Histopathological approach for differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita

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Histopathological approach for differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

<ABSTRACT>

Histopathological approach for differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita

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Autoimmune subepidermal bullous diseases (ASBDs) are characterized by the presence of autoantibodies against dermal–epidermal junction structural components. These diseases include several disorders for which molecular target antigens have been identified, and these diseases share clinical characteristics including tense blisters and erosions. However, ASBDs cannot generally be differentiated with clinical features alone. In particular, differential diagnosis of bullous pemphigoid (BP) and inflammatory type epidermolysis bullosa acquisita (EBA) is challenging as the diseases share similar clinical and histopathological findings. The purpose of this study was to examine the reliability of histopathological diagnostic methods for differentiating BP and EBA.

Immunohistochemical staining of type IV collagen (COL4) was performed using 24 BP and 12 inflammatory type EBA skin biopsy samples. The site of COL4 staining was checked to determine if it appeared on blister roof or base. The composition of inflammatory cells including eosinophils, neutrophils, basophils, and mast cells was analyzed in each tissue specimen. Electron microscopic examination of skin biopsy specimens was performed for 11 patients with BP and 3 patients with EBA.

COL4 staining for BP diagnostic sensitivity and specificity were 100% and 82.8%,

respectively. For EBA, sensitivity and specificity were 58.3% and 100%, respectively. There was a statistically significant difference in inflammatory cell infiltration. BP had more mast cell (p=0.030) and eosinophil (p=0.002) infiltration than EBA, while EBA had more neutrophil (p=0.049) infiltration. There was no significant difference in basophil infiltration. Basal cell damage and, inflammatory cell infiltration into the epidermis was seen in BP biopsy tissue on both electron microscopy and H&E staining. In addition, these inflammatory cells were attached to necrotizing basal epidermal cells, appearing to 'hang' onto the basal cells. However, these 'hanging' cells and infiltration findings were not seen in EBA.

COL4 immunostaining is a reliable diagnostic marker for BP, but not EBA. Inflammatory cell composition analysis,especially for neutrophils and eosinophils is a reliable diagnostic tool. The histopathologic feature of basal cell damage with inflammatory cell attachment to basal cellsmay also be a characteristic diagnostic features of BP.

KeyWords:Bullous pemphigoid, Epidermolysis bullosa acquisita, Type IV collagen

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I. Introduction

Autoimmune subepidermal bullous diseases (ASBDs) are characterized by the presence of autoantibodies againstdermal–epidermal junction structural components.[1]

ASBDs include several disorders for which molecular target antigens have been identified including bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), mucous membrane pemphigoid, linear IgA dermatosis, and anti-p200 pemphigoid. ASBDs cannot be easily distinguished with clinical features alone as these diseases commonly show similar clinical features such as erythematous tense blisters. In particular, differential diagnosis of BP and inflammatory EBA is challenging because these diseases share similar clinical and histopathological features.

BP is the most common ASBD characterized by autoantibodies targeting the 180-kDa BP antigen (BP180, BPAG2, or type XVII collagen) and/or the 230-kD BP antigen (BP230 or BPAG1).[2] EBA is caused by autoantibodies against type VII

collagen (COL7) which is a major anchoring fibril component in the sublamina densa.[3] EBA has two major clinical subtypes, the mechanobullous and inflammatory variants. The mechanobullous subtype presents with tense blisters and skin fragility preferentially localized to extensor skin surfaces at trauma-prone areas while the inflammatory subtype resembles other autoimmune bullous diseases. BP and inflammatory EBA possess overlapping clinical presentations including erythematous pruritic blisters and upon histopathological examination, both show subepidermal separation with inflammatory cell infiltrations. Moreover, direct immunofluorescence (DIF) shows linear deposition of immunoglobulin G and complement along the basement membrane zone in both diseases. Therefore, the differential diagnosis for these two diseases is commonly based on a combination of autoantibody serologic detection with indirect immunofluorescence (IIF) using salt split skin, and identification of autoantigens with immunoblotting or enzyme-linked immunosorbent assay (ELISA). However, these autoantigen detection methods are expensive, time consuming, and require special technical skills which are not available in many institutions. More practical and easier alternative methods for differentiating these two diseases are required.

