

The use of immunohistochemistry in the evaluation of the nail matrix in biopsies of ingrown toenails

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Abstract

The success of surgical approaches to ingrown toenails depends on the extraction (either partial or total) of the nail matrix. The identification of the nail matrix in specimens taken from ingrown toenails is not always easy because of the fragmentation of the biopsies, difficulties in matrix orientations and the heavy inflammatory infiltrate. In biopsies taken from polydactyly surgeries, the matrix shows a peculiar pattern of expression of the CD10 and CD34 markers that differs from the one shown by the lateral nail fold. We investigated whether such a pattern was also found in biopsies from ingrown toenails, which can be greatly distorted through inflammation and fibrosis. We examined 15 biopsies from cases of ingrown toenails at different clinical stages. We performed routine Hematoxylin–Eosin studies, as well as immunohistochemical studies with CD10, CD34, HMB-45 and Melan-A. The morphologic changes in all cases were typical of those found in ingrown toenails and their intensities correlated with the clinical stages. Matrical keratinization was identified in all of the biopsies. Morphologic features that are compatible with the lateral nail fold were also seen in seven of the 15 biopsies. In five cases, an intermediate area of transition between matrix and lateral nail folds was heavily distorted by inflammatory changes. Melanocytic markers showed scattered intra-epidermal cells in all but one case. HMB-45 and Melan-A were equally good in demonstrating the melanocytic population. We concluded that the expression of CD10 and CD34 in cases of ingrown toenails is preserved and it follows the pattern described in nails from polydactyly. Therefore, both markers can be useful in fragmented specimens taken from surgeries for ingrown toenails, in order to confirm the removal of the nail matrix.

Keywords: ingrown toenail, CD10, CD34, onychofibroblast.

Introduction

Ingrown toenails can become an incapacitating problem and have been therapeutically approached in a number of ways. One of these is surgical, through extraction of the nail matrix. However, if nail extraction (either partial or total) is performed without removing the matrix, the rate of recurrence ranges between 64% and 83% [1–4]. Matrix phenolization has been used as an alternative method [5] and has also been done in combination with wedge resection of the matrix [6]. Partial matrix excision has shown more favorable outcomes in terms of postoperative morbidity and is the method preferred by some because of the toxicity of phenols [7]. The morphologic evaluation of the specimens from ingrown toenails is of interest, not only in ruling out any additional pathology (malignant or not), but also in confirming that the nail matrix has actually been removed. However, determining whether the matrix is present in specimens that are sent for evaluation is not always an easy task. Although it is not difficult to distinguish the nail matrix from the cuticle, nor from the plate, differentiating between the nail matrix and the lateral nail fold is not as easy. Classic textbooks emphasize that the matrix epithelium has a cuboidal appearance and goes through the matrical keratinization,

with no granular cell layer. However, the biopsies that are sent for evaluation have either fragmented many times or are not easy to orientate (Figure 1).

Fragments of the epithelium from the eponichium and the ventral proximal nail fold are mixed with fragments of both the matrix and the lateral nail fold. An additional morphologic complication is that the proximal matrix branches into several rootlets [8, 9]. Also, inflammatory changes in the biopsy can be so powerful that they greatly distort the architecture (Figure 1).

Immunostaining for CD10 and CD34 can, however, play a diagnostic role. Some authors have demonstrated that nail mesenchymal cells beneath the nail matrix and the proximal bed express CD10 [10] and these same authors have coined the term “onychofibroblasts” to refer to these phenotypically (and maybe functionally) specialized fibroblasts [11, 12]. On the contrary, CD10 is not expressed by either the dermal fibroblasts or the surrounding extracellular matrix of the lateral nail fold, except around blood vessels and eccrine structures [10]. Also, the expression of CD34 varies from the nail matrix to the lateral nail fold: CD34 is expressed by the endothelia of blood vessels in the nail matrix and in a perieccrine and perivascular pattern in the lateral nail fold [10].

