Optimum Biopsy Site for Direct Immunofluorescence

Subjects: Dermatology

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Direct immunofluorescence of skin and oral mucosa is a vital diagnostic test for assessing vesiculobullous disorders, vasculitides, and connective tissue diseases. It is a robust and valuable technique that offers essential diagnostic information for many critical dermatoses. Dermatopathologists depend heavily on the data obtained from direct immunofluorescence evaluation to confirm final diagnoses.

Keywords: direct immunofluorescence ; vesiculobullous disorder ; vasculitis ; connective tissue disease ; oral mucosa

1. Introduction

Laboratory immunodermatology studies provide an excellent set of tools that can help diagnose various skin conditions. Direct immunofluorescence (DIF) is the primary diagnostic ancillary test used to evaluate vesiculobullous disorders (VBD), vasculitides, and connective tissue diseases (CTD). This technique targets abnormal proteins in the patient's tissue using antibody–fluorophore conjugates. Compared to indirect immunofluorescence and enzyme-linked immunosorbent assay, DIF is more sensitive in detecting bullous pemphigoid (BP) [1].

2. Vesiculobullous Disorder

Direct immunofluorescence is a crucial diagnostic tool for autoimmune VBD. A 4 mm punch biopsy is the recommended method for DIF. If a shave biopsy must be done, it should be deep enough to include the reticular dermis. In the case of BP, DIF biopsy is traditionally taken from perilesional skin, and the center of active blisters and distant uninvolved or never-involved sites are avoided [2][3]. Anstey et al. concluded in their study that a single perilesional biopsy is usually sufficient to provide results in both pre-treatment and post-treatment cases of BP [4]. More recently, Sladden et al. demonstrated a higher probability of positive DIF results in BP from lesional non-bullous (urticarial or pre-bullous) skin than perilesional or normal skin [5]. Other techniques have been suggested for subepidermal VBD, where the biopsy is taken from the edge of an active blister to contain part perilesional skin and part bullous lesion [6]. This technique can provide a bonus salt-split-like analysis in the bullous zone; however, if not correctly executed, the epidermis can completely separate from the dermis and compromise the evaluation.

For BP and other autoimmune subepidermal VBDs (i.e., pemphigoid gestationis, lichen planus pemphigoides, epidermolysis bullosa (EB) acquisita, bullous lupus erythematosus (LE), and linear IgA bullous dermatosis), all the approaches mentioned above can provide the desired results. The recommended site of DIF biopsy is perilesional erythematous or uninvolved skin, about 3 mm away from the edge of a blister but no more than 10 mm away, or non-bullous lesional skin, or the edge of an active blister (2/3 perilesional and 1/3 lesional). If in doubt, two biopsies can be taken for DIF, one from the blister's edge and the other from perilesional erythematous or uninvolved skin. Also, the trunk and flexural skin of the forearms are generally preferred.

In the pemphigus group, the convention is to perform a DIF biopsy on perilesional erythematous or uninvolved skin, about 3 mm away from the edge of a blister but no more than 10 mm away. In addition, positive DIF results have been seen with high sensitivity utilizing the outer root sheaths of hair in patients with pemphigus [7]. For this economical, non-invasive technique, anagen hairs from the scalp are forcibly plucked using simple forceps, and immunofluorescence studies are performed on the outer root sheath.

Direct immunofluorescence results are crucial in diagnosing dermatitis herpetiformis (DH). Zone et al. found more intense IgA staining in perilesional normal-appearing, uninvolved skin compared to erythematous skin and distant, never-involved skin [8]. Their study defined perilesional skin as normal-appearing skin located 1 mm to 10 mm from a papulovesicle or area of erythema. The recommended site of DIF biopsy in DH is perilesional normal-appearing, uninvolved skin about 3 mm from the edge of the lesion. If IgA DIF results are negative, but there is a high clinical suspicion of DH, a repeat biopsy for DIF should be considered [9].

When evaluating porphyria and drug-induced pseudoporphyria, it is best to utilize lesional skin for DIF. Skin from the hands or face is preferred, and the biopsy is taken from the edge of an active blister to contain 1/3 perilesional skin and 2/3 lesional skin.

