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Uncovering a new risk factor for subungual melanoma: Clinicopathological profiles with cadaveric study on the nail apparatus

조갑하 흑색종의 새로운 위험인자 규명: 병리임상학적 고찰 및 조갑부위 해부학적 연구

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Uncovering a new risk factor for subungual melanoma: Clinicopathological profiles with cadaveric study on the nail apparatus

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A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medicine (Plastic Surgery) at Seoul National University College of Medicine

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Abstract

Background

Subungual melanoma (SUM) is a rare subtype of melanoma originating from the melanocytes in the nail matrix. Recently, there is growing attention toward a more conservative functional surgery in order to escape functional deficit from amputation. However, the consensus on the surgical treatment is still controversial. Also, progression of SUM and correlation with clinical outcomes remain unclear.

Objective

The purpose of the present study was to provide objective measurements by analyzing the anatomy of the nail apparatus and to identify the pattern of dermal invasion in different locations of the nail bed with its relationship with clinical prognosis.

Methods & Materials

The nailbed was divided into 5 subunits; hyponychium (H), sterile matrix (SM), germinal matrix (GM), ventral floor of proximal nail fold (VFPNF), and dorsal roof of proximal nail fold (DRPNF). From 21 cadavers, nailbed thickness was measured in 5 landmark points. Microvessel and lymphatic density was histologically measured in each subunit. Retrospective data from 44 SUM cases between January 2011 and April 2019 were reviewed regarding invasion pattern in each subunit histopatholgically, correlating with clinical outcomes to assess risk factors.

Results

The nailbed thickness was the thinnest at the most proximal point of the nail matrix (thumbs, 1.10±0.42 mm; big toes, 1.15±0.37 mm) and the thickest at the hyponychium (thumbs, 2.86±0.82 mm; big toes, 2.72±0.84 mm). The median microvessel and lymphatics density was the highest at the hyponychium (25.74 vessels/mm^2, 7.55 vessels/mm^2) and lowest at the germinal matrix (16.26 vessels/mm^2, 4.14 vessels/mm^2), respectively ($p<0.05$). Dermal invasion of SUM was shown mostly in the distal areas of nail apparatus, with 11, 30, 18, 7, and 4 in the H, SM, GM, VFPNF, and DRPNF, respectively. The patients with hyponychial invasion showed a significantly greater Breslow depth ($p=0.009$), higher rate of lymph node metastasis ($p=0.019$), distant metastasis ($p=0.036$), and shorter disease-free survival ($p=0.001$).

Conclusion

Nailbed thickness is the thinnest at the proximal nail matrix, and the thickest at the hyponychium. Microvessel or lymphatic density was highest at the hyponychium. Hyponychial invasion is an important prognostic predictor of SUM, given its strong association with invasion depth, metastatic progression, and disease-free survival. Patients with invasion in the hyponychium should undergo stricter workup, treatment, and surveillance.

Keywords: malignant melanoma, nail melanoma, risk factors, functional surgery, prognosis

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Table of Contents

Chapter 1. Introduction

1.1. Study Background

Subungual melanoma (SUM) is a rare form of malignant melanoma that originates from the melanocytes in the nail matrix, comprising 0.7−3.5% of all melanoma subtypes [1−9]. At the early stage, subungual melanoma may only feature longitudinal dark pigmentation under the nail and is frequently misdiagnosed as other diseases such as striate melanonychia and onychomycosis. Approximately 15% to 65% of subungual melanomas are known to be amelanotic, which delays the patients in seeking medical advice [10, 11]. Thus, a diagnosis of subungual melanoma is commonly delayed, and most of the times, the disease is diagnosed at an advanced stage.

Historically, many patients with diagnostic delay had to undergo aggressive treatment to obtain clear safety margins [7, 8]. Radical amputation was recommended as Das Gupta *et al.* reported that any resection distal to the metacarpophalangeal joints increased the local recurrence and decreased survival [12]. However, because of the great functional loss and cosmetic defects caused by amputation, more conservative surgical approaches have been accepted by many surgeons recently. Park *et al.,* in their review of 100 cases of SUM, found no difference in the survival of patients treated with local proximal interphalangeal joint amputation compared with those

with more proximal amputations [13]. In a retrospective review of 124 cases, Nguyen *et al.* concluded that the resection level does not influence outcomes if histologically free margins are obtained [14]. The most recent meta-analyses suggested that amputation was not significantly beneficial in terms of prognosis and survival over more conservative treatments in early lesions [15, 16].

Despite the increasing trend towards non-amputative surgeries, the proper treatment remains controversial regarding the requirement for amputation, level of amputation, or resection margin and depth during functional surgery [2]. In order to determine whether to perform amputation or to delineate an exact excision margin during functional surgery, the knowledge of normal nailbed anatomy and the pattern of tumor invasion in SUM is essential.

Previously, there have been a few cadaveric studies, evaluating the anatomy of the nail apparatus [17, 18]. However, these studies did not measure the distances between multiple anatomical structures of the nail apparatus, and therefore, did not fully provide the information essential to achieve a safe resection margins. With insufficient anatomical knowledge, precise surgical planning is challenging. Further, very little is known about the sequence of development of SUM. There is a strong evidence that SUM originates from the proximal germinal matrix and grows in radial direction in early stages, with dermal invasion occurring at more advanced stages [19-23]. However, sufficient evidences have not been provided to elucidate the dermal invasion pattern, and the

correlation between the disease progression and clinical outcomes.

1.2. Purpose of Research

In this study, we collected specimens from 21 cadavers and performed a histological analysis including nailbed thickness, microvessel density (MVD), and lymphatic density (LD) according to the nail subunit. In order to investigate invasion pattern of SUM in relation with clinical outcomes, dermal invasion in each subunit of the nail apparatus was reviewed pathologically. Also, its relationship with prognostic findings was evaluated, such as local recurrence, lymph node metastasis, and distant metastasis. This will help us to understand the invasion pattern of SUM associated with unique anatomical structure of the nailbed. Pattern of tumor invasion gives us essential information in delineating resection margin during functional surgery. Further, correlation between the disease progression and clinical outcomes will provide a new risk factor of SUM, as well as giving us clues to establish a modified staging system for SUM.

Chapter 2. Body

Materials and methods

2.1 Cadaveric anatomical study

2.1.1 Subject selection

Specimens were obtained from 21 cadavers, and those that were inappropriately processed were excluded, resulting in a total of 55 specimens (thumbs, 27; big toes, 28). Two cadavers were female and the rest were male. The age at death ranged between 49 and 91 years, with the average age being 71.5 years. Among the 55 specimens, 13 were of the right thumb; 14, left thumb; 14, right big toe; and 14, left big toe. No specimens had a history of trauma or disease on the digit involved.