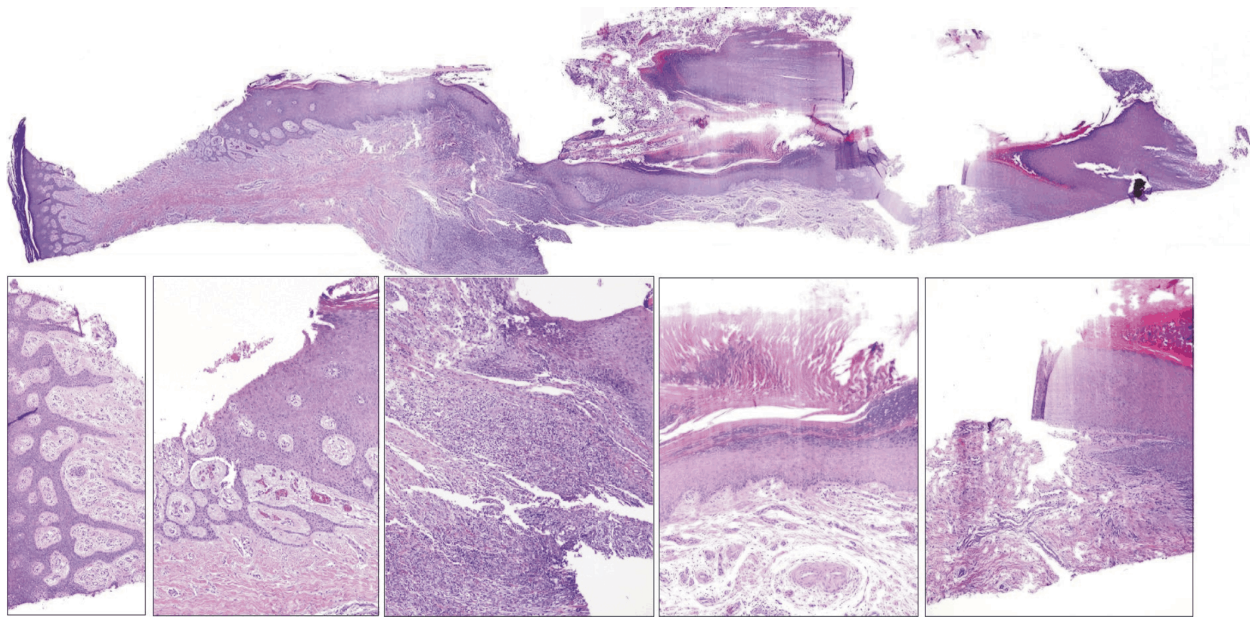


Figure 1 – The picture shows the aspects that most specimens from ingrown toenails present. The entire specimen is seen at the top of the figure. The bottom left shows intense changes resulting from ingrown toenails (first and second bottom insets on the left). The central areas of the specimen are highly distorted by the inflammatory infiltrate, which can be so dense that evaluating the presence of the matrix in the biopsy becomes extremely difficult (HE stain: $\times 2$ top, $\times 4$ bottom).

However, one main limitation of the immunohistochemical studies that have been published about CD10 and CD34 in onychofibroblasts is that they were performed on specimens from polydactyly [10, 11]. Although it is assumed a priori that the immunoeexpression of such markers would be the same in specimens from ingrown toenails, such assertions need to be demonstrated. The fibroinflammatory changes seen in ingrown toenails have been noted [13] but no tests have determined whether such inflammatory responses alter the immunohistochemical pattern that is described by Lee KJ *et al.*, in relation to polydactyly. Therefore, the goal of the current paper is to study the expression of CD10 and CD34 in specimens from ingrown toenail surgery and to prove, or dismiss, the role of both markers in the identification of the nail matrix in these types of specimens.

We have also included the melanocytic markers HMB-45 and Melan-A in our study. The matrix epithelium is said to be where most of the melanocytes in the nail are found; they are found in only very small amounts in the lateral nail fold. We have therefore decided to study the expression of melanocytic markers through the intra-epidermal melanocytes of the biopsies from our ingrown toenail surgeries and to check whether the number of positive cells could also be correlated to the matrix nature of some of the fragments.