3. Oral Mucosal Biopsy for Vesiculobullous Disorder

The definitive diagnosis of oral mucosal autoimmune VBD requires DIF analysis. However, due to the fragility of the mucosa and the challenging access to certain parts of the mouth, the biopsy procedure can be complicated. As a result, it is advisable to reserve DIF biopsy only for patients with a strong clinical suspicion of autoimmune VBD [10]. Elliptical/wedge incisional scalpel and punch biopsies are utilized, with punch biopsy providing better diagnostic yield and sensitivity [11]. It is crucial to handle the biopsy gently, as mucosal tissue is easily susceptible to crush artifacts. It is vital to obtain a sample that retains its structural integrity with the epithelium attached to the subepithelial connective tissue.

When performing a biopsy of oral VBD for DIF, the traditional practice is to take a perilesional mucosa sample, about 3 mm away from the area of a blister or ulceration but no more than 10 mm away [12][13]. However, normal-looking non-lesional mucosa more than 10 mm away from the lesions can also provide accurate results. A study of a large group of patients with oral pemphigus vulgaris and multi-site mucous membrane pemphigoid (MMP) showed that a 4 mm punch biopsy of normal buccal mucosa delivers DIF results with sensitivity equivalent to perilesional biopsy [14]. This suggests that the immunoreactants may be present in the affected and unaffected oral mucosa. Other analyses confirm that normal-looking mucosa displays similar rates of DIF positivity to perilesional mucosa for MMP; however, perilesional mucosa shows slightly better results for oral pemphigus vulgaris [15]. Normal buccal mucosal punch biopsy offers an uncomplicated and sensitive method that various physicians can dependably employ, particularly in cases where the perilesional sites are technically challenging to access [16]. Due to chronic inflammation and fragility, the gingiva is considered an inferior biopsy site and should be avoided. For localized gingival MMP, neighboring normal reflected alveolar mucosa can be biopsied for DIF.

Performing multiple and repeated biopsies can increase the sensitivity of DIF in diagnosing MMP [17]. To achieve an accurate diagnosis, it is recommended to perform two biopsies for DIF, one from the perilesional area and the other from normal-appearing buccal mucosa. However, this approach can sometimes be redundant and impractical and should only be used selectively. Diagnosing ocular MMP can be challenging since up to 50% of cases do not meet the immunopathological criteria necessary for the diagnosis. This often leads to delayed diagnosis and poor outcomes for patients. To improve the sensitivity of DIF in complex cases, it is suggested to perform DIF biopsies of both bulbar conjunctiva from non-inflamed areas and normal buccal mucosa [18]. As the DIF of buccal mucosa can be positive even when the conjunctiva is negative, this approach can help establish an accurate and timely diagnosis.

4. Hereditary Epidermolysis Bullosa

Inherited EB is diagnosed and subtyped with the help of immunofluorescence antigen mapping and transmission electron microscopy. However, testing existing blisters (over 12 h old) is not recommended due to proteolytic antigenic degradation and artifacts caused by reepithelization. Instead, it is suggested to induce a fresh blister for testing purposes. Normal intact skin is selected, preferably neighboring where the patient usually gets blisters. The upper inner arm skin is preferred for this purpose, while the glabrous skin of palms and soles is avoided.

To induce a fresh blister, the skin is rubbed using a pencil eraser (or gloved finger, or cotton swab) with firm downward pressure followed by rotating it 180 degrees in each direction 3 to 5 times [19]. The skin is rubbed at least twenty times until it turns red. After the skin turns red, pause for about 5 min to allow for the development of a microscopic blister. Two punch biopsies are performed from the red area, one for immunofluorescence antigen mapping (submit in Michel's or Zeus medium) and the second for electron microscopy (submit in 2.5% glutaraldehyde solution). If a blister develops at the site of rubbing, execute the biopsies so that each sample contains part of the blister's edge and part of the perilesional skin (i.e., 2/3 perilesional and 1/3 lesional). In severe cases with extreme skin fragility, rubbing may not be required as the cleavage plane may form simply by the twisting motion of the punch biopsy procedure. It is recommended to avoid topical anesthetics as they may cause artefactual blistering, and injectable anesthetics are preferred.