Two hemidesmosomal proteins, BP180 and BP230 are target antigens in BP. BP230 is an intracellular plakin family protein in the hemidesmosomal plaque and BP180 is a transmembrane collagenous protein that ultrastructurally spans the lamina lucida. BP180 binds to laminin 332, which binds to type IV collagen (COL4), a major component of the lamina densa.[4] Below the lamina densa, anchoring fibrils, composed of type VII collagen, are connected to instantiate dermal-epidermal junction integrity.

Ultrastructurally, separation occurs in the lamina lucida in BP and the sublaminadensa in EBA. Immunohistochemical staining for COL4 could help identify the cleavage site in ASBDs. Therefore, it might be a useful diagnostic tool for differentiating BP and EBA. If blisters occur within the lamina lucida, COL4 generally lines the floor of the blister cavity and this indicates BP. If the site of

separation is deep to the lamina densa, it is immunolocalized to the roof of the blister cavity and most likely indicates EBA.[5]

In addition, inflammatory cell populations, including neutrophils, eosinophils, basophils and mast cells found in skin biopsy tissue from BP and EBA could provide cluesfor differential diagnosis. Complement activation at the dermal–epidermal junction, neutrophils, macrophages, mast cells, and various proteases are known to be important for blister formation.[6] Differences in these cellular infiltrations may be helpful for differential diagnosis. Additionally, electron microscopic findings could reveal ultrastructural differences between these two diseases.

II. Materials and methods

1. Clinical materials

Histological examinations were performed on paraffin-embedded biopsy specimens from 24 patients with BP and 12 patients with EBA. Diagnosis was confirmed by autoantibodies detected DIF, salt-split IIF, immunoblotting, and enzyme-linked immunosorbent assay (ELISA).

2. Immunohistochemical staining

COL4 staining was performed using anti-COL4 monoclonal antibodies (ab6586) (Abcam, Cambridge, MA, USA). COL4 stained sites were checked for location (dermal-epidermal separation roof or base). Diagnostic sensitivity and specificity were measured.

Inflammatory cell composition was analyzed for each tissue specimen. Toluidine blue staining was performed to detect mast cells, and anti-basophil antibody (ab155577) (Abcam, Cambridge, MA, USA) was used to detect basophil. Eosinophils and neutrophilswere detected on H&E stained slides. The absolute number of infiltrating cells in the bulla cavity and dermis was counted using two different light microscopic fields at 400x magnification.

3. Electron microscopic examination

Electron microscopic examinations of skin biopsy specimen were performed for 11 patients with BP and 3 patients with EBA. Biopsy specimens for electron microscopic examination were obtained from blister margin, and fixed in 4% formaldehyde and 1% glutaraldehyde in 0.1M PBS (pH 7.4). Specimens were post-fixed in 1% osmium tetroxide, and then dehydrated in ethanol. Ultrathin sections at

60-90 nm thick on the grids were stained with uranyl acetate, and were observed with an electron microscope (GEOL, Japan, Tokyo).

4. Statistical analysis

Descriptive statistics for quantitative values were expressed as mean (±SD) or median (±Q) in accordance with the data distribution. Frequencies and percentages were used to describe categorical variable data. COL4 staining level diagnostic sensitivity and specificity for BP and EBA identification was measured. The statistical significance for differences in inflammatory cell infiltrations between the two diseases was assessed using a t-test. Statistical significance was defined as $p< 0.05$. Statistical analyses were performed using SPSS version 21.0 (SPSS, Inc)

III. RESULTS

1. COL4 immunohistochemical staining

All 24 BP tissue biopsies (100%) showed COL4 staining at the blister base. However, for EBA biopsy tissues, 1 biopsy tissue (8.3%) showed COL4 staining at the blister roof, 6 (50%) showed staining both at the roof and base, and 5 tissues (41.6%) showed COL4 staining at the blister base (Table 1, Figure 1). COL4 staining diagnostic sensitivity and specificity for BP were 100% and 82.8% respectively. For EBA, sensitivity and specificity were 58.3% and 100%, respectively (Table 2).