Materials and Methods

We studied the biopsies of 15 cases of ingrown toenails. The clinical stages of the patients were determined according to the Mozena classification [14].

A podiatrist performed the biopsies, aiming to remove the plate, the cuticle and the matrix. In all cases, we examined the routine slides (Hematoxylin–Eosin) and

performed the immunohistochemical study with CD34 (Dako, clone QBEnd 10, code M7165), CD10 (Dako, clone 56C6, code 7308), HMB-45 (Dako, clone HMB-45, code M0634) and Melan-A (Dako, clone A103, code M7196).

With respect to the expression of CD10 and CD34, we evaluated the evidence of a “zonation” pattern, defined as follows: (a) expression of CD10 in a perivascular and perieccrine pattern in the lateral nail fold, and in a disorganized diffuse pattern in the stroma of the nail matrix, and (b) expression of CD34 only by the endothelia of blood vessels in the nail matrix and in a perivascular and perieccrine pattern in the lateral nail fold.

Results

Table 1 shows the clinical details of all of the patients, including their ages and genders and the location of the ingrown process.

Table 1 – Clinical details of all of the patients

Case No.	Age [years]	Gender	Stage	Evolution time [months]	Toe	Side of the toe
1.	25	M	2a	12	1 st left	Int.
2.	46	F	2a	48	1 st left	Ext.
3.	29	F	1	18	1 st left	Int.
4.	46	F	2a	48	1 st right	Ext.
5.	24	F	2a	12	1 st left	Ext.
6.	75	F	2a	12	1 st right	Ext.
7.	19	M	2b	36	1 st left	Ext.
8.	19	M	3	24	1 st right	Ext.
9.	18	F	2	36	1 st left	Int.
10.	16	F	3	24	1 st right	Ext.

Case No.	Age [years]	Gender	Stage	Evolution time [months]	Toe	Side of the toe
11.	18	F	1	36	1 st right	Ext.
12.	21	F	2b/3	4	1 st left	Ext.
13.	52	M	1	6	1 st right	Ext.
14.	20	F	2a	24	1 st right	Ext.
15.	20	F	1	6	1 st left	Ext.

F – Female; M – Male; Ext. – External; Int. – Internal.