It is important to note that these tests for hereditary EB are not routine at all laboratories and are frequently sent to specialized centers. As standards of specimen acceptance may vary, before scheduling a biopsy, the clinician should collaborate with the laboratory regarding additional collection and transport-related instructions [20].

5. Cutaneous Vasculitis

When it comes to diagnosing cutaneous vasculitis, the timing and location of the biopsy are critical factors for accurate interpretation. In immune complex-mediated vasculitis, diagnostic immunofluorescence highlighting immunoreactant deposition is inversely related to the duration of the lesions [21][22]. Immunoglobulin deposits tend to fade in older purpuric lesions and the highest percentage of biopsies showing the presence of immunoglobulins are observed within the initial 48 h after the lesion first appears. Between 48 and 72 h, the percentage of biopsies that show immunoglobulin positivity decreases to 70%, and after 72 h, no immunoglobulins are detected. Complement deposition lasts longer than immunoglobulins, with more than 50% of cases still showing positivity after 72 h. A study by Nandeesh and Tirumalae demonstrated that biopsy timing can affect the positivity of DIF in vasculitis [23]. They found that 85% of biopsies performed within the first 7 days were positive, 14% were positive between 1 and 2 weeks, and only 1% were positive between 2 weeks and 1 month.

In a study conducted by Giangiacomo and Tsai, 14 pediatric patients suffering from Henoch–Schönlein purpura were analyzed for DIF of purpuric lesional and normal-appearing uninvolved skin [24]. The study found that 93% of the biopsies from the lesional skin displayed vascular IgA positivity compared to only 43% of the biopsies from uninvolved skin, with half of the positive cases from uninvolved skin showing only weak staining. However, a study by Van Hale et al. displayed comparable vascular IgA positivity between lesional and uninvolved skin [25].

In vasculitis, the biopsy for DIF should be obtained from the edge or active border of a fresh pink blanchable macule, as immunoglobulins degrade with time in the center of older lesions [26]. The lesion selected should be newly evolved, ideally between 8 and 24 h, and no older than 48 h. DIF should not be performed on old necrotic or ulcerated lesions and hemorrhagic blisters. It is suggested to avoid sampling from distal lower legs if the patient has long-standing venous stasis-induced changes [27]. Normal-appearing uninvolved skin adjacent to lesions can also provide desired DIF results; however, it has a low sensitivity and high false negative rate.

6. Connective Tissue Disease

Direct immunofluorescence is used to investigate CTD, particularly the various expressions of LE. CTD is a diverse group of disorders with distinct clinical presentations, histopathological features, and immunological profiles. The immunological changes lead to autoantibodies, immune complexes, and immune deposits in the skin. Serological tests can identify the existence of circulating autoantibodies, while DIF can determine cutaneous immune deposits. Although DIF has been used to diagnose CTD, serological testing is considered more reliable. In fact, with the availability of improved serum serological tests, DIF may be unwarranted and only required in selected cases.

The sensitivity of DIF results is affected by various factors in LE, including clinical morphology, the duration of lesions, biopsy location, and the patient's treatment status. As the lesion's age increases, the frequency of positive DIF also rises. DIF positivity is higher in untreated lesions than in treated ones [28]. Treatment up to three weeks before biopsy reduces the frequency of DIF positivity. The positivity rate is also influenced by the location of the biopsy site on the body, with sun-exposed skin showing a higher frequency of DIF positivity than sun-protected skin. Sun-exposed healthy skin of up to 20% of normal adults can show non-specific false-positive staining at the dermoepidermal junction [29].

