

DIAGNOSTIC ACCURACY OF TZANCK SMEAR AND HISTOLOGY COMPARED TO DIRECT IMMUNOFLUORESCENCE IN AUTOIMMUNE BLISTERING DISEASES

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Received : 29/05/2025
Received in revised form : 17/07/2025
Accepted : 05/08/2025

Keywords:
Pemphigus, Direct Immunofluorescence, Histopathology, Tzanck Smear.

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DOI: 10.47009/jamp.2025.7.4.143

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (4); 764-769



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ABSTRACT

Background: Intraepidermal autoimmune blistering diseases such as pemphigus vulgaris involve autoantibody-mediated disruption of keratinocyte adhesion, leading to acantholysis and blister formation. While Direct Immunofluorescence (DIF) is considered the gold standard for diagnosis, it is not always accessible, especially in resource-limited settings. Therefore, alternative methods such as Tzanck smear and histopathology remain clinically relevant. The objective is to evaluate the diagnostic accuracy of Tzanck smear and histopathology in comparison with DIF in the diagnosis of intraepidermal bullous disorders. **Materials and Methods:** This prospective observational study included 50 patients with clinically suspected intraepidermal blistering diseases, conducted between November 2015 and May 2017. All patients underwent Tzanck smear, histopathology, and DIF, and the performance of each method was assessed using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). **Result:** DIF confirmed 36 cases of pemphigus. Tzanck smear identified 30 true positives with 4 false positives and 6 false negatives, yielding a sensitivity of 83.3% and specificity of 71.4%. Histopathology showed 32 true positives with only 1 false positive and 4 false negatives, resulting in a sensitivity of 88.9% and specificity of 92.9%. Concordance across all three modalities was observed in 28 cases. Tzanck smear showed typical acantholytic cells, while histology demonstrated hallmark features like suprabasal clefting and the tombstone appearance. DIF revealed intercellular IgG in a fishnet pattern. **Conclusion:** While DIF remains the most sensitive and specific method for confirming pemphigus, histopathology serves as a reliable alternative, especially where DIF is unavailable. Tzanck smear is a useful rapid screening tool. An integrated approach using clinical features, cytology, and histology ensures effective diagnosis and early treatment in resource-limited settings.

INTRODUCTION

Autoimmune blistering disorders, particularly pemphigus vulgaris (PV) and its variants, persist as formidable diagnostic and therapeutic challenges in dermatology and pathology worldwide.^[1] These disorders are driven by pathogenic IgG autoantibodies targeting desmosomal adhesion proteins—notably desmoglein 1 and 3—causing loss of keratinocyte cohesion (acantholysis) and leading to suprabasal intraepidermal bullae formation.^[2] The definitive diagnosis of pemphigus is crucial, not only for initiating appropriate immunosuppressive therapy but also to avoid unnecessary exposure to such therapies in patients without autoimmune etiologies. Accurate differentiation of pemphigus

from other blistering dermatoses is critical, as treatment regimens and prognoses differ markedly across entities.^[3] Direct immunofluorescence (DIF) of perilesional skin remains the gold standard for diagnosing pemphigus, revealing a granular or “chicken-wire” deposition pattern of IgG and complement C3 between epidermal keratinocytes, with reported sensitivity approaching 100% in active lesions.^[4] Despite its diagnostic precision, the deployment of DIF is often limited in low-resource settings due to factors such as specialized equipment, high cost, and technical expertise constraints.

Given these limitations, histopathology using routine H&E-stained sections and Tzanck smear cytology continue to be essential modalities,