Table 1. Level of type IV collagen staining in bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA)

Level	Number of patients (%)	
	BP	**EBA**
Base	24 (100)	5 (41.7)
Roof	0 (0)	1 (8.3)
Roof and base	0 (0)	6 (50)
Total	24 (100)	12 (100)

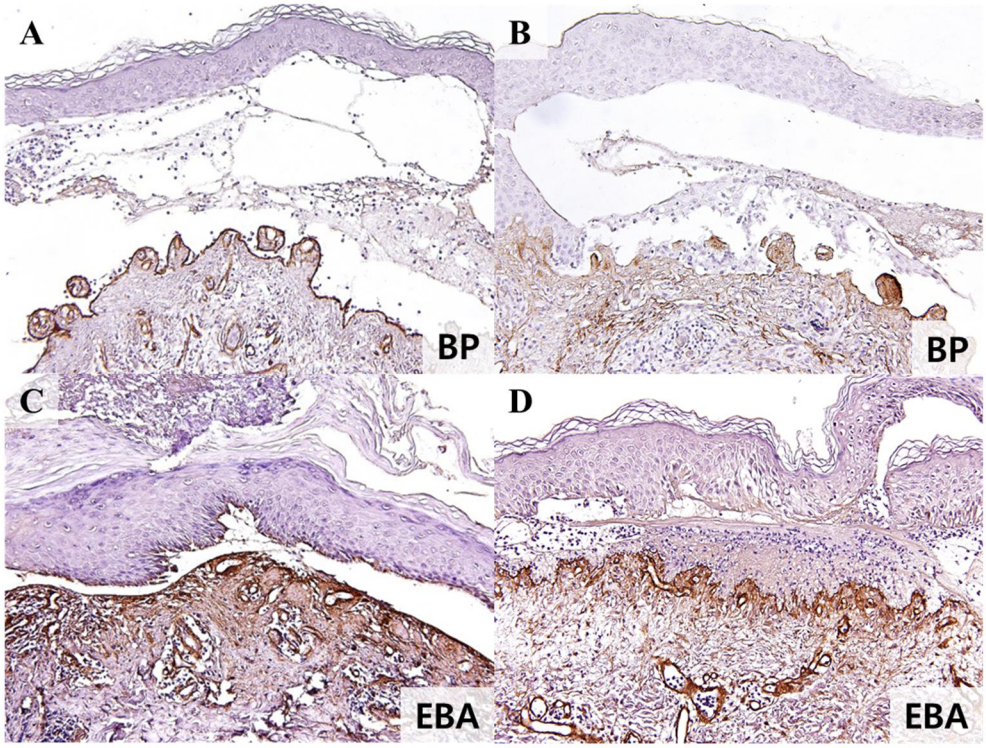

Figure 1. Histopathologic analysis of bullous pemphigoid (BP) and epidermolysis bullosa acquisiata (EBA) biopsy tissue. COL4 staining is shown **(A), (B)** on the base of BP biopsy tissue and **(C)** on the both roof and base, or **(D)**on the base of EBA biopsy tissue. (x200)

Table 2. COL4 staining diagnostic sensitivity and specificity for BP and EBA

	BP	EBA
Sensitivity	100%	58.3%
Specificity	82.8%	100%

2. Immunohistochemical staining

There was a statistically significant difference in inflammatory cell infiltration. BP tissue showed more eosinophil infiltration than EBA tissue in both the bulla cavity (p=0.02) and dermis (p<0.001) and more mast cell infiltration than EBA tissue in the dermis (p=0.030). EBA tissue showed more neutrophil infiltration in the bulla cavity than BP tissue (p=0.049). Only a few basophils were detected in both BP and EBA tissues, and there was no statistically significant difference in basophil infiltration (Figure 2, 3).