For evaluating CTD, lesional and uninvolved non-lesional sun-protected skin can be utilized for DIF analysis. In cases where discoid LE, subacute cutaneous LE, or dermatomyositis are suspected, a biopsy is taken from the erythematous active border of an established lesion. To ensure accurate results, the lesion should be older and have a duration of at least three months. Clinically, a 'red and angry' lesion is targeted, while 'burnt-out' lesions are avoided. An untreated lesion from an area not chronically exposed to sunlight is preferred if possible.

In cases where systemic LE or acute cutaneous LE is suspected, two biopsies for DIF are obtained. One biopsy is taken from the erythematous active border of an established lesion, while the second biopsy for the lupus band test is obtained from non-lesional sun-protected skin like the buttocks, abdomen, and inner thigh [30]. It is worth noting that the DIF of perilesional skin does not provide any additional value in CTD diagnosis.

References

1. Sárdy, M.; Kostaki, D.; Varga, R.; Peris, K.; Ruzicka, T. Comparative study of direct and indirect immunofluorescence and of bullous pemphigoid 180 and 230 enzyme-linked immunosorbent assays for diagnosis of bullous pemphigoid. J. Am. Acad. Dermatol. 2013, 69, 748–753.

2. Elston, D.M.; Stratman, E.J.; Miller, S.J. Skin biopsy: Biopsy issues in specific diseases. J. Am. Acad. Dermatol. 2016, 74, 1–16.
3. Cocks, M.M. A Starter Guide to Immunofluorescence Testing in Dermatology. Cutis 2021, 108, E23–E26.
4. Anstey, A.; Venning, V.; Wojnarowska, F.; Bhogal, B.; Black, M.M. Determination of the optimum site for diagnostic biopsy for direct immunofluorescence in bullous pemphigoid. Clin. Exp. Dermatol. 1990, 15, 438–441.
5. Sladden, C.; Kirchhof, M.G.; Crawford, R.I. Biopsy location for direct immunofluorescence in patients with suspected bullous pemphigoid impacts probability of a positive test result. J. Cutan. Med. Surg. 2014, 18, 392–396.
6. Braswell, M.A.; McCowan, N.K.; Schulmeier, J.S.; Brodell, R.T. High-yield biopsy technique for subepidermal blisters. Cutis 2015, 95, 237–240.
7. Tanasilovic, S.; Medenica, L.; Popadic, S. Direct immunofluorescence of the outer root sheath in anagen and telogen hair in pemphigus vulgaris and pemphigus foliaceus. Australas. J. Dermatol. 2014, 55, E74–E76.
8. Zone, J.J.; Meyer, L.J.; Petersen, M.J. Deposition of granular IgA relative to clinical lesions in dermatitis herpetiformis. Arch. Dermatol. 1996, 132, 912–918.
9. Caproni, M.; Antiga, E.; Melani, L.; Fabbri, P.; Italian Group for Cutaneous Immunopathology. Guidelines for the diagnosis and treatment of dermatitis herpetiformis. J. Eur. Acad. Dermatol. Venereol. 2009, 23, 633–638.
10. Bresler, S.C.; Bavarian, R.; Granter, S.R.; Woo, S.B. Direct immunofluorescence is of limited utility in patients with low clinical suspicion for an oral autoimmune bullous disorder. Oral. Dis. 2020, 26, 81–88.
11. Gilvetti, C.; Collyer, J.; Gulati, A.; Barrett, A.W. What is the optimal site and biopsy technique for the diagnosis of oral mucosal autoimmune blistering disease? J. Oral Pathol. Med. 2019, 48, 239–243.
12. Suresh, L.; Neiders, M.E. Definitive and differential diagnosis of desquamative gingivitis through direct immunofluorescence studies. J. Periodontol. 2012, 83, 1270–1278.