particularly in peripheral or resource-constrained centers.^[5] Histopathologic hallmarks of PV include suprabasalclefting, acantholytic keratinocytes, and the “tombstone appearance” of basal cells adhering to the dermoepidermal junction—features that help distinguish PV from other intraepidermal bullous conditions.^[6] The Tzanck smear, a rapid cytological technique introduced in 1947, permits bedside identification of acantholytic keratinocytes (Tzanck cells) scraped from the base of fresh blisters.^[7] Although not subtype- or disease-specific, this method has regained interest for its value in early triage. A study of bullous lesions including pemphigus reported 89% sensitivity and 100% specificity of the Tzanck smear compared with histopathology in identifying PV case.^[8] In recent years, a growing body of literature has highlighted that while molecular and serological advancements—such as ELISA assays for desmoglein antibodies, indirect immunofluorescence, and biochip mosaics—have significantly improved the accuracy of disease monitoring and confirmation, they cannot replace tissue-based methods in establishing a definitive primary diagnosis. Particularly in resource-constrained settings, where access to specialized immunodermatopathology services is limited, the continued reliance on practical and accessible diagnostic tools remains essential. These traditional methods, including histopathology and Tzanck smear, play a pivotal role in ensuring timely intervention and guiding appropriate clinical management.^[9,10] Furthermore, clinical differentiation of blistering diseases can be challenging. Many of these conditions, including pemphigus vulgaris, pemphigus foliaceus, and bullous impetigo, may present with clinically indistinguishable erosions and vesicles. Therefore, solely relying on clinical judgment is insufficient. Despite considering overlap in clinical and histological features, careful analysis of combined clinical, cytological, histopathological, and immunological data enables a high level of diagnostic accuracy in most cases.^[2,11] Hence, especially in resource-limited regions, a diagnostic workflow that begins with clinical evaluation and Tzanck smear, followed by histopathology, and ultimately DIF confirmation, remains both practical and effective. This prospective institutional study evaluates the diagnostic performance metrics—sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—of the Tzanck smear and H&E histopathology, using DIF as the reference standard, in a cohort of clinically suspected intraepidermal bullous diseases.

MATERIALS AND METHODS

The present prospective observational study was carried out in the Department of Pathology in

association with the Department of Dermatology at Bangalore Medical College and Research Institute, Bengaluru. The duration of the study was 18 months, extending from November 2015 to May 2017. Institutional ethical clearance was obtained before commencing the study. Written informed consent was taken from all the patients prior to sample collection and diagnostic procedures. The study was designed to include 50 patients presenting to the dermatology outpatient department with clinical features suggestive of intraepidermal blistering disorders, including pemphigus and its variants.

Patients of any age and gender, presenting with clinical signs of intraepidermal blistering disease and who had not initiated prior treatment, were included in the study. The diagnosis was suspected clinically based on the morphology of the lesions, their distribution, and associated symptoms like mucosal erosions and flaccid bullae. Patients already on immunosuppressive therapy, or those diagnosed with subepidermal blistering diseases such as bullous pemphigoid, were excluded from the study.

Tzanck Smear Examination

In each case, a Tzanck smear was obtained by gently unroofing the blister using a sterile scalpel blade and scraping the base of the lesion. The material was spread on a clean glass slide, air-dried, and stained with Giemsa stain. The slides were examined microscopically for the presence of acantholytic cells, characterized by rounded keratinocytes with a large nucleus and pale cytoplasm, which are indicative of intraepidermal acantholysis.

Histopathological Examination

A skin biopsy was taken from the lesional site for histopathological examination. The tissue was fixed in 10% buffered formalin and subjected to routine paraffin processing. Sections of 4–5 µm thickness were stained with hematoxylin and eosin. The stained slides were evaluated for characteristic histological features such as suprabasal cleft formation, acantholysis, tombstone appearance of basal cells, and inflammatory cell infiltrate in the dermis.

Direct Immunofluorescence (DIF)

A separate perilesional skin biopsy was collected and preserved in Michel’s medium for DIF studies. Frozen sections were cut and stained with fluorescein isothiocyanate (FITC)-conjugated antisera specific to human IgG, IgA, IgM, and complement C3. The stained sections were examined under a fluorescent microscope for intercellular and/or basement membrane zone deposition of immunoreactants. The presence of a fishnet pattern of IgG and/or C3 was considered diagnostic of pemphigus.

Data Analysis: The results obtained from Tzanck smear and histopathology were compared with the DIF findings, which served as the gold standard. Statistical measures including sensitivity, specificity, positive predictive value (PPV), and

negative predictive value (NPV) were calculated to assess the diagnostic performance of the two modalities.

RESULTS

A total of 50 patients clinically diagnosed with intraepidermal blistering diseases were evaluated using Tzanck smear cytology, histopathology, and Direct Immunofluorescence (DIF). [Table 1] summarizes the demographic profile. The age distribution showed that 30% of patients were between 31–40 years, followed by 22% in the 21–30 and 41–50 age groups each, and 26% above 50 years. A female preponderance was observed (64% females vs. 36% males). Most cases presented with diffuse blister distribution (80%) and predominantly involved both the skin and mucous membranes (52%). Flaccid blisters were the most common morphology (86%). Tzanck smear positivity was noted in 68% of cases, while DIF positivity was confirmed in 72% of cases. Most patients presented with erosions, flaccid bullae, and crusted plaques. The average duration of disease was 3–6 months in the majority of cases.