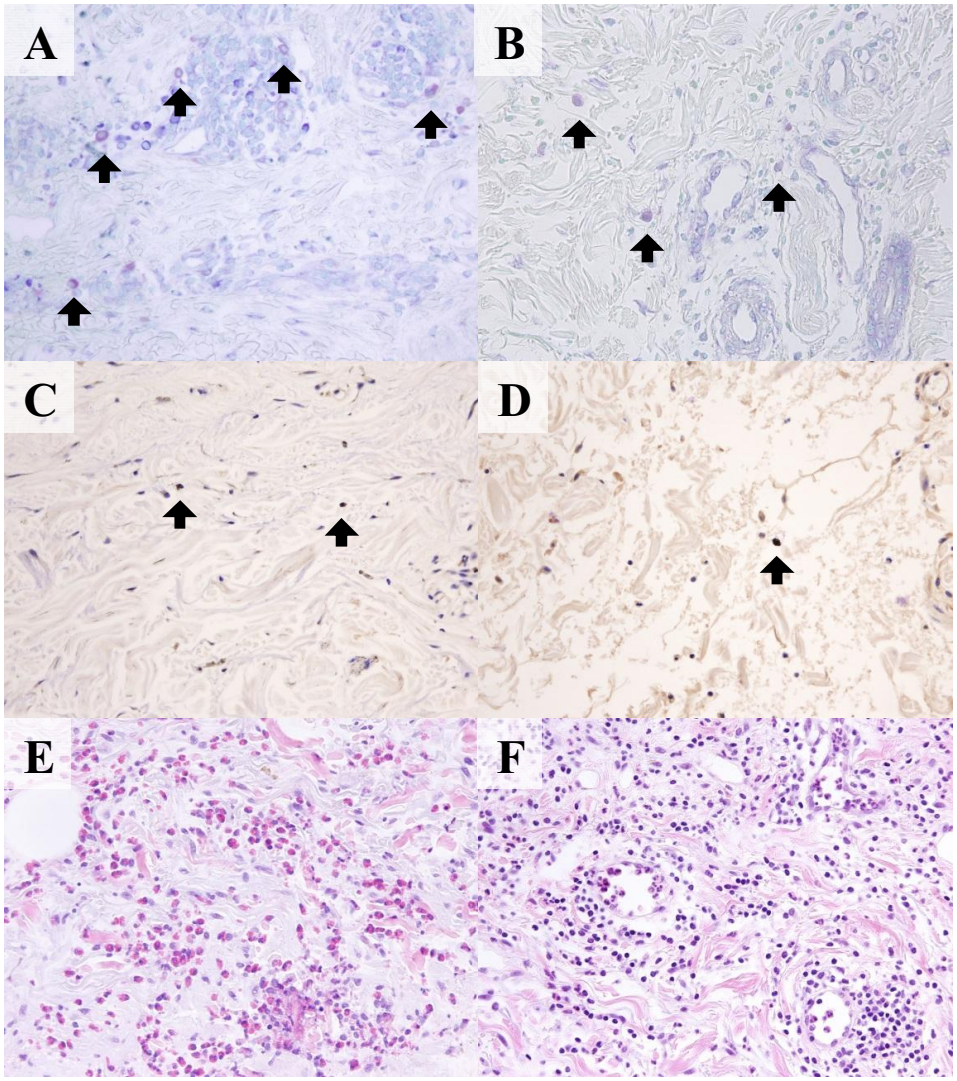

Figure 2. Histopathologic analysis of bullous pemphigoid (BP) and epidermolysis bullosa acquisiata (EBA) biopsy tissue. Toluidine blue staining shows mast cell infiltration in **(A)** BP and **(B)** EBA biopsy tissue. Basophil infiltration on the **(C)** BP and **(D)** EBA biopsy tissue (Cells are remarked with black arrows). Eosinophil and neutrophil count was done with H&E stained slide for **(E)** BP and **(F)** EBA biopsy tissue, respectively. (x400)