13. Sano, S.M.; Quarracino, M.C.; Aguas, S.C.; González, E.J.; Harada, L.; Krupitzki, H.; Mordoh, A. Sensitivity of direct immunofluorescence in oral diseases. Study of 125 cases. Med. Oral. Patol. Oral Cir. Bucal. 2008, 13, E287–E291.
14. Carey, B.; Joshi, S.; Abdelghani, A.; Mee, J.; Andiappan, M.; Setterfield, J. The optimal oral biopsy site for diagnosis of mucous membrane pemphigoid and pemphigus vulgaris. Br. J. Dermatol. 2020, 182, 747–753.
15. Zeng, Q.; Liu, J.; Mu, J.; Yang, J.; Gao, Q.; Wu, F.; Zhou, H. Optimal biopsy site for the diagnosis of oral pemphigus vulgaris and mucous membrane pemphigoid: A systematic review and meta-analysis. Int. J. Oral Maxillofac. Surg. 2023, 52, 1162–1172.
16. Kamaguchi, M.; Iwata, H.; Ujiie, I.; Ujiie, H.; Sato, J.; Kitagawa, Y.; Shimizu, H. Direct Immunofluorescence Using Non-Lesional Buccal Mucosa in Mucous Membrane Pemphigoid. Front Med. 2018, 5, 20.
17. Shimanovich, I.; Nitz, J.M.; Zillikens, D. Multiple and repeated sampling increases the sensitivity of direct immunofluorescence testing for the diagnosis of mucous membrane pemphigoid. J. Am. Acad. Dermatol. 2017, 77, 700–705.
18. Schmidt, E.; Rashid, H.; Marzano, A.V.; Lamberts, A.; Di Zenzo, G.; Diercks, G.F.H.; Alberti-Violetti, S.; Barry, R.J.; Borradori, L.; Caproni, M.; et al. European Guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European Academy of Dermatology and Venereology—Part II. J. Eur. Acad. Dermatol. Venereol. 2021, 35, 1926–1948.
19. Intong, L.R.; Murrell, D.F. How to take skin biopsies for epidermolysis bullosa. Dermatol. Clin. 2010, 28, 197–200.
20. Epidermolysis Bullosa Sample Submission Instructions. Available online: https://stanfordlab.com/anatomic-pathology/dermatopathology.html#submit-a-specimen (accessed on 10 December 2023).
21. Sais, G.; Vidaller, A.; Jucglà, A.; Servitje, O.; Condom, E.; Peyri, J. Prognostic factors in leukocytoclastic vasculitis: A clinicopathologic study of 160 patients. Arch. Dermatol. 1998, 134, 309–315.
22. Carlson, J.A. The histological assessment of cutaneous vasculitis. Histopathology 2010, 56, 3–23.
23. Nandeesh, B.; Tirumalae, R. Direct immunofluorescence in cutaneous vasculitis: Experience from a referral hospital in India. Indian J. Dermatol. 2013, 58, 22–25.
24. Giangiacomo, J.; Tsai, C.C. Dermal and glomerular deposition of IgA in anaphylactoid purpura. Am. J. Dis. Child. 1977, 131, 981–983.
25. Van Hale, H.M.; Gibson, L.E.; Schroeter, A.L. Henoch-Schönlein vasculitis: Direct immunofluorescence study of uninvolved skin. J. Am. Acad. Dermatol. 1986, 15, 665–670.
26. Davin, J.C.; Weening, J.J. Diagnosis of Henoch-Schönlein purpura: Renal or skin biopsy? Pediatr. Nephrol. 2003, 18, 1201–1203.

27. Helander, S.D.; De Castro, F.R.; Gibson, L.E. Henoch-Schönlein purpura: Clinicopathologic correlation of cutaneous vascular IgA deposits and the relationship to leukocytoclastic vasculitis. Acta Derm. Venereol. 1995, 75, 125–129.
28. Dahl, M.V. Usefulness of direct immunofluorescence in patients with lupus erythematosus. Arch. Dermatol. 1983, 119, 1010–1017.
29. Fabré, V.C.; Lear, S.; Reichlin, M.; Hodge, S.J.; Callen, J.P. Twenty percent of biopsy specimens from sun-exposed skin of normal young adults demonstrate positive immunofluorescence. Arch. Dermatol. 1991, 127, 1006–1011.
30. Reich, A.; Marcinow, K.; Bialynicki-Birula, R. The lupus band test in systemic lupus erythematosus patients. Ther. Clin. Risk Manag. 2011, 7, 27–32.