recommending clinical follow up and /or repeat deeper biopsy. This diagnosis is obviously frustrating to all parties involved and we seek ways to be more unequivocal with our recommendation. In our study, we investigate the use of morphometric quantitative image analysis (IA) as a tool to aide in the diagnosis of transected squamous neoplasms. IA involves using computer software to objectively measure histologic image characteristics. The exact software and technical approaches may vary from study to study, but in the end, objective measurements are made. Studies have investigated prognostic implications of IA in various carcinomas such as colon, renal cell, bladder, ovarian, and breast among others [1-11], and other studies have looked at the diagnostic applications of IA [12-22]. In addition, correlation of IA measurements to genetic molecular alterations has been explored [13,23-26]. We used IA to evaluate specific analytical variables in diagnostically clear neoplasms including the mean and median nuclear sizes (NS), standard deviation of nuclear sizes as a correlate of nuclear pleomorphism, and cellularity. We then used the information obtained from known neoplasms to construct IA diagnostic ranges that can be used to categorize histologically challenging transected neoplasms on superficial skin biopsies as either being benign (ISK and VV) or pre-malignant/malignant (HAK and SCC).

Materials and Methods

60 diagnosed cases of ISK, VV, HAK, and SCC (15 cases of each neoplasm) were retrieved from our archives. Also, 10 cases of transected SA cannot rule out malignancy were retrieved.

Using a Nikon camera (Nikon DS-Fi1) attached to an Olympus microscope (BX40), representative images of the neoplasms (H&E slides) were taken at 20x magnification in .tiff format at file size of 3.7 megabytes. Adobe Photoshop software (version 11.0) and Image J software (version 1.46r) were used to analyze the histologic image characteristics of the neoplasms.

For the 60 cases of ISK, VV, HAK, and SCC, a representative area of the stratum spinosum comprising 210,000 pixels was selected. The stratum basale was excluded as it would not be present on transected SAs

When analyzing the transected SAs, the largest possible area of the stratum spinosum was selected with areas ranging from 148,000 to 383,000 pixels.

Mean and median nuclear sizes (NS), pleomorphism (measured by the standard deviation), and cellularity were determined on the known neoplasms. Standard deviation of the NS can be performed in morphometric studies to measure the degree of nuclear pleomorphism [10,12,23,24,27,28]. Cellularity measurements were based on the number of cells per 210,000 pixels. For the transected SAs, the cellularity measurements were adjusted mathematically to the predicted number per 210,000 pixels.

Using IA attributes of the known neoplasms, diagnostic ranges were created. The IA attributes of the transected neo-

plasms were then placed in these ranges to categorize them as being either benign (ISK and VV) or pre-malignant/malignant (HAK and SCC). Chart review and clinical follow-up information was obtained on the transected neoplasms to confirm our diagnostic categorizations. Statistical analysis was carried out using Graphpad (GraphPad Prism version 5.04).

Figure 1: Example of Image Anaysis performed on a Known Neoplasm
1a: H&E image of a known hypertrophic actinic keratosis neoplasm (original magnification x 20).
1b: A representative area of stratum spinosum is selected comprising 210,000 pixels.
1c: Nuclei are selected out.
1d: Image analysis carried out using Image J software.

Results

There were statistically significant differences between the benign (ISK and VV) and the pre-malignant/malignant neoplasms (HAK and SCC) when analyzing NS and cellularity. Moving from ISK, VV, HAK, and to SCC, there was a progressive increase in the NS as well as in the pleomorphism (Table 1). With cellularity, ISK had the highest cellularity, VV had a lower cellularity, and HAK/SCC had the lowest cellularities.

Neoplasm	Mean NS[5]	Median NS	Pleomorphism[6]	Cellularity
ISK[1]	117	110	57	398
VV[2]	216	201	92	127
HAK[3]	223	210	111	83
SCC[4]	231	218	126	66

1: Irritated seborrheic keratosis
2: Verruca vulgaris
3: Hypertrophic actinic keratosis
4: Squamous cell carcinoma
5: Nuclear Size
6: Pleomorphism measured via the standard deviation of the nuclear size
Table 1: Image Analysis Characteristics of the Known Neoplasms

Diagnostic ranges were created using the IA measurements of the known neoplasms (Table 2). Using this table, the unknown transected neoplasms were classified as either benign or pre-malignant/malignant [Tables 3a-3c]. Some of the transected neoplasms could not be reliably classified due to the overlap of some diagnostic ranges, and were classified as indeterminate in these instances. These classifications were clinically correlated.