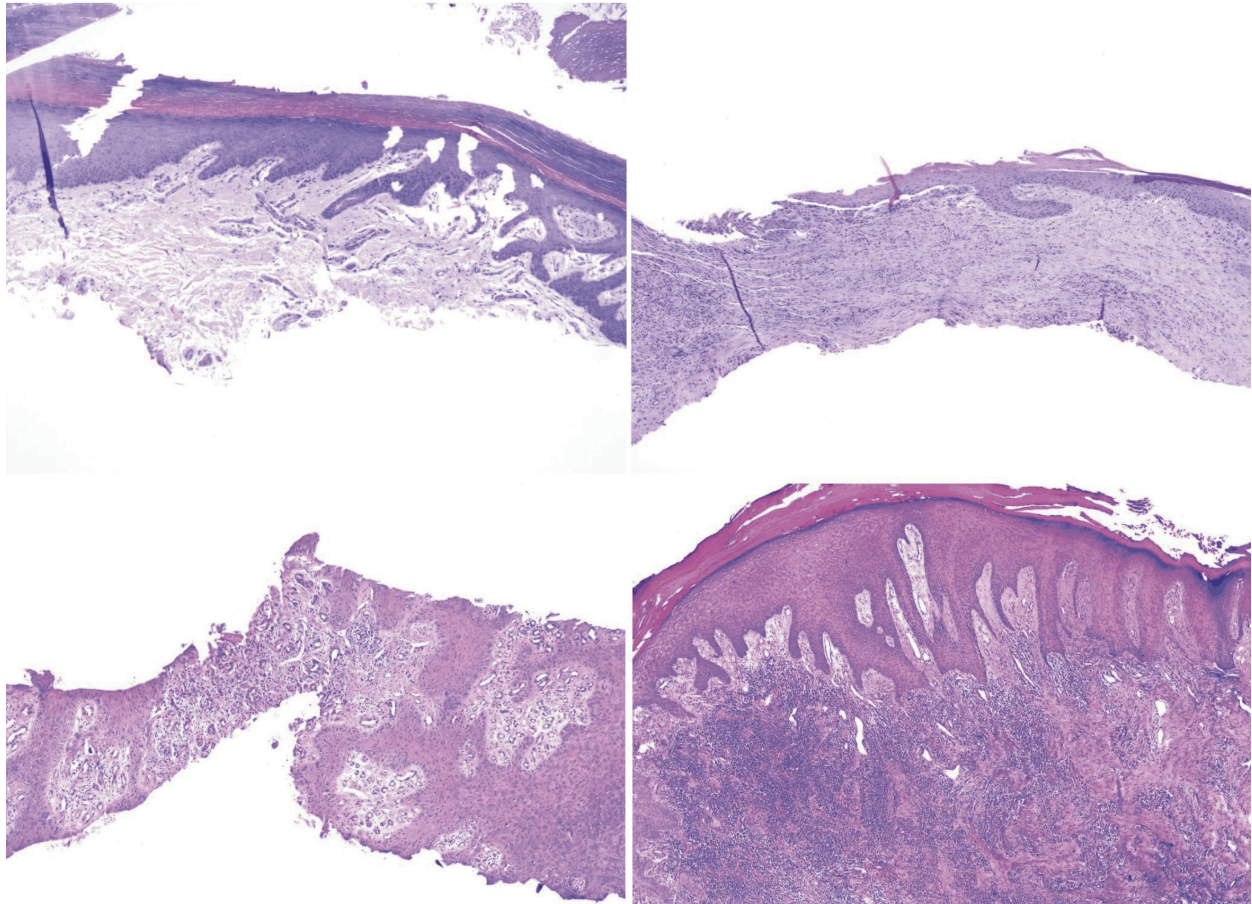


Figure 2 – Morphologic changes seen in our specimens of ingrown toenails. Changes were less intense in the lower stages (1, top left; 2a, top right) than in the advanced ones (2b, bottom left; 3, bottom right) (HE stain, ×10).

Matrical keratinization, which is typical of the nail matrix, was identified in all of the biopsies. Morphologic features compatible with lateral nail fold were also seen in seven out of the 15 biopsies (Table 2). In five cases (Cases No. 6, 8, 7, 10, and 12), an intermediate area of

transition between the matrix and the lateral nail fold was heavily distorted by inflammatory changes. It would have been very difficult to determine whether such areas included any segments of the matrix if these had been the only parts that were removed.

Table 2 – Immunohistochemical findings. We call “zonation” the distinct expression between matrix and lateral ungueal fold

Case No.	Evidence of matrix	CD10	CD34	HMB-45	Melan-A
1.	Yes	Zonation	Zonation	Scattered cells in matrix as well as in lateral nail fold	Scattered cells in matrix as well as in lateral nail fold
2.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells
3.	Yes	Zonation	Zonation	Scattered cells in matrix as well as in lateral nail fold	Scattered cells in matrix as well as in lateral nail fold
4.	Yes	Only matrix was evidenced	Only matrix was evidenced	Negative	Negative
5.	Yes	Zonation	Zonation	Scattered cells in matrix; no cells in the lateral fold	Scattered cells in matrix; no cells in the lateral fold