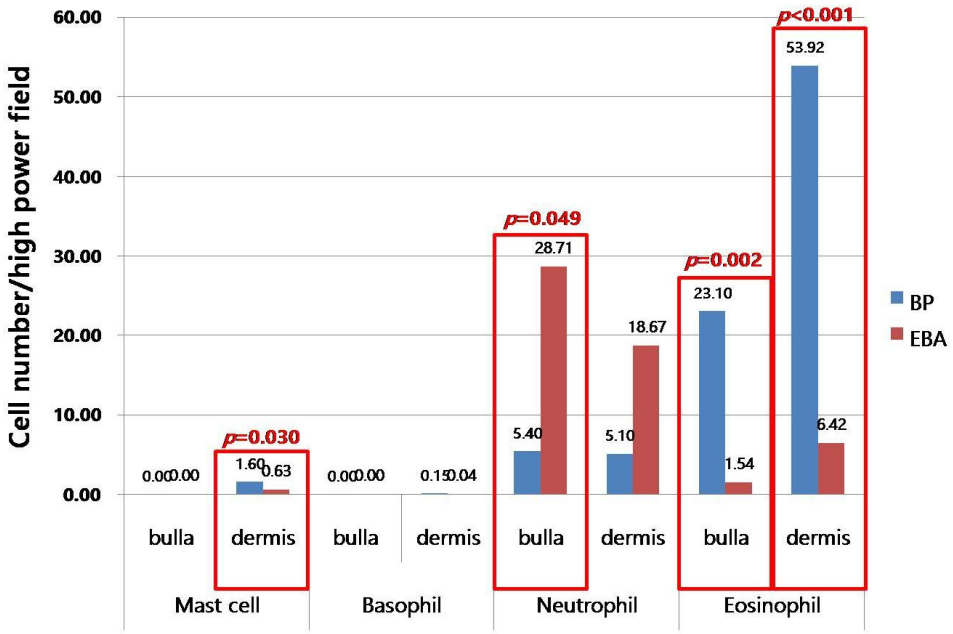

Figure 3. Inflammatory cell composition in bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) skin biopsy tissue. BP tissue has more mast cell infiltration than EBA tissue in dermis (p=0.030) and more eosinophil infiltration in both bulla cavity (p=0.02) and dermis (p<0.001). EBA tissue had more neutrophil infiltration in bulla cavity than BP tissue (p=0.049). There was no significant difference in basophil infiltration between BP and EBA tissue.

3. Electron microscopic findings

BP specimens showed separation at the lamina densa, whereas EBA specimens showed separation at the sublaminadensa level. BP specimens showed several distinguishing features: (i) basal cell damage and (ii) inflammatory cell infiltration into the epidermis breaking the lamina densa (e.g. lymphocytes and eosinophils). These findings were not remarkable in EBA specimens. (Figure 4)

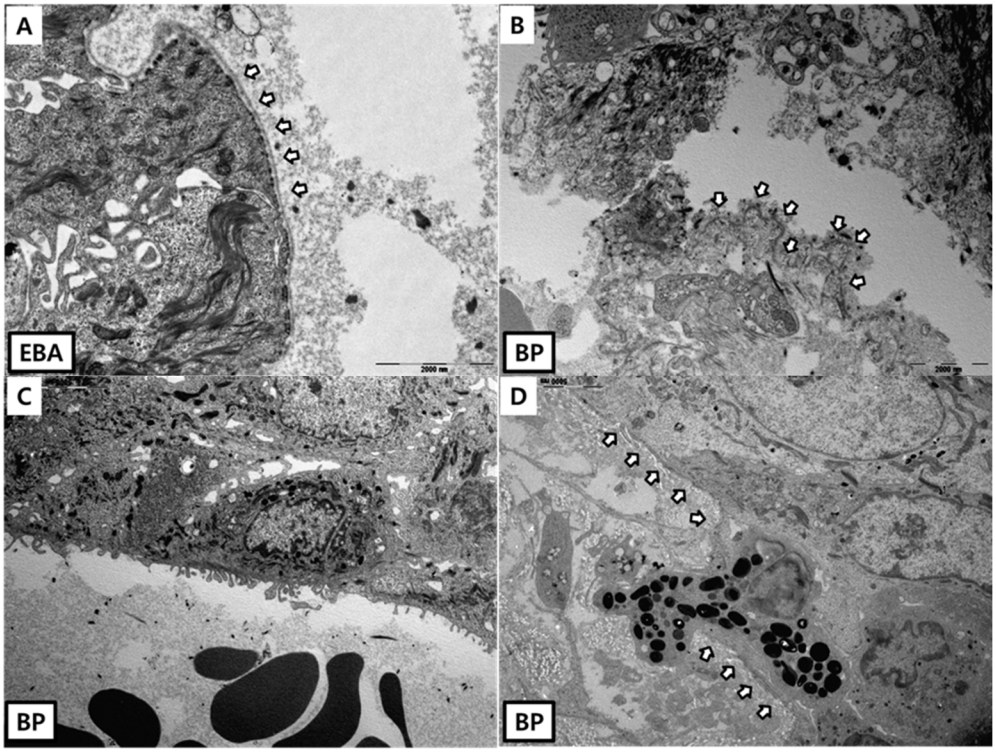

Figure 4. Electron microscopic findings. (Lamina densa is remarked with white arrows.) In epidermolysis bullosa acquisita, **(A)** lamina densa is seen below the epidermis. Separation occurred on the sublaminadensa level. Bullous pemphigoid showed **(B)** separation occurred over the lamina densa. **(C)** Basal cell damage with herniation of cytoplasm and **(D)** inflammatory cells, such as lymphocytes and eosinophils infiltration into the epidermis breaking the lamina densa were seen.