2.1.2 Histological analysis

The specimens were cut along the longitudinal midline in 10μm thick sections. Hematoxylin and Eosin (H&E) staining was performed for cross-sectional analysis. Eleven landmarks were selected as shown in figure 1 as follows. M, most proximal point of nail matrix; B1, bony cortex closest to point M; B2, bony cortex closest to S2; B3, bony cortex closest to S3; B4, processus unguicularis; B5, bony cortex closest to S5; S1, surface of skin closest to M; S2, eponychium; S3, surface midpoint of S2 and S4; S4, surface of nailbed at B4; and S5, Hyponychium. The vertical distance of the nailbed was measured at multiple sites using ImageScope™ (Aperio Technologies, Inc., Vista, CA).

2.1.3 Immunohistochemical staining and calculation of MVD and LD

Along with the H&E-stained slides, 16 additional slides were labeled using CD31 and D2-40 antibodies to evaluate MVD and LD, respectively. Two slides were discarded because of inadequate staining. Subsequently, the stained slides were scanned using Aperio ScanScope® CS instrument (Aperio Technologies, Inc., Vista, CA) at ×400 magnification. Following this, the slides were analyzed with ImageScope™ using the Microvessel Analysis v1 algorithm (Aperio Technologies, Inc., Vista, CA). The algorithm counted vessels within the area range of 50 μm to 200,000 μm. In each slide, the nail apparatus was divided into five different subunits; hyponychium (H), sterile matrix (SM), germinal matrix (GM), ventral floor of proximal nail fold (VFPNF), and dorsal roof of proximal nail fold (DRPNF) [24]. The subunit H is the distal free margin of the nail, which unlike the nailbed, comprises the stratum granulosum layer [25]. The SM subunit includes nail matrix distal to the lunula and proximal to subunit H. GM is the portion of the nail matrix underlying the lunula. From the eponychium, the proximal nail fold forms a blind pocket encasing the nail plate. The ventral portion of the pocket indicates the VFPNF, and DRPNF is the dorsal skin layer of the pocket. The MVD and LD of each subunit was calculated by dividing the microvessel count (number) by the area of the particular subunit (mm^2).

2.1.4 Statistical analysis

For MVD and LD according to its nail subunit, normality test was performed using SPSS version 26.0 (IBM, Armonk, NY). Because the data did not meet the assumption of normality, Kruskal–Wallis test was performed to determine if there were significant differences between MVDs or LDs calculated according to the nail subunits. Following this, Wilcoxon Mann–Whitney test was performed as a *post hoc* test to confirm whether the data collection was statistically significant.

2.2 Pathologic evaluation with clinical prognosis

2.2.1. Study design

A retrospective review was performed of 60 surgically-treated patients who were diagnosed with malignant melanoma in the fingers or toes between January 2011 and April 2019. Nine patients diagnosed as non-subungual melanoma and 7 patients without available pathologic specimen were excluded. A final of 44 patients were incorporated in the study, including 24 pathologically diagnosed from outside clinics and 20 diagnosed at our hospital. The biopsy methods used for diagnosis included punch biopsy in 30 patients, wedge biopsy in 3, tangential biopsy in 4, and excisional biopsy in 7. The study was approved by the Institutional Review Board (reference: H-2004-132-1117) and was performed in accordance with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects.

2.2.2. Histopathological analysis

The slides of 44 SUM cases stained by HE were reviewed by two pathologists. The specimens were obtained in sagittal, longitudinal 4mm-interval sections. The nail plate was decalcified prior to the slide production to smooth the hard tissue such as the nail plate and underlying bone. The slide which showed the deepest invasion was chosen in order to analyze dermal invasion pattern of SUM. The histopathological analysis was performed according to the aforementioned five anatomical subunits of the nail apparatus; H, SM, GM, VFPNF, and DRPNF.

The invasion of SUM in each subunit was categorized using three criteria: no tumor, in situ, or dermal invasion. The vertical invasion was evaluated by measuring the Breslow depth at locations with the deepest invasion. To evaluate the radial invasion, the total involvement score was calculated by adding scores

from the five subunits, 0 as no tumor, 1 as melanoma in situ, and 2 as invasive melanoma. For example, in a patient with invasion of the H, N, and GM, but in situ in the VFPNF, and no tumor in the DRPNF, the score was calculated as 7 (2+2+2+1+0=7).

2.2.3. Clinical analysis

Clinical data on demographics, clinical presentation, surgical method, follow-up period, disease-free survival, and prognostic factors including local recurrence, lymph node metastasis, and distant metastasis were obtained from the electronic medical records. The high-risk group was defined as patients who presented with local recurrence, lymph node metastasis, or distant metastasis, whereas the low-risk group was defined as patients with none of them.

2.2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Version 23.0 (IBM Corp., Armonk, NY). The Fisher's exact test and chi-square test was used to assess the relationship between subunit invasion and clinical findings. The Mann-Whitney U test was used to compare the relationship between subunit invasion and Breslow depth, total involvement score. The Log rank test was used to evaluate the disease-free survival. The statistical significance level was set at the p-values less than 0.05.

Results

3.1 Cadaveric anatomical study

3.1.1. Vertical distances of the nail apparatus

The nailbed thickness was defined as the vertical distance between the nailbed surface and the underlying phalangeal bone. Among these measurements, the distance between the hyponychium and the underlying bony cortex (S5−B5) was the largest, measuring 2.86±0.82 mm in the thumb and 2.72±0.84 mm in the big toe. The distance from the eponychium to the underlying bony cortex (S2−B2) was 1.91±0.49 mm at the thumb and 2.08±0.49 mm at the big toe. The distance between the surface midpoint of S2 and S4 and the bony cortex closest to S3 (S3−B3) measured 1.84±0.50 mm at the thumb and 2.40±0.58 mm at the big toe. The distance from the surface of the nailbed at B4 to the processus unguicularis (S4−B4) was 1.11±0.52 mm at the thumb and 1.40±0.58 mm at the big toe. The shortest vertical distance was observed from the most proximal point of the nail matrix to the bony cortex (M−B1), which measured 1.10±0.42 mm at the thumb and 1.15±0.37 mm at the big toe (Table 1).

3.1.2. MVD and LD of nail apparatus

In all, 14 slides were made for MVD and LD (vessels/mm^2) analysis. A representative slide is shown in figures 2 and 3, respectively. The median MVD was 25.74±8.42 vessels/mm^2 in subunit H, 17.99±9.33 vessels/mm^2 in SM, 16.26 ±3.82 vessels/mm^2 in GM, 19.88±11.07 vessels/mm^2 in VFPNF, and 18.62±6.21 vessels/mm^2 in DRPNF. The differences were statistically significant between MVD of subunit H and those of subunit GM ($p < 0.05$) and DRPNF ($p < 0.05$). No statistically significant differences were observed between other subunits. The median LD was 7.55±3.87 vessels/mm^2 in subunit H, 5.84±3.82 vessels/mm^2 in SM, 5.18±2.67 vessels/mm^2 in GM, 5.91±2.85 vessels/mm^2 in VFPNF, and 4.14± 1.46 vessels/mm^2 in DRPNF. The differences were statistically significant between LD of subunit H and those of subunit DRPNF ($p < 0.05$). No statistically significant differences were observed between other subunits.