Neoplasm	NS[5] Range[6]	Pleomorphism Range[7]	Cellularity Range[8]
ISK[1]	61-173	49-65	200-596
VV[2]	124-307	74-110	52-202
HAK[3]	112-334	89-133	50-116
SCC[4]	105-357	95-157	32-100

1: Irritated seborrheic keratosis
2: Verruca vulgaris
3: Hypertrophic actinic keratosis
4: Squamous cell carcinoma
5: Nuclear Size
6: Created by taking the NS +/- 1 standard deviation
7: Created by taking the NS standard deviation +/- 1 standard deviation
8: Created by taking the Cellularity +/- 1 standard deviation
Table 2: Diagnostic Ranges

Case	Mean NS[1]	Possibilities	IA Diagnosis	Clinical Correlation
Case 1	115	ISK[2]	Benign	Benign
Case 2	297	VV[3], HAK[4], SCC[5]	Indeterminate *	Benign
Case 3	536	SCC	Malignant	Malignant
Case 4	209	VV, HAK, SCC	Indeterminate	Benign
Case5	300	VV, HAK, SCC	Indeterminate	Benign
Case 6	267	VV, HAK, SCC	Indeterminate	Malignant
Case 7	342	SCC	Malignant	Malignant
Case 8	355	SCC	Malignant	Benign
Case 9	197	VV, HAK, SCC	Indeterminate	Benign
Case 10	412	SCC^	Malignant	Malignant

1: Nuclear Size
2: Irritated seborrheic keratosis
3: Verruca vulgaris
4: Hypertrophic actinic keratosis
5: Squamous cell carcinoma
*: When cases could not be reliably classified as Benign or Pre-malignant/

Malignant, they were classified as Indeterminate.
^: In instances where the NS exceeded the highest value of the NS diagnostic ranges, the IA diagnosis rendered was that of SCC.
Blue: Designates an IA diagnosis that correctly correlates with the clinical course.
Red: Designates an IA diagnosis that incorrectly correlates with the clinical course.
Table 3a: Diagnosing Transected Neoplasms using the Nuclear Size Diagnostic Range

Case	Pleomorpism	Possibilities	Plemorphism Diagnosis	Clinical Correlation
Case 1	42	ISK[1*]	Benign	Benign
Case 2	83	VV[2]	Benign	Benign
Case 3	191	SCC[3]	Malignant	Malignant
Case 4	50	ISK[4]	Benign	Benign
Case5	94	VV, HAK	Indeterminate	Benign
Case 6	108	VV, HAK, SCC	Indeterminate	Malignant
Case 7	125	HAK, SCC	Malignant	Malignant
Case 8	65	ISK	Benign	Benign
Case 9	82	VV	Benign	Benign
Case 10	144	SCC	Malignant	Malignant

1: Irritated seborrheic keratosis
2: Verruca vulgaris
3: Hypertrophic actinic keratosis
4: Squamous cell carcinoma
*: In instances where the pleomorphism fell below the lowest value of the Pleomorphism diagnostic ranges, the IA diagnosis rendered was that of ISK.
Table 3b: Diagnosing Transected Neoplasms using the Pleomorphism Diagnostic Range

Case	Cellularity	Possibilities	Cellularity Diagnosis	Clinical
Case 1	215	ISK[1]	Benign	Benign
Case 2	42	SCC	Malignant	Benign
Case 3	36	SCC	Malignant	Malignant
Case 4	93	VV[2], HAK[3], SCC[4]	Indeterminate	Benign
Case5	86	VV, HAK, SCC	Indeterminate	Benign
Case 6	108	VV	Benign	Malignant
Case 7	71	VV, HAK, SCC	Indeterminate	Malignant
Case 8	69	VV, HAK, SCC	Indeterminate	Benign
Case 9	216	ISK	Benign	Benign
Case 10	63	VV, HAK, SCC	Indeterminate	Malignant