4. Additional findings

Basal cell damage (21 of 24 patients, 87.5%) and inflammatory cell infiltration (18 of 24 patients, 85%) into the epidermis were seen in BP tissue biopsies not only on electron microscopy but in H&E stained samples. In EBA biopsy tissue, basal cell damage was seen in 3 of 12 patients (25%). Inflammatory cells attached to necrotizing basal epidermal cells (13 of 24 patients, 54.2%) were observed in BP, and appeared to be‘hanging’ on the basal cells. However, this ‘hanging’ or infiltration finding was not seen in EBA (Table 3, Figure 5).

Table 3. Histopathological findings of basal cell necrosis, cell 'hanging' and infiltration

Findings	Number of patients (%)	
	BP	**EBA**
1. Basal cell damage	21 (87.5)	3 (25)
2. Inflammatory cell 'hanging'	13 (54.2)	0 (0)
3. Cell infiltration into epidermis	18 (85)	0 (0)
4. None	1 (4.2)	9 (75)
Total	24 (100)	12 (100)

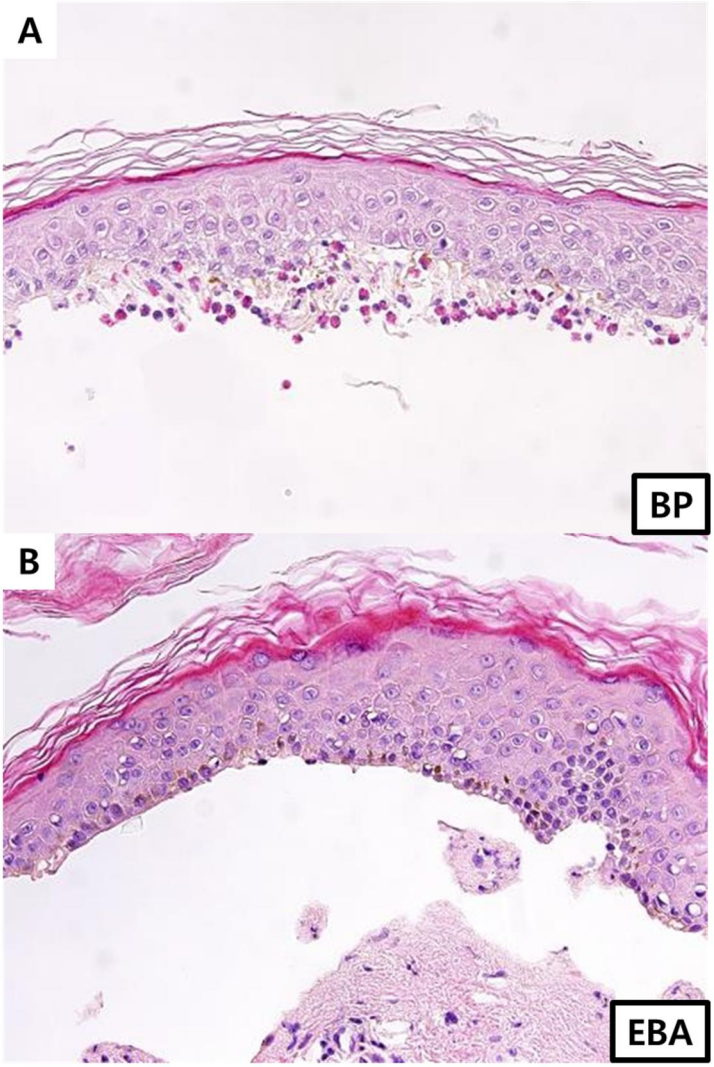

Figure 5. (A) In bullous pemphigoid, damaged basal cells are seen. Inflammatory cells are attached to the necrotizing basal epidermal cells. It seems like they are 'hanging' on the basal cells. **(B)** These findings are not remarkable in EBA. (H&E, x400)

IV. Discussion

Differential diagnosis for autoimmune vesicobullous diseases depends on the combination of clinical findings, histological features, and immunofluorescence study results. Although recent studies have led to better understanding of their pathophysiology, diagnosis remains challenging due to complex pathogenic mechanisms and morphological overlap. BP and EBA share common clinical characteristics and even histopathological analysis with H&E staining shows similar findings for subepidermal blister formation, which makes diagnosis more difficult. In this study, we examined the reliability of COL4 immunohistochemical staining of biopsy specimens as a useful diagnostic tool for BP and EBA.