3.2 Pathologic evaluation with clinical prognosis

3.2.1. Patient characteristics

SUM was present in 44 cases, with an average age of 61.07years. Thirty-four cases were diagnosed with invasive melanoma (77.3%), and 10 with melanoma in situ (22.7%). The clinical presentation was mostly total melanonychia (n=33, 75.0%), followed by longitudinal melanonychia (n=6, 13.6%), Hutchinson's sign only (n=3, 6.8%), and amelanotic lesions with nail mass or nail deformity (n=2, 4.5%). The Hutchinsons' sign was present in 36 cases (81.8%), and ulceration was present in 11 cases (25.0%). Twenty-four patients underwent amputation (54.5%) while 20 underwent functional surgeries (45.5%). Sentinel lymph node biopsy was performed in 26 cases of invasive melanoma according to the previous guideline [26], of which 3 cases were present with metastasis. During the mean follow-up period of 3.04 years, lymph node metastasis was found in 12 cases (27.3%), distant metastasis in 13 cases (29.5%), and local recurrence in 3 cases (6.8%) (Table 2).

3.2.2. Distribution of SUM in each nail subunit and correlation with high-risk factors

Twenty-nine patients were at low risk, while the other 15 were at high-risk. In the DRPNF, 4 patients presented with dermal invasion (9.1%) while 17 presented with in situ lesions (38.6%). In the VFPNF, 10 patients were diagnosed with invasive melanoma (22.7%) and 27 were diagnosed with melanoma in situ (61.4%). Eighteen patients had dermal invasion in the GM (40.9%) while 21 had in situ lesions (47.7%). In the SM, 30 patients showed dermal invasion (68.2%) and 11 patients showed in situ (25.0%). In the hyponychium, 11 patients were diagnosed with invasive melanoma (25.0%) while 21 were diagnosed with melanoma in situ (47.7%).

When the relationship between the tumor involvement at each subunit, including both melanoma in situ and invasive melanoma, and the high-risk of metastasis and recurrence was analyzed, no statistically significant correlation was found in any area of the nail apparatus. However, when we examined the correlation between tumor involvement with only dermal invasion and the high-risk group, a significantly higher risk of lymph node metastasis, distant metastasis, or local recurrence was observed in patients with dermal invasion in the hyponychium (p=0.028) (Table 3).

3.2.3. Dermal invasion in the hyponychium and its correlation with clinicopathological factors

Eleven cases included dermal invasion in the hyponychium (25.0%), 21 involved in situ invasion (47.7%), and 12 had no tumor in the area (27.3%) (Table 4). There was no statistically significant association with most variables, including age, sex, tumor location, Hutchinson's sign, invasion in other subunits, and operation. Amputation was performed in 8 of 11 patients with hyponychial invasion (72.7%), while only 16 of 33 patients without hyponychial invasion underwent amputation (48.5%), although the difference was not statistically significant (p=0.162). The patients with hyponychial invasion showed a significantly higher rate of lymph node metastasis (p=0.045) and tendency of distant metastasis (p=0.057), but there was no statistical difference in the local recurrence (p=1.000).

3.2.4. Histological landscape of invasion in each nail subunit and risk factors

Figure 4 illustrates the pattern of dermal invasion, categorized according to the presence of hyponychial invasion. Invasion occurred mostly in the distal portion, with 11 cases in the hyponychium, 30 in SM, 18 in GM, 7 in VFPNF, and 4 in DRPNF. The deepest invasion was frequently found in the distal areas, with 7

cases in the hyponychium and 21 in SM, whereas very few were found in the proximal areas. The continuity of invasion between adjacent subunits was observed, as there were few cases of invasive or in situ lesions at an isolated subunit.

Patients with hyponychial invasion showed a greater tendency to have lymph node metastasis and distant metastasis. In 11 cases of hyponychial invasion, lymph node metastasis was discovered in 6 (54.5%), distant metastasis in 6 (54.5%), and local recurrence in 1 (9.1%). In the 21 cases with in situ lesions in the hyponychium, 3 patients had lymph node metastasis (14.3%), 4 distant metastasis (19.0%), and no local recurrence (0.0%). In the 12 patients with no tumor in hyponychium, 3 cases were identified with lymph node metastasis (25.0%), 3 with distant metastasis (25.0%), and 2 with local recurrence (16.7%). Amputation was performed in 8 of 11 patients with hyponychial invasion (72.7%), 11 of 21 patients with in situ lesions (52.4%), and 4 of 12 patients with no tumor in the area (33.3%). The average total involvement score was 7.27 for hyponychial invasion, 6.10 for in situ, and 4.58 for cases with no tumor in the hyponychium.

3.2.5. Dermal invasion in each nail subunit and correlation with Breslow depth, total involvement score, and disease-free survival

Dermal invasion proximal to the hyponychium, including the SM, GM, VFPNF, and DRPNF, showed a strong correlation with higher total involvement scores ($p<0.001$), but no association with the Breslow depth. On the contrary, invasion in the hyponychium showed a statistically significant association with greater Breslow depth ($p=0.009$), but no significant association with the total involvement score. The disease-free survival was significantly shorter in patients with invasion in the hyponychium ($p=0.001$) and in SM ($p=0.047$) (figure 5).

Discussion

Because the tumor depth is essential for the determination of the resection margin and whether to perform sentinel lymph node biopsy, vertical distances are crucial. Accordingly, Kim *et al.* measured the shortest distance between the nail matrix and the underlying bone [17]. Their measurement values (thumb, 0.90±0.27 mm; big toe, 0.87±0.27 mm), however, were slightly lower compared with ours (thumb, 1.10±0.42 mm; big toe, 1.15±1.37 mm); this may be because over 90% (19/21) of the cadaver in our study were males, who tend to have larger sized digits, compared to only 60% (9/15) in the previous study. Additional studies have also noted that invasion of SUM may not occur adjacent to the primary location of subungual melanoma [23, 24]. Therefore, in this study, nailbed thickness was measured in each subunit to establish accurate deep surgical margins.

Current consensus of resection margin for the treatment of malignant melanoma is based on the NCCN guideline. Briefly, 5mm safety margin is recommended for the in situ lesions, 10mm for lesions of melanoma depth less than 1mm, 10–20mm for lesions of 1.0–2.0mm, and 2.0mm safety margin for lesions of melanoma depth more than 2mm [26]. However, nailbed depth was just around 1.1mm in the proximal nail matrix or processus unguicularis. Also, nailbed is located adjacent to hard tissue, between overlying nail plate and underlying phalangeal bone. The activity of melanocytes can be influenced by dynamic growth of nail plate from proximal to distal direction. These mean that conventional NCCN guideline cannot be applied for the SUM. Independent staging system or guideline for SUM should be made in the future with accumulated evidence including our data.