Case No.	Evidence of matrix	CD10	CD34	HMB-45	Melan-A
6.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells
7.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells
8.	Yes	Zonation	Zonation	Scattered cells in matrix as well as in lateral nail fold	Scattered cells in matrix as well as in lateral nail fold
9.	Yes	Zonation	Zonation	Scattered cells in matrix as well as in lateral nail fold	Scattered cells in matrix as well as in lateral nail fold
10.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells
11.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells
12.	Yes	Only matrix was evidenced	Only matrix was evidenced	Negative	Scattered cells
13.	Yes	Zonation	Zonation	Scattered cells in matrix; no cells in the lateral fold	Scattered cells in matrix; no cells in the lateral fold
14.	Yes	Zonation	Zonation	Scattered cells in matrix as well as in lateral nail fold	Scattered cells in matrix as well as in lateral nail fold
15.	Yes	Only matrix was evidenced	Only matrix was evidenced	Scattered cells	Scattered cells

Table 2 also summarizes the immunohistochemical results. In seven of the 15 biopsies, we noted a zonation pattern, and the expression of both markers was the same as had been described in the methods. CD10 immun-expression was nicely organized in a perivascular and perieccrine pattern in the lateral nail fold. However, a powerful, disorganized and diffuse expression of CD10 was seen in the stroma of the nail matrix (Figure 3B), and CD34 was only expressed by the endothelia of the blood vessels in the nail matrix and in a perivascular and perieccrine pattern in the lateral nail fold (Figure 4). When such immunohistochemical criteria were applied to the five specimens that showed a dense inflammatory infiltrate and a distorted architecture, the pattern of

expression of CD34 and CD10 identified such areas as belonging either to the matrix or to the lateral nail fold, in all cases. Figure 5 shows one example of such controversies.

With respect to the expression of melanocytic markers, we found positive intra-epidermal melanocytic cells in all but one case (Case No. 4). In general, the results with HMB-45 and Melan-A were the same. Only Case No. 12 showed discordance (negativity with HMB-45 and positivity with Melan-A). Both matrix and lateral nail folds were seen in the fragments, and melanocytes were found as scattered cells in the epidermis of both areas. Therefore, melanocytic markers did not help in separating both anatomic areas.