COL4 is a nonfibrillar collagen which is a major component of the lamina densa. Self-assembly of type IV collagen suprastructure and laminin polymer drives basement membrane assembly.[7] BP180 and BP230, target autoantigens for BP locates upper side of the lamina densa, thus blister formation in BP leaves COL4 and laminin on the floor of the blister. On the other hand, COL7 which is an autoantigen for EBA locates below the lamina densa, leaving COL4 on the roof of the blister.

In this study all BP skin biopsy specimens showed COL4 staining on the blister base as expected. However, almost all (11 of 12) EBA skin biopsies showed COL4 staining on the blister base. Moreover, 5 of them showed COL4 deposition only on the blister base. This might be because the proteolytic enzymes secreted by inflammatory cells can damage the lamina lucida, which is fragile enough to be broken by these enzymes. In EBA, the secretion of reactive oxygen species and matrix metalloproteinases (MMPs) from immune complex-activated neutrophils directly damages the dermal-epidermal junction.[8] This enzymatic damage influences the weak lamina lucida layer, and separation can occur at the lamina lucida level. Another possible explanations is that the COL7-NC1 domain, which anchors COL7 to the basal lamina, works as a binding site for EBA autoantibodies,

and therefore epidermal separation in EBA occurs adjacent to the lamina densa.[9] This might result in irregular separation above and below the lamina densa. The results of this study suggest that COL4 immunohistochemical staining in biopsy tissue might not be a reliable diagnostic marker, at least for EBA.

Inflammatory cells presence in each biopsy (e.g. neutrophils, eosinophils, mast cells, and basophils) was also analyzed in this study. BP biopsies showed more eosinophil infiltration in both the bulla cavity (p=0.02) and dermis (p<0.001) and more mast cell infiltration compared to EBA biopsies in the dermis (p=0.030). In contrast, EBA biopsiesshowed more neutrophil infiltration in the bulla cavity than compared to BP biopsies (p=0.049). Only scant infiltration of basophils was observed in both BP and EBA biopsies and there was no significant difference in basophil infiltration between BP and EBA biopsies.

The presence and degranulation of mast cells at BP lesion sites were first reported by Wintroub et al. in 1978.[10] Subepidermal blistering induced by pathogenic anti-BP180 antibodies depends on mast cells, which play an essential role in recruiting neutrophils to the target tissue via degranulation.[11] This suggests that mast cells are a key player in the inflammatory cascade leading to blister formation in BP.[12] However, Kasprick et al.[13] revealed that mast cells do not contribute to immune-mediated tissue injury in EBA. In the murine EBA model, mast cell activation and mast cell-dependent edema formation were observed, but mast cells did not contribute to blister induction and depletion of mast cells had no impact on disease severity.[13] These studies imply that mast cells play a key role in blister formation in BP, but are less important in EBA blister pathogenesis. This is consistent with our result that BP biopsies showed more mast cell infiltration than EBA.

Neutrophil recruitment and activation occur in both BP and EBA. It has been known that complement activation, such as C5a, mediates neutrophil recruitment into the skin during the early inflammation phase.[14] Skin inflammation is amplified by the recruitment of additional neutrophils, mediated by liberation of several

proteases, which are detected in lesional skin and blister fluid, resulting in local tissue damage in the basement membrane.[9] Further studies are needed to investigate the pathophysiology of the difference in neutrophil infiltration density seen between BP and EBA.

Eosinophils appear to play an essential role in BP initiation and progression, while few have been known in EBA.[15] Eosinophils are often found scattered throughout the upper dermis or aggregated at the edge of the dermal–epidermal junction in BP.[16] With regards to the pathogenesis of BP, studies have shown T-lymphocytes react against the BP180 ectodomain[17] and subsequently recruit eosinophils via production of interleukin-5 (IL-5) and eotaxin.[18] IL-5 and eotaxin are thought to increase inflammatory reactions and contribute to granulocyte influx. This lead to proteinase or cytotoxic agent release, including eosinophil major basic protein (MBP) and eosinophilic cationic protein (ECP) which results in separation of the epidermis and dermis.[18]

In this study, few basophil infiltrations were detected in both BP and EBA specimens and there was no statistically significant difference in the number of infiltrating cells. Basophil function is not well characterized in autoimmune bullous diseases. A pathological ultrastructural study done by Dvorak et al.[19] in 1982 revealed that there was no difference in basophil number and activity between normal and lesional skin tissue. Although basophils are known as a major source of Th2 cytokines, it remains unknown whether they mediates pathological mechanisms in Th2-dominant autoimmune diseases.