1: Irritated seborrheic keratosis
2: Verruca vulgaris
3: Hypertrophic actinic keratosis
4: Squamous cell carcinoma
Table 3c: Diagnosing Transected Neoplasms using the Cellularity Diagnostic Range

The pleomorphism range turned out to be the most diagnostically useful (Table 3b). 5/5 categorized benign lesions were clinically benign. 3/3 categorized pre-malignant/malignant neoplasms were clinically pre-malignant/malignant. 2 lesions could not be categorized and deemed indeterminate. One of these was clinically benign and the other pre-malignant/malignant. As a general rule, cases that had a pleomorphism <89 could be correctly classified as benign, and cases that had pleomorphism >110 could be correctly classified as pre-malignant/malignant.

When placing the IA attributes of the transected neoplasms into the NS ranges and cellularity ranges, a significant number of the cases were indeterminate and some were misclassified (Tables 3a,3c). Using the NS range, 4 cases were correctly classified, but one case was misclassified. Using the cellularity range, 3 cases were correctly classified, but 2 cases were misclassified.

Discussion

Using IA, we discovered that the most reliable range to distinguish the benign neoplasms from the malignant ones was the pleomorphism range. While the NS and cellularity of the known benign and pre-malignant/malignant neoplasms were significantly different from one another, the diagnostic ranges created were not useful to reliably distinguish the transected benign neoplasms from the pre-malignant/malignant ones.

The pleomorphism range is the also most useful range of the three we investigated because it is independent of any specific technical or methodological approach. We used certain software (Image J and Photoshop) and took the image at a certain resolution (3.7 megabytes) prior to analysis. If another study used different software or took the image at a higher or lower resolution, the NS and cellularity measurements could easily be different than ours. This lack of standardization among IA studies is a problem that needs to be addressed [17,29]. However, as the pleomorphism range is resistant to methodological variation, it has the best clinical utility and can be easily comparable to other potential IA studies.

As technology advances, pathology will become increasingly digitally based. It is foreseeable that one day the microscope will be abandoned in favor of digital computer images. As this happens, morphometric IA measurements will become easier to perform [1,29,30] and will play a greater role as an aid in diagnosis. While some may advance the idea that IA could potentially replace the pathologist and make a diagnosis solely based on morphometric measurements, this is highly unlikely. As pathologists, we make numerous 'measurements' that are not easily quantifiable and measured by IA. Also, much of what we do is informed by our acquired medical knowledge and clinico-pathological correlation [20,30]. Still, IA can play an important role as a tool in diagnosis analogous to the role of immunohistochemistry, especially in histologically challenging cases [8] such as ours. IA helps to decrease subjectivity and helps increase inter-observer agreement [1,3,4,6,12,17,23].

One of main drawbacks to IA analysis in our study is the time spent to perform the analysis. [1,29,30]. For a given case, the average time for analysis was approximately 30-45 minutes which included the time needed to capture the image, manipulate it to ready it for analysis, and then perform the analysis. While we may have been hindered by our technical prowess, faster methods to select cells and perform the analysis would make IA more clinically applicable. As digital pathology advances, the speed will surely increase and clinical studies such as ours will help form the basis of the diagnostic ranges needed for accurate diagnosis.

In summary, in our study we found statistically significant differences between the IA attributes of benign versus pre-malignant neoplasms. Using the table of diagnostic ranges and excluding indeterminate cases, the unknown transected neoplasms were correctly classified benign or pre-malignant/malignant 80%, 60%, and 100% of the time respectively based on NS, cellularity, and pleomorphism ranges. The pleomorphism diagnostic range was the most useful and reliable. As a general rule, cases that had a pleomorphism <89 could be correctly classified as benign, and cases that had pleomorphism >110 could be correctly classified as pre-malignant/malignant. The pleomorphism range accurately categorized ambiguous transected squamous neoplasms as being either benign or pre-malignant in 8/10 cases. As such, the SD range can help pathologists diagnose otherwise ambiguous transected neoplasms and assist the pathologist in creating a more decisive treatment recommendation for the patient.

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