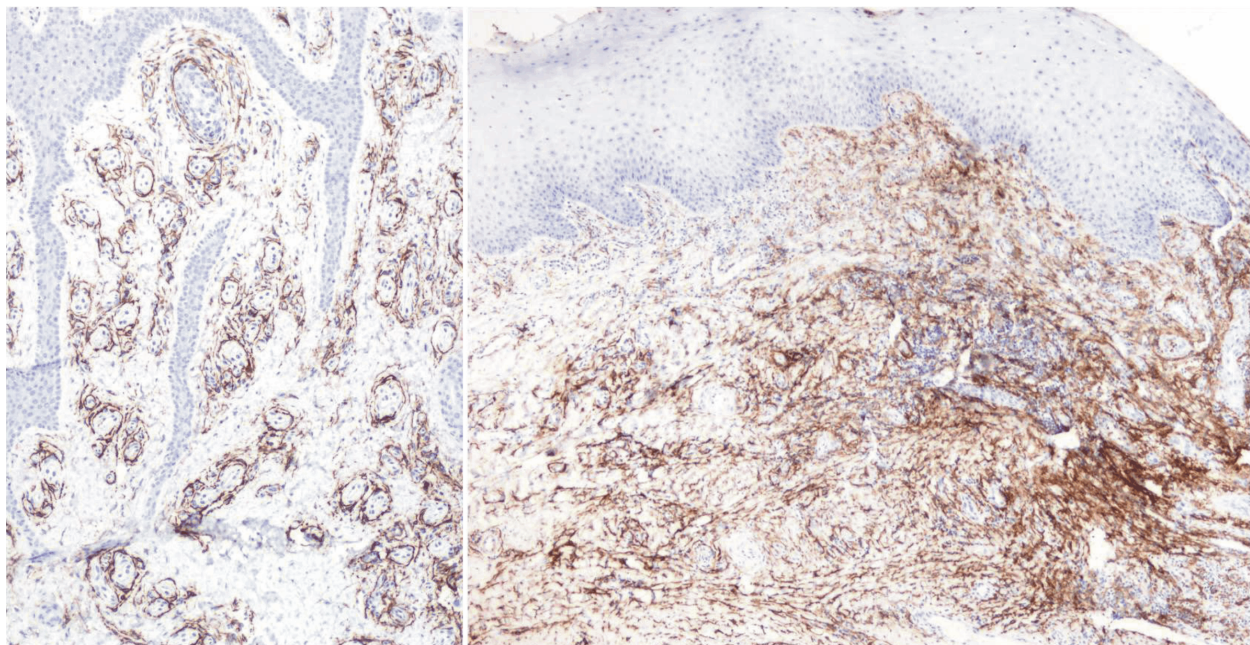


Figure 3 – The zonation pattern for CD10 expression. Left: the lateral nail fold expressed CD10 in a perivascular and perieccrine pattern. Right: the matrix showed a disorganized and diffuse pattern of expression in its stroma ($\times 20$).