We also analyzed MVD and LD according to the nail subunit. MVD and LD were highest in subunit H. Previous studies have shown that a high MVD is associated with poor prognosis in various cancer types [27, 28]. This was also consistent in melanomas, in which MVD is associated with tumor recurrence, particularly in melanomas with tumor depths greater than 2 mm [29]. Our results may indicate

that once subungual melanomas infiltrate the microvessel or lymphatic-rich regions such as the hyponychium, they may become more susceptible to metastasis [30]. Therefore, we suggest that a more meticulous resection should be performed once the melanoma has invaded the hyponychium.

In this study, both the dermal invasion and the deepest invasion of SUM occurred mostly in the distal portion of the nail apparatus. This is consistent with previous studies on the possibility of dermal invasion starting in subunits other than the GM where tumors are known to originate from. *Shin et al.* reported that dermal invasion in SM is much less or occurs later than in other areas of the nail unit [22]. Izumi *et al.* also showed that the proliferation of tumor cells is more prominent in the hyponychium than in GM in the early stages [23]. They suggested that the invasion of dermal layer starts not in the GM but in SM or in hyponychium as the atypical melanocytes continually move in a distal direction towards the hyponychium where they accumulate at last. The lower tendency of invasion in GM can be explained by the physiologic environment of the distal direction of nail plate growth and the upward direction of cell growth to produce the nail plate, which inhibits the downward invasion.

However, there were few cases in our study with tumors in isolated subunits with no continuity to GM, even though SUM is known to begin from GM [20]. Three patients presented with invasion in the hyponychium but no tumor in GM, and two cases presented with *in situ* in the hyponychium or SM but no involvement in GM. Such cases show the possibility that malignant proliferation of melanocytes could initiate not only in GM but also in the distal subunits such as the hyponychium. In an immunohistochemical review of the anatomic distribution of melanocytes, active melanocytes are present throughout the nail apparatus and especially active in the VFPNF and hyponychium [31]. The active melanocytes in the hyponychium could initiate malignant proliferation and spread to the adjacent areas in a proximal direction. Such an unusual case of melanoma originating from the hyponychium has

been reported in the literature previously [32]. Further histological studies with large samples are needed to identify the accurate mechanism of progression of SUM.

Among the five subunits of nail apparatus, hyponychium was the only area in which the invasion has a significant association with the high-risk group ($p=0.028$), with more metastasis in lymph nodes ($p=0.045$) and in distant organs ($p=0.057$). The 5-year disease-free survival was significantly poorer in patients with invasion in the hyponychium ($p<0.001$) and in SM ($p=0.047$). The hyponychium could be vulnerable to metastatic progression because of the high density of microvessel and lymphatics and the absence of underlying bone [33], which is compatible with our cadaveric study. Hyponychial invasion also showed a strong association with greater Breslow depth ($p=0.009$) but no association with the total involvement score ($p=0.093$). Invasion in the hyponychium was associated with greater ulceration and vertical tumor burden regardless of the amount of radial progression, and this greater invasion depth could be correlated with poorer prognoses.

Although the delayed diagnosis has often resulted in the requirement for amputation traditionally [5, 7, 8, 16, 17, 34-36], conservative surgeries are equally beneficial in terms of prognosis and survival [1, 13-16, 37-47]. However, most studies are limited to case series rather than randomized controlled trials, and they lack information on tumor characteristics, including the depth at presentation, which is one of the most important prognosticators of SUM. In our study, the patients with hyponychial invasion underwent more amputation than those without, although the association was not statistically significant ($p=0.162$). The higher susceptibility of invasion in the hyponychium and its strong correlation with unfavorable clinical findings, including greater tumor depth, more metastasis, and poorer disease-free survival, suggests the need for more meticulous preoperative evaluation, treatment approach, and frequent surveillance. Further well-designed

controlled studies are needed to propose an exact treatment guideline.

High-resolution imaging tools could be helpful for evaluating tumor invasion pattern, preoperatively [48-50]. Preoperative punch biopsy in the hyponychium can also provide crucial information in deciding surgical options. Staged operation for SUM could be another option. At first stage, SUM is widely excised with proper resection margin. The open wound is kept with wet dressing until permanent biopsy results come out for around two weeks. At second stage, surgeons can decide whether to proceed the reconstructive surgery, or to perform amputation, according to the invasion pattern in the hyponychium from the permanent biopsy.

There are limitations to our study. In comes of cadaver study, our cadavers were all of Korean origin and were mostly male (90%). A future study including specimens from more diverse ethnicities and genders may reinforce our study's result. Also, the measurements were also performed at the very midline of the digits, which may be considered to be a limitation because we were not able to evaluate the cross-sections in different planes. Also, the number of patients with SUM were small without control group due to its retrospective nature. Serial observation of the development and progression of the disease was unavailable because of the limitations of a cross-sectional study.

Chapter 3. Conclusion

To the best of our knowledge, this is the first study to combine anatomical, pathological, and clinical evaluation to assess the risk factors of SUM, and to predict the clinical course and prognosis. Nail bed thickness was measured between 1.0 and 3.0mm according to subunits, with highest microvessel and lymphatic density in the hyponychium. Also, hyponychial invasion is an important prognostic predictor of SUM because of its strong association with invasion depth,

metastatic progression, and disease-free survival. Robust microvessel and lymphatic densities in the hyponychium could contribute to tumor spreading and poor prognosis. Our findings will help us planning treatment, and surveillance for SUM. With more accumulated evidence of SUM, staging system and treatment guideline should be independently offered for SUM in the future.

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Tables

Table 1. Nail bed thickness and surface anatomy of nail apparatus

Landmarks		Thumb		Big toe	
Vertical distances	M-B1	1.10±0.42	(0.90±0.27)*	1.15±0.37	(0.87±0.27)*
	S2-B2	1.91±0.49		2.08±0.49	
	S3-B3	1.84±0.50		2.40±0.58	
	S4-B4	1.11±0.52		1.40±0.58	
	S5-B5	2.86±0.82		2.72±0.84	

*[17]

483 Table 2. Patient characteristics

Variables	Value (%)
Number of patients	44
Age, year	
<65	27 (61.4)
≥65	17 (38.6)
Mean ± 95% CI	61.07 ± 3.88
Sex	
M	23 (52.3)
F	21 (47.7)
Location	
Finger	26 (59.1)
Toe	18 (40.9)
Thumb/Big toe	33 (75.0)
Others	11 (25.0)
Clinical presentation	
Total melanonychia	33 (75.0)
Longitudianl melanonychia	6 (13.6)
Hutchinson's sign only	3 (6.8)
Amelnotic lesions	2 (4.5)
Hutchinson's sign	
Present	36 (81.8)
Absent	8 (18.2)
Ulceration	
Present	11 (25.0)
Absent	33 (75.0)
Operation	
Amputation	24 (54.5)
Functional surgery	20 (45.5)
Sentinel lymph node biopsy	
Performed	26 (59.1)
Not performed	18 (40.9)
Invasion	
In situ	10 (22.7)
Dermal invasion	34 (77.3)
Lymph node metastasis	
Present	14 (31.8)
Absent	30 (68.2)
Distant metastasis	
Present	13 (29.5)
Absent	31 (70.5)
Local recurrence	
Present	3 (6.8)
Absent	41 (93.2)
Follow up period, yr	
Mean ± SD	3.04 ± 1.79