Findings upon electron microscopic examination were consistent with different pathophysiology and separation levels for the two diseases. In BP, separation was seen between the lamina densa and basal cells, whereas separation in EBA was seen below the lamina densa.

One other notable finding is that, the damaged basal cells with cytoplasm

herniation were seen in BP specimens. This finding was also seen in H&E stained sections. Moreover, inflammatory cells were adjoining these damaged basal cells, as if hanging on the basal cell layer. Interestingly, these findings were not observed in EBA specimens. This histological finding might be related to BP pathophysiology, and further investigation is needed to reveal the mechanism.

V. Conclusion

In conclusion, this study indicated that COL4 immunostaining offered an alternative means to assist in the diagnosis of BP, but may not be a reliable diagnostic tool for EBA. In addition, inflammatory cell composition analysis, especially for neutrophils and eosinophils, and the histopathological features of basal cell damage with inflammatory cell attachment to basal cells could represent characteristic BP diagnostic features.

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ABSTRACT (IN KOREAN)

유천포창과 후천성 수포성 표피박리증의 감별진단을 위한 조직학적 연구

<지도교수 김수찬>

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홍원진

자가면역성표피하수포성 질환 (Autoimmune subepidermal bullous disease, ASBDs)은 표피진피경계부의 구조적 구성 성분들에 대한 자가 항체로 인해 발생하는 질환군이다. 여기에는 몇 종류의 자가항원이 밝혀진 질환이 포함되어 있으며, 단단한 수포와 미란 등의 비슷한 임상적 특징을 가지고 있다. 그러나 임상적 소견만으로는 ASBD에 속하는 질환들의 감별 진단이 어려운데, 특히 유천포창(Bullous pemphigoid, BP)과 염증성 후천성 수포성 표피박리증(Epidermolysis bullosa acquisita, EBA)의 경우 임상뿐 아니라 조직학적으로도 매우 유사한 소견을 보여 감별 진단이 어렵다. 본 연구에서, 저자들은 두 주요 ASBD인 BP와 EBA의 감별 진단을 위해 새로운 조직학적 방법을 연구하고자 하였다.

이미 진단이 내려진 24개의 BP 피부 조직검사 검체와 12개의 EBA 피부 조직검사 검체를 이용하여, 4형 콜라겐 (Type 4 collagen, COL4) 염색을 시행하였으며, COL4가 수포의 지붕에 염색되는지 바닥에 염색되는지를 확인하였다. 또한 조직마다

침윤하고 있는 호산구, 호중구, 호염구, 비만 세포의 구성을 분석하였으며, 11개의 BP 및 3개의 EBA 피부 조직에 대한 전자현미경 소견도 분석하였다.

COL4 특수염색을 이용하였을 때 BP에 대한 진단의 민감도와 특이도는 각각 100%와 82.8%였으며, EBA에 대한 민감도와 특이도는 58.3%와 100%였다. 침윤한 염증 세포의 구성에 있어서도 차이를 보였는데, BP에서 더 많은 비만 세포(p=0.030)와 호산구(p=0.002)의 침윤을 보였고, EBA에서 더 많은 호중구(p=0.049) 침윤을 보였으며 호염구의 경우 차이를 보이지 않았다. 전자현미경과 H&E 염색 모두에서 BP 조직의 기저세포 손상과 표피로의 염증세포 침투가 관찰되었는데, 이러한 염증세포들은 괴사하는 기저층 표피 세포에 마치 매달려 있는 듯한 소견을 보였다. 그러나 이러한 소견은 EBA 조직에서는 관찰되지 않았다.

따라서 COL4의 면역화학 염색은 표피하의 어느 위치에서 분리가 일어나는지를 확인하여 BP의 진단에 유용한 방법이 될 수 있으나, EBA의 경우 진단 가치가 떨어진다고 볼 수 있다. 또한, 침윤한 염증세포의 분석과 기저층 세포의 괴사, 염증 세포가 이러한 기저층 세포에 매달려있는 조직학적 소견이 BP의 진단에 도움이 됨을 알 수 있었다.

핵심되는 말 : 유천포창, 후천성 수포성 표피박리증, 4형 콜라겐