Out of the 50 cases examined, DIF confirmed 36 cases as pemphigus and was considered the gold standard in this study. Among the DIF-positive cases, the Tzanck smear yielded positive results in 30 patients, while it failed to detect 6 cases (false negatives) and showed 4 false positives in DIF-negative patients. This translated to a sensitivity of 83.3% and a specificity of 71.4% for the Tzanck smear. In contrast, histopathological examination showed greater diagnostic reliability, with 32 of the 36 DIF-positive cases confirmed on H&E slides. There were only 4 false negatives and a single false positive, resulting in a sensitivity of 88.9% and a specificity of 92.9%.

The characteristic cytological finding on Tzanck smear was the presence of acantholytic cells- large,

rounded keratinocytes with hyperchromatic nuclei and perinuclear halo. In histopathological sections, the hallmark features observed were suprabasal cleft formation, acantholysis, and the tombstone pattern of basal cells seen especially in pemphigus vulgaris. These findings substantiate the diagnostic utility of both techniques. The statistical parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of both tests are summarized in table 2.

When comparing diagnostic agreement across the three methods, it was observed that 28 cases were positive by all three modalities, indicating strong concordance. Six cases were negative by Tzanck smear but positive by histopathology and DIF, while two cases were only positive by DIF. This comparative agreement is shown in table 3, which outlines the distribution of positive and negative results across the diagnostic tools.

The photomicrographs presented in [Figure 1a] illustrate typical findings on Giemsa-stained Tzanck smears, including acantholytic cells (100x), and [Figure 1(b,c,d)] represents H&E-stained sections showing suprabasal blistering. [Figure 2] provides a representative image of DIF showing a classic fishnet pattern of IgG deposition in the intercellular spaces of the epidermis.

Taken together, the results from this study demonstrate the complementary roles of Tzanck smear and histopathology in the diagnostic workup of intraepidermal blistering disorders. While DIF remains the gold standard, the accessibility, speed, and diagnostic correlation provided by cytology and histopathology justify their ongoing use, especially in centers with limited access to immunofluorescence facilities. These findings validate that while DIF is irreplaceable for confirmation, histopathology is a highly reliable standalone diagnostic tool and Tzanck smear serves as an effective rapid screening modality.

Table 1: Demographic characteristics

Characteristics	Frequency	Percentage
Age Group		
21-30	11	22.0
31-40	15	30.0
41-50	11	22.0
>50	13	26.0
Sex		
Male	18	36.0
Female	32	64.0
Blister Distribution		
Localized	10	20.0
Diffuse	40	80.0
Site of Blister		
Skin	16	32.0
Mucus membrane	8	16.0
Both	26	52.0
Type of blister		
Tense	6	12.0
Flaccid	43	86.0
Both	1	2.0
Tznack smear		

Positive	34	68.0
Negative	16	32.0
DIF for Pemphigus		
Positive	36	72.0
Negative	14	28.0

Table 2: Diagnostic accuracy of Tzanck smear and histopathology using DIF as gold standard.

Test	Gold Standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Tzanck Smear	DIF	83.3	71.4	88.2	62.5
Histopathology	DIF	88.9	92.9	97.0	76.5

Table 3: Diagnostic Agreement between Tzanck Smear, Histopathology, and DIF.

Diagnostic Pattern	Tzanck Smear	Histopathology	DIF (Gold Standard)
Positive by all three modalities	Positive	Positive	Positive (n = 28)
Negative by Tzanck smear; positive by HPE and DIF	Negative	Positive	Positive (n = 6)
Positive by DIF only	Negative	Negative	Positive (n = 2)

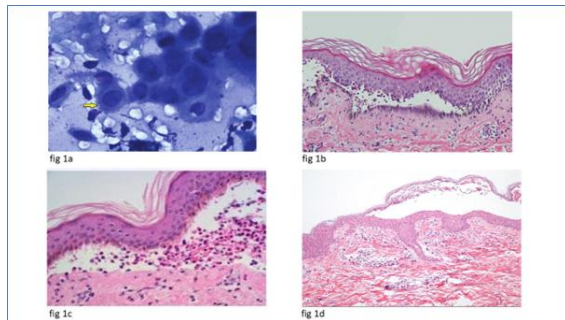


Figure 1: (a) Tzanck smear showing acantholytic cells (100x). (b) Histopathology of pemphigus vulgaris showing eosinophilic spongiosis with intraepidermal bullae and acantholytic cells (Scanner view). (c) Bullous pemphigoid with subepidermal bullae (Scanner view). (d) Pemphigus foliaceus showing subcorneal bullae (Scanner view).