Table 3. Distribution of SUM in each nail subunit and correlation with high risk factors

Melanoma in situ or Invasive melanoma	Low risk (%)	High risk (%)**	Total (%)	p-value	Only Invasive melanoma	Low risk (%)	High risk (%)**	Total (%)	p-value
Dorsal roof of proximal nail fold									
Present	16 (76.2)	5 (23.8)	21 (47.7)	0.169†	Present	3 (75.0)	1 (25.0)	4 (9.1)	1.000*
Absent	13 (56.5)	10 (43.5)	23 (52.3)		Absent	26 (65.0)	14 (35.0)	40 (90.9)	
Ventral floor of proximal nail fold									
Present	25 (67.6)	12 (32.4)	37 (84.1)	0.675*	Present	6 (60.0)	4 (40.0)	10 (22.7)	0.714*
Absent	4 (57.1)	3 (42.9)	7 (15.9)		Absent	23 (67.6)	11 (32.4)	34 (77.3)	
Germinal matrix									
Present	26 (66.7)	13 (33.3)	39 (88.6)	1.000*	Present	11 (61.1)	7 (38.9)	18 (40.9)	0.576†
Absent	3 (60.0)	2 (40.0)	5 (11.4)		Absent	18 (69.2)	8 (30.8)	26 (59.1)	
Sterile matrix									
Present	26 (63.4)	15 (46.6)	41 (93.2)	0.540*	Present	17 (56.7)	13 (43.3)	30 (68.2)	0.089*
Absent	3 (100.0)	0 (0.0)	3 (6.8)		Absent	12 (85.7)	2 (14.3)	14 (31.8)	
Hyponychium									
Present	21 (65.6)	11 (34.4)	32 (72.7)	1.000*	Present	4 (36.4)	7 (63.6)	11 (25.0)	0.028*
Absent	8 (66.7)	4 (33.3)	12 (27.3)		Absent	25 (75.8)	8 (24.2)	33 (75.0)	
Total	29 (65.9)	15 (34.1)	44 (100.0)			29 (65.9)	15 (34.1)	44 (100.0)	

** Fisher's exact test p-value*

† Chi-square test p-value

*** High risk : Patients with lymph node metastasis, distant metastasis, local recurrence*

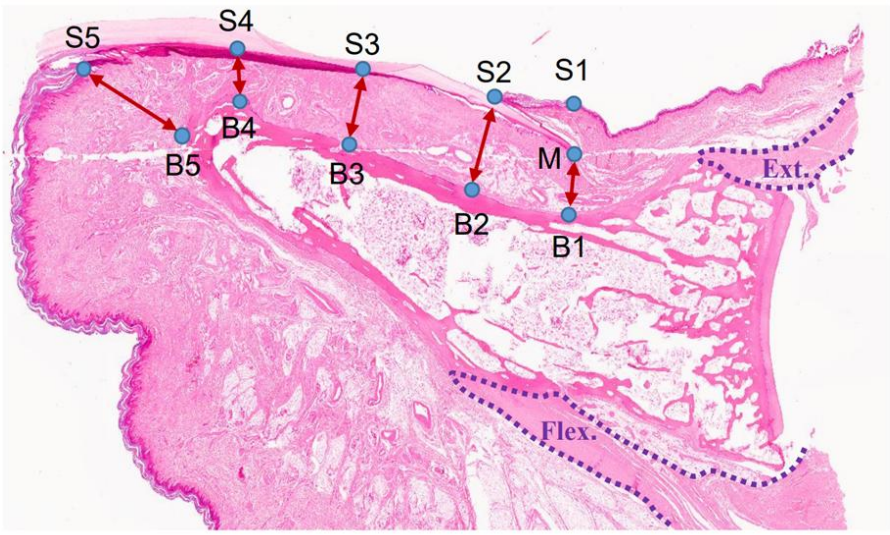
Table 4. Dermal invasion in the hyponychium and correlation with clinicopathological factors

Variables		No invasion in hyponychium (%)	Invasion in hyponychium (%)	Total (%)	p-value
Age					
	<65yr	22 (66.7)	5 (45.5)	27 (61.4)	
	≥65yr	11 (33.3)	6 (54.5)	17 (38.6)	0.289*
Sex					
	Male	17 (51.5)	6 (54.5)	23 (52.3)	
	Female	16 (48.5)	5 (45.5)	21 (47.7)	0.862†
Thumb/Big toe					
	Thumb/Big toe	25 (75.8)	8 (72.7)	33 (75.0)	
	Others	8 (24.2)	3 (27.3)	11 (25.0)	0.841†
Hutchinson's sign					
	Present	26 (78.8)	10 (90.9)	36 (81.8)	
	Absent	7 (21.2)	1 (9.1)	8 (18.2)	0.367†
Invasion in DRPNF					
	Present	2 (6.1)	2 (18.2)	4 (9.1)	
	Absent	31 (93.9)	9 (81.8)	40 (90.9)	0.256*
Invasion in VFPNF					
	Present	6 (18.2)	4 (36.4)	10 (22.7)	
	Absent	27 (81.8)	7 (63.6)	34 (77.3)	0.237*
Invasion in GM					
	Present	13 (39.4)	5 (45.5)	18 (40.9)	
	Absent	20 (60.6)	6 (54.5)	26 (59.1)	0.738*
Invasion in SM					
	Present	21 (63.6)	9 (81.8)	30 (68.2)	
	Absent	12 (36.4)	2 (18.2)	14 (31.8)	0.456*
Operation					
	Amputation	16 (48.5)	8 (72.7)	24 (54.5)	
	Functional surgery	17 (51.5)	3 (27.3)	20 (45.5)	0.162†
Lymph node metastasis					
	Present	6 (18.2)	6 (54.5)	12 (27.3)	
	Absent	27 (81.8)	5 (45.5)	32 (72.7)	0.045*
Distant metastasis					
	Present	7 (21.2)	6 (54.5)	13 (29.5)	
	Absent	26 (78.8)	5 (45.5)	31 (70.5)	0.057*
Local recurrence					
	Present	2 (6.1)	1 (9.1)	3 (6.8)	
	Absent	31 (93.9)	10 (90.9)	41 (93.2)	1.000*
Total		33 (75.0)	11 (25.0)	44 (100.0)	

DRPNF, Dorsal roof of proximal nail fold; VFPNF, Ventral floor of proximal nail fold; GM, Germinal matrix; SM, Sterile matrix

** Fisher's exact test p-value, † Chi-square test p-value*

Figures



S1 : Surface of the skin closest to M
S2 : Eponychium
S3 : Surface midpoint of S2 and S4
S4 : Surface of nailbed at B4
S5 : Hyponychium
M : Most proximal point of nail matrix

B1 : Bony cortex closest to point M
B2 : Bony cortex closest to S2
B3 : Bony cortex closest to S3
B4 : Processus unguicularis
B5 : Bony cortex closest to S5
Ext. : Extensor tendon (contour marked in dotted line)
Flex. : Flexor tendon (contour marked in dotted line)

Figure 1. Cross-sectional landmarks and measured distances (mm)
Representative cross-sectional histologic slide of specimens stained with Hematoxylin and Eosin depicting 11 landmarks.