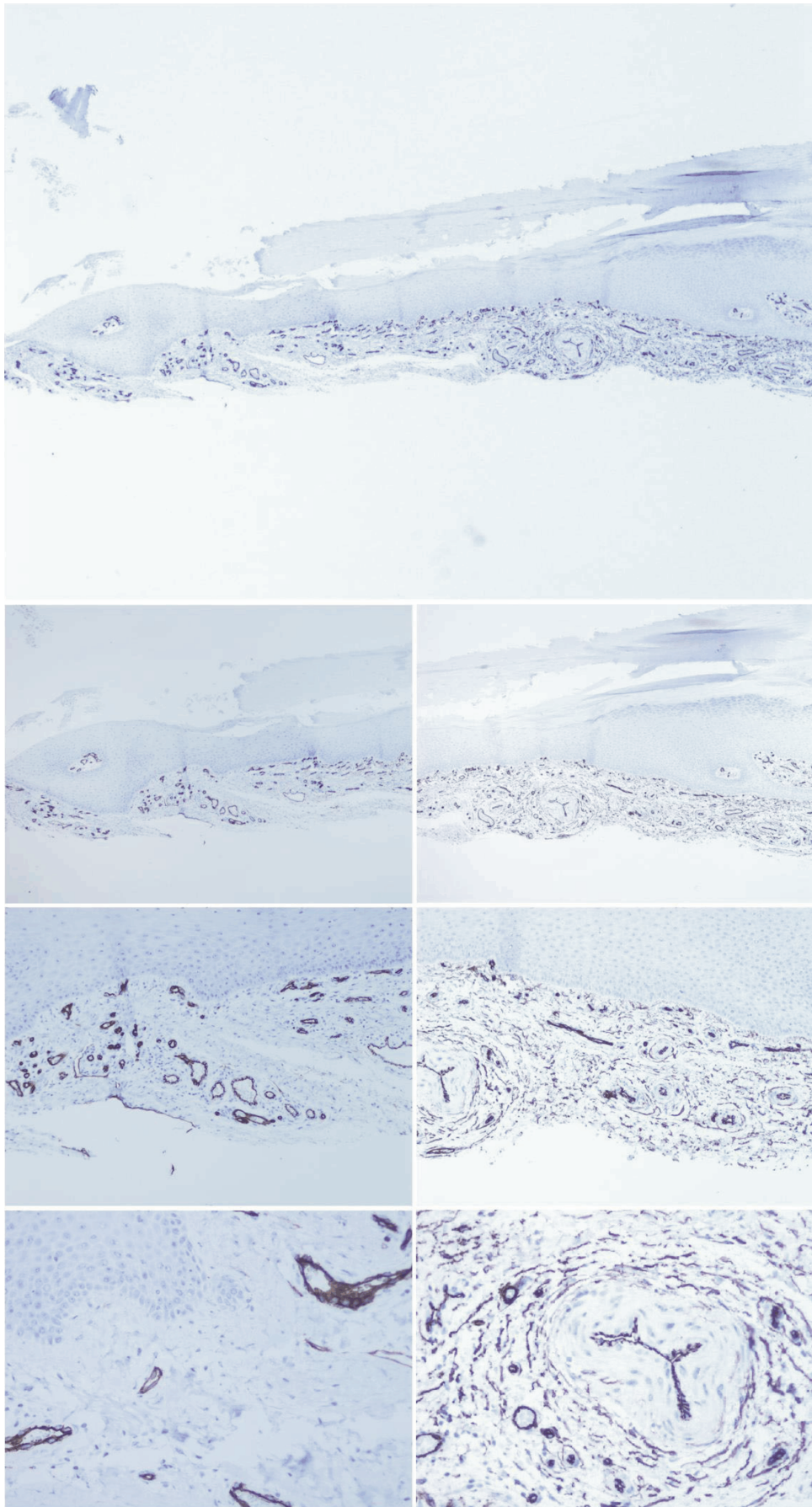


Figure 4 – The zonation pattern for CD34 expression. Top: the picture shows transition from the lateral nail fold (left) to the matrix (right). CD34 is only expressed by the endothelia of the vessels in the lateral nail fold (insets on the left column), while it is expressed in a perieccrine and perivascular pattern in the matrix (insets in the right column) ($\times 2$ top; $\times 4$ middle; $\times 10$ bottom left; $\times 20$ bottom right).