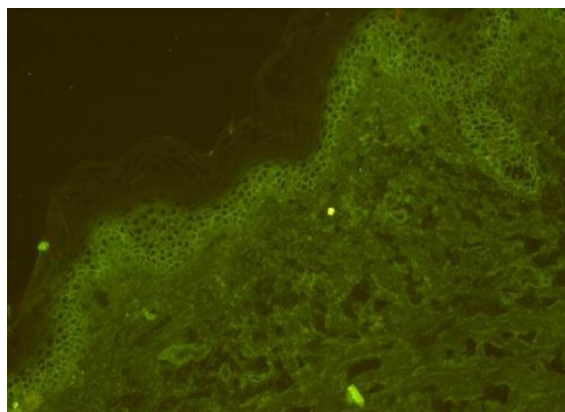


Figure 2: Pemphigus vulgaris showing intracellular deposition of IgG showing fish net pattern

DISCUSSION

This study highlights the diagnostic utility and comparative performance of Tzanck smear and histopathology when measured against the gold standard, direct immunofluorescence (DIF), in the evaluation of intraepidermal bullous lesions.^[12] The findings underscore a pertinent clinical reality in dermatopathology: although DIF remains indispensable in confirming autoimmune blistering diseases, especially pemphigus, histopathology and

cytological techniques such as the Tzanck smear still retain considerable value in resource-constrained settings.

Direct immunofluorescence showed intercellular deposits of IgG in a net-like pattern in confirmed cases, aligning with classical presentations of pemphigus.^[13] This pattern is considered pathognomonic and confirms the autoimmune etiology of intraepidermal blistering. However, DIF requires infrastructure, fluorescent microscopy, specific antibodies, and frozen section processing, limiting its use in peripheral or under-resourced centers. Consequently, a validated, accessible alternative that yields high diagnostic accuracy is critical for prompt treatment initiation.^[14,15] Histopathology demonstrated a higher diagnostic accuracy than Tzanck smear in this study. The sensitivity and specificity observed—88.9% and 92.9%, respectively—support its role as a robust tool, capable of identifying key architectural features such as suprabasalclefting, acantholysis, and the ‘tombstone’ basal layer. These metrics closely mirror other institutional studies: in a multicenter evaluation, suprabasal acantholysis and a tombstone basal pattern yielded histopathological sensitivity around 85–90%.^[16,17] The presence of eosinophilic infiltrate, spongiosis, and inflammatory features further aided subtyping, making histopathology invaluable not only for primary diagnosis but also for subclassification.^[18]

Tzanck smear, though less specific, presented a reliable bedside or clinic-based technique with a sensitivity of 83.3% and specificity of 71.4%, these values are consistent with contemporary findings; Panwar et al. (2017) reported sensitivity up to 90% but caution about decreased specificity in mixed cases.^[8] The cytologic identification of acantholytic cells serves as a quick screening tool, particularly in acute presentations. When used judiciously in combination with clinical features and histology, it adds immediate value by supporting an early presumptive diagnosis.^[19,20] In many regions, especially developing countries, healthcare infrastructure may not support routine DIF testing.^[21] In such contexts, our study reaffirms the

practical utility of combining Tzanck smear and histopathology. In our concordance analysis, 28 of the 36 DIF-confirmed cases were also positive on both Tzanck and histopathology, establishing that dual positivity substantially increases diagnostic confidence. Only two cases showed DIF positivity without corresponding cytology or histology positivity—demonstrating that while DIF is the most sensitive, it may not always be feasible or necessary for all patients.^[22] The demographic findings, showing a higher frequency in middle-aged females, are consistent with global trends in pemphigus epidemiology.^[23] The predominance of mucocutaneous involvement also mirrors the typical distribution pattern seen in pemphigus vulgaris, the most common form of intraepidermal bullous disease. Several studies have explored immunofluorescence on cell smears, with sensitivities ranging from 82–100% and excellent specificity. One report found Kappa agreement of 0.77 with conventional DIF (~82% sensitivity), underscoring its future value in cytology-based immunodiagnosis.^[24]

While the study confirms the diagnostic accuracy of histopathology and cytology, it also reveals the limitations inherent to each. Tzanck smear lacks specificity and is susceptible to observer bias, as acantholytic cells may be missed or confused with other cytological features.^