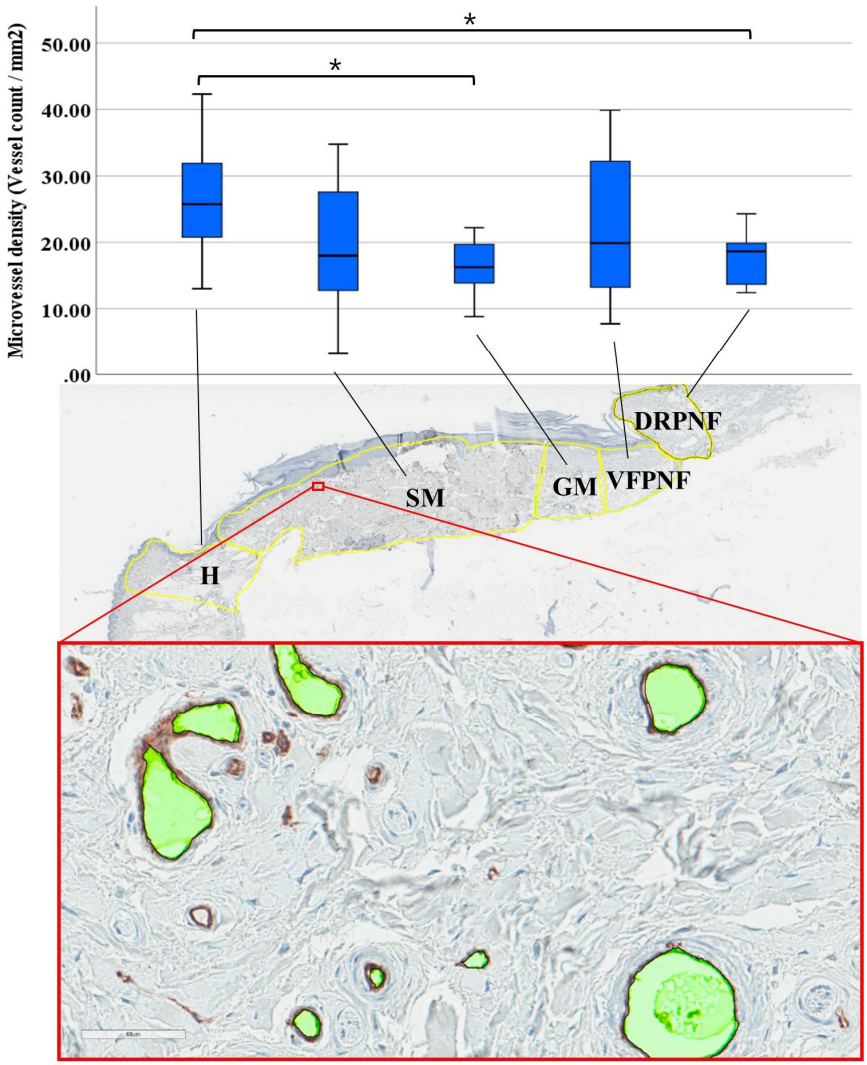


Figure 2. Microvessel density according to anatomic subunits

Top: Graph showing microvessel density (vessel count / area of subunit) according to the nail subunit. The asterix indicate differences that were statistically significant ($p < 0.05$) Middle: Representative slide of CD31 immunohistochemical staining of microvessels. The yellow lines demarcate the subunits. (H: Hyponychium, SM: Sterile matrix, GM: Germinal matrix, VFPNF: Ventral floor of proximal nail fold, DRPNF: Dorsal roof of proximal nail fold)

Bottom: Magnified figure of the CD31 IHC stained slide. The green area depicts the counted vessels at x400 magnification.

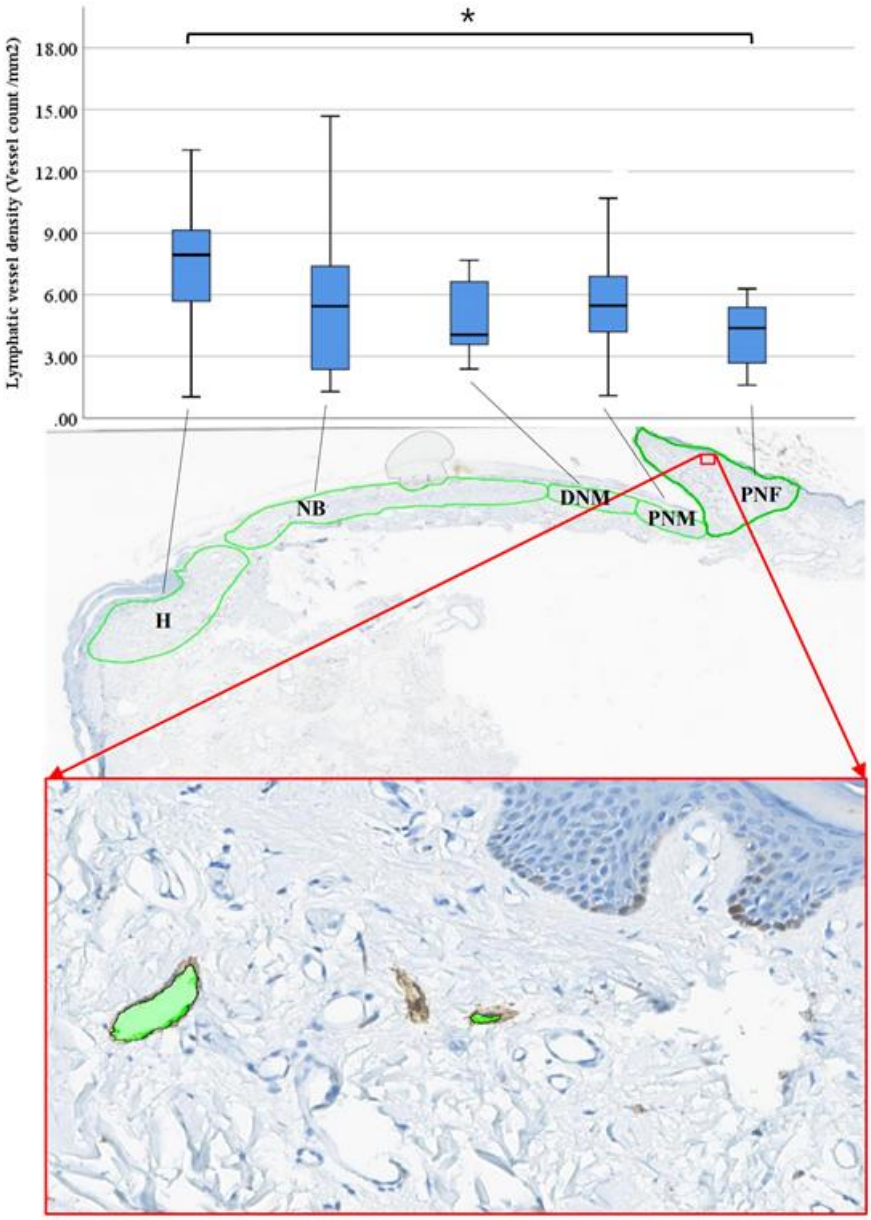
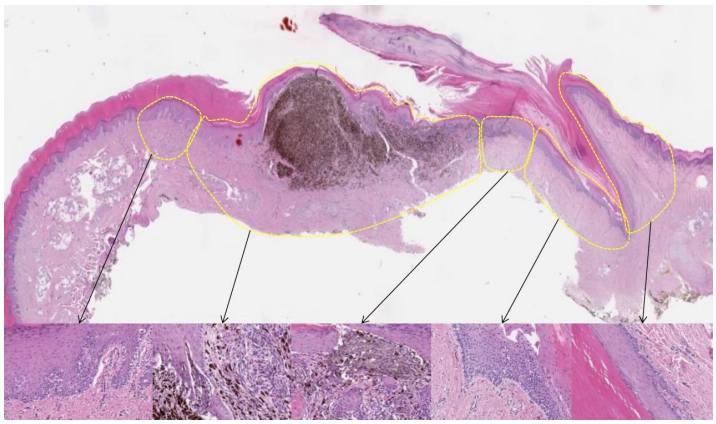