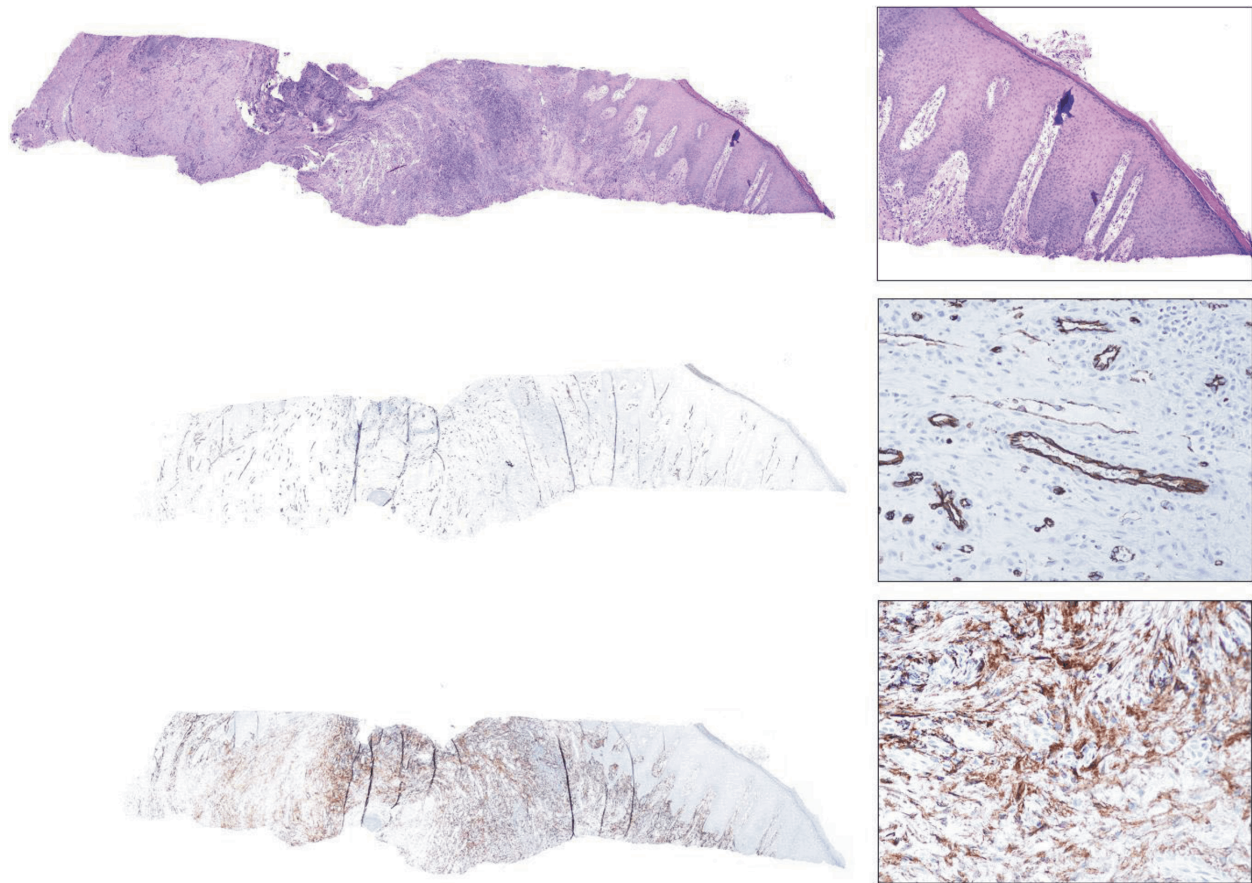


Figure 5 – *Is there any matrix in this fragment? In this segment of Case No. 6, the architecture was greatly distorted by the inflammatory infiltrate and its categorization as either a lateral nail fold or a nail matrix is difficult in the routine slide. In addition, the only epithelium that is shown reveals a granular layer that speaks against a matrical area. The immunohistochemical study is of enormous assistance in cases like this one. CD34 shows expression only of the endothelia of the blood vessels (middle row), while CD10 is diffusely expressed in the stroma, in a somewhat disorganized pattern. Both features allow for confirmation that the central segment belongs to the nail matrix (×2 left; ×4 top right; ×10 middle and bottom right).*

Discussion

There has been debate in the literature over the most appropriate therapeutic method for treating ingrown toenails. The most frequent methods are surgery and phenolization, either used separately or combined [7, 15–18]. When combined, the recurrence rate is lower than with any of the methods when they are used separately [6]. Those who are opposed to phenolization insist on its toxicity [7]. Whether treatment is surgical or chemical, the goal seems to be to destroy the nail matrix. Performing only surgical extraction of the nail is not satisfactory because the recurrence rate with this procedure ranges between 64% and 83% [1–4]. As noted in the introduction, identifying the matrix in specimens from ingrown nails through routine morphological study (Hematoxylin–Eosin) is not easy. Lee KJ *et al.* give an interesting description of the immunohistochemical pattern they found for CD10 and CD34 immunostaining in the nail matrix. In their studies, CD10 was expressed in the normal skin in periadnexal mesenchymal cells [19–21]. The expression of CD10 by the mesenchyme of the nail matrix has been presented as proof of the special nature of fibroblasts in the matrix, which have been called onychofibroblasts [10]. By 2007, Lee DY *et al.* had already mentioned the implications that onychofibroblasts might have in the surgery for ingrown

nails [11]. However, they had never tested this expression on ingrown toenail specimens since they demonstrated the onychofibroblasts in 2006, in nail units from specimens obtained during operations for polydactyly [11]. In 2011, they again emphasized their findings on paraffin-embedded tissue from specimens obtained during operations for polydactyly [10].

Our findings corroborate those of Lee KJ *et al.* and demonstrate that the zonation pattern is preserved even when the inflammatory changes of ingrown toenails are intense. Our report also supports the use of CD10 and CD34 in confirming the existence of the nail matrix in the specimens of ingrown toenails, especially in highly distorted specimens where Hematoxylin–Eosin is not totally convincing.

Conclusions

The expression of CD10 and CD34 in cases of ingrown toenails is preserved, and it follows the pattern described in the normal nail. Therefore, both markers can be useful in fragmented specimens from surgery for ingrown toenails, to confirm the successful removal of the nail matrix.

Acknowledgments

We thank Josefina Prada Valle, our technician, for her tremendous help with the immunohistochemical stainings.

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Received: February 7th, 2013

Accepted: May 17th, 2013