Figure 3. Lymphatic density according to anatomic subunits

Representative slide of D2-40 immunohistochemical staining of lymphatics. (lymphatic count / area of subunit). Magnified figure of the D2-40 IHC stained slide. The green area depicts the counted lymphatics at x400 magnification. (H: Hyponychium, SM: Sterile matrix, GM: Germinal matrix, VFPNF: Ventral floor of proximal nail fold, DRPNF: Dorsal roof of proximal nail fold)



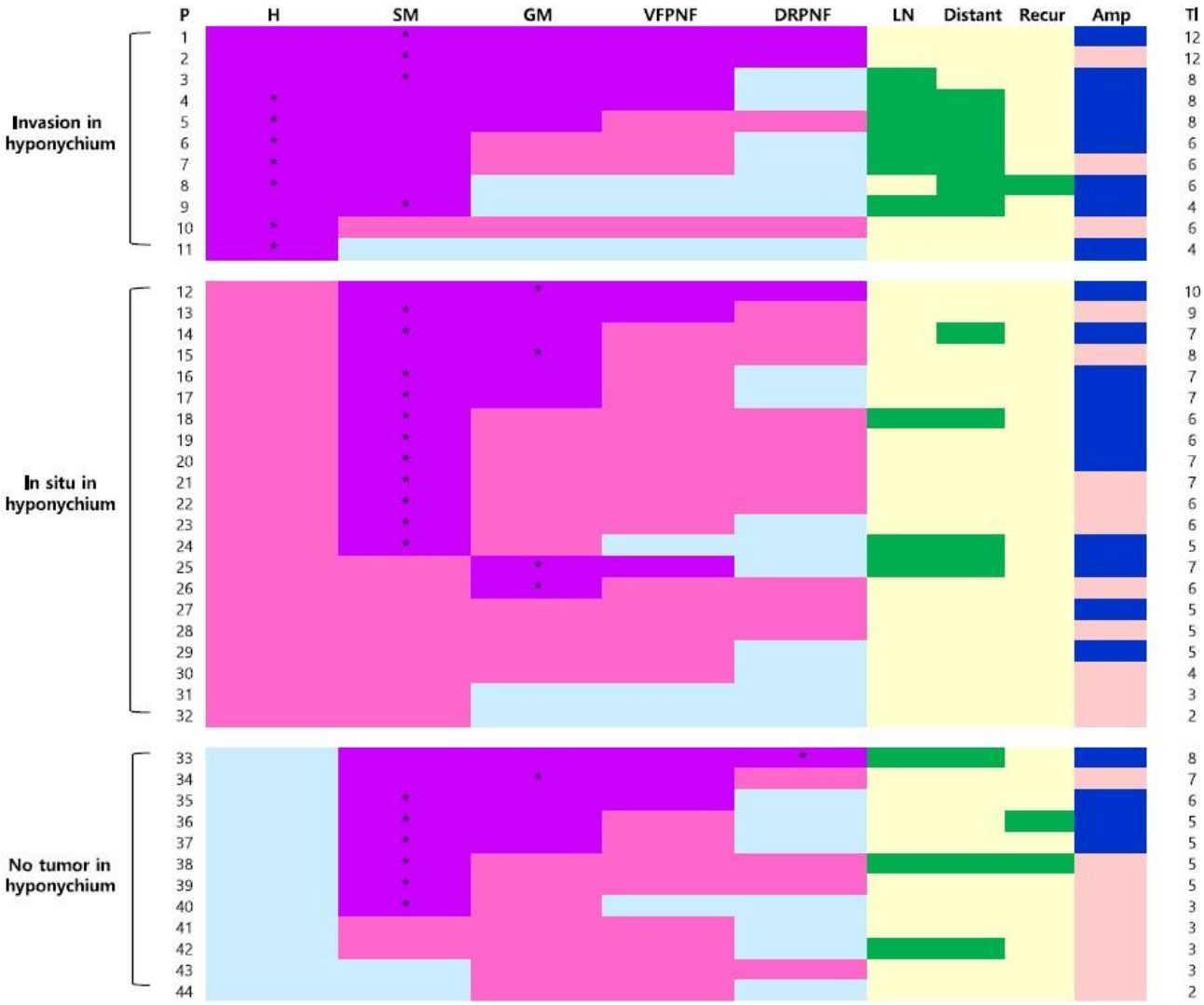

P
H
SM
GM
VFPNF
DRPNF
LN
Distant
Recur
Amp
TI
Invasion in hyponychium
In situ in hyponychium
No tumor in hyponychium

Figure 4. Subungual melanoma

(A) Histological slide of specimen *(upper)*

(B) Landscape of invasion in nail subunit and risk factors *(lower)*

Abbreviations: P, patient; H, hyponychium; SM, sterile matrix; GM, germinal matrix; VFPNF, ventral floor of proximal nail fold; DRPNF, dorsal roof of proximal nail fold; LN, lymph node metastasis; Distant, distant metastasis; Recur, recurrence; Amp, Amputation

The purple color indicates invasive lesions, the pink in situ lesions, and the light blue no tumors. The green indicates positive, whereas the yellow indicates negative. The dark blue indicates amputation, and the light pink indicates functional surgery.

* Lesion with the deepest invasion

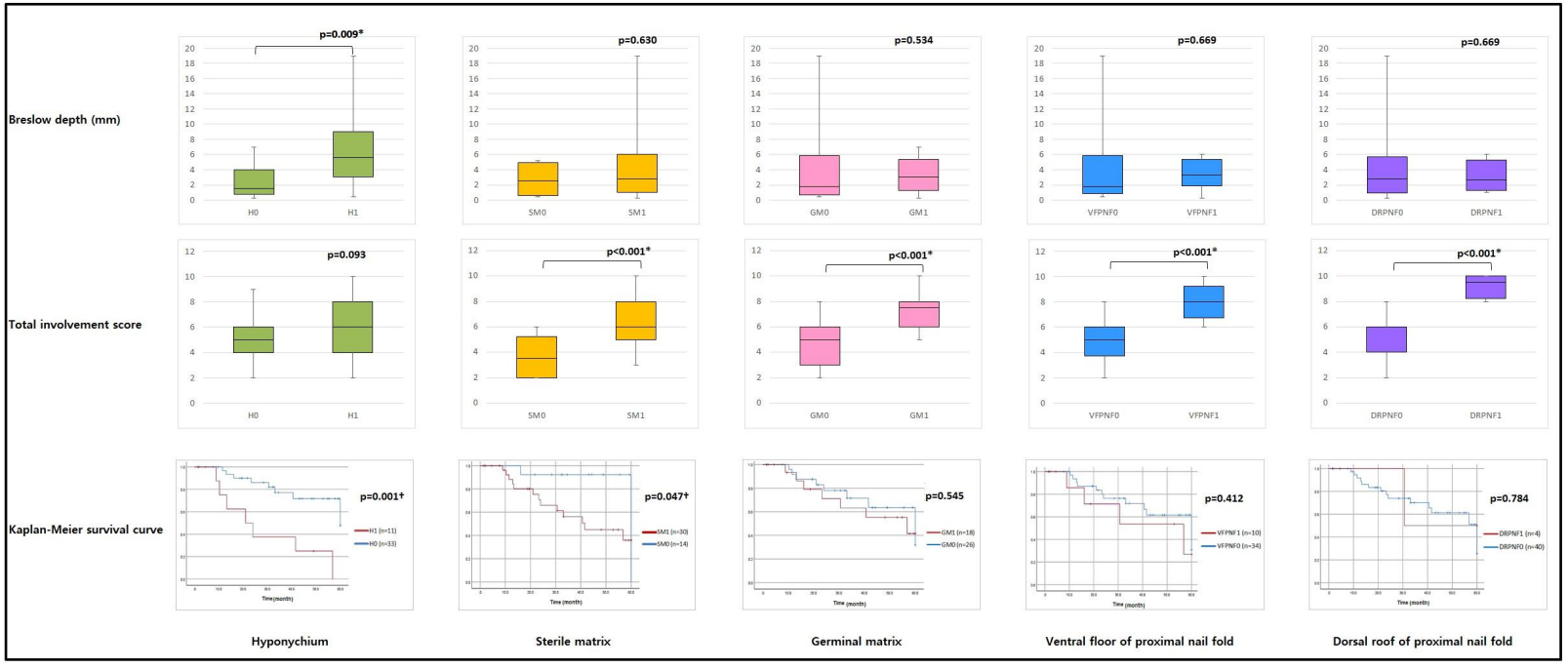


Figure 5. Dermal invasion of SUM in each nail subunit and correlation with Breslow depth, the total involvement score, and disease free survival

Abbreviations: H0, No hyponychium invasion; H1, Hyponychium invasion; SM0, No sterile matrix invasion; SM1, sterile matrix invasion; GM0, no germinal matrix invasion; GM1, germinal matrix invasion; VFPNF0, no ventral floor of proximal nail fold invasion; VFPNF1, ventral floor of proximal nail fold invasion; DRPNF0, no invasion in dorsal roof of proximal nail fold; DRPNF1, invasion in dorsal roof of proximal nail fold

* indicates significant differences according to the Mann-Whitney U test with $p < 0.05$

† indicates significant differences in survival curve according to the log-rank test with $p\text{-value} < 0.05$

Abstract

연구의 배경

조갑하흑색종은 진단이 지연되는 경우가 많아 예후가 좋지 않은 것으로 알려져 있다. 조갑하흑색종에 대해 절단술을 시행하는 경우가 많았으나, 이로 인한 기능적 장애를 최소화하고자 기능적 절제술이 최근에 많이 시행되고 있는 추세이다. 하지만 이를 위한 수술적 방법의 의견이 일치되지 않으며 실제 조갑하흑색종의 침윤 양상이나 관련된 예후에 대해서도 연구가 부족한 실정이다.

연구의 목표

이번 연구의 목표는 정상 손톱의 해부학적인 데이터를 측정하는 것이다. 또한 실제 환자에서 병리학적인 평가를 통해 손톱바닥의 소단위 별로 조갑하흑색종의 침윤 양상을 규명하고 이를 임상적인 예후와 연계시켜 분석하는 것이다.

재료 및 방법

손톱바닥을 다음과 같이 총 5개의 소구획으로 나누었다; hyponychium, sterile matrix, germinal matrix, ventral floor of proximal nail fold, dorsal roof of proximal nail fold. 21구의 시신연구를 통해서 손톱의 소단위별로 손톱바닥의 두께를 측정하였다. 면역조직화학검사를 통해 손톱 소단위별 미세혈관과 림프관의 분포를 조사하였다. 총 44명의 조갑하흑색종 환자의 임상데이터 및 병리슬라이드 리뷰를 진행하여 손톱 소단위 별 흑색종의 침윤 양상을 분석하고 이와 연관된 예후인자를 규명하였다.

결과

손톱바닥의 두께는 proximal nail matrix에서 가장 짧았고(엄지손톱; 1.10±0.42 mm 엄지발톱; 1.15±0.37 mm) hyponychium에서 가장 길었다(엄지손톱, 2.86±0.82 mm; 엄지발톱, 2.72±0.84 mm). 미세혈관 및 림프관의 밀도는 hyponychium에서 가장 높았고 (25.74 vessels/mm^2, 7.55 vessels/mm^2) germinal matrix에서 가장 낮았다(16.26 vessels/mm^2, 4.14 vessels/mm^2) ($p<0.05$). 조갑하흑색종은 대부분 원위부에서 침윤이 관찰되었다. Hyponychium에서 침윤이 관찰된 환자의 경우 통계적으로 유의하게 Breslow깊이가 깊었으며, 임파선 전이($p=0.019$) 및 원격 전이($p=0.036$)가 빈번하게 관찰되었고 무병생존율이 짧았다($p=0.001$).

결론

손톱바닥의 두께는 proximal nail fold에서 가장 얇았고, hyponychium에서 가장 두껍게 나타났다. 미세혈관 및 림프관은 hyponychium에서 가장 밀도가 높게 나타났다. Hyponychium에서 조갑하흑색종의 침윤이 있는 경우 침윤의 깊이가 깊고 임파전이, 원격전이, 짧은 무병생존율 등을 보여 중요한 예후인자로 생각된다.

Keywords: 악성흑색종, 조갑하흑색종, 위험인자, 기능적 수술, 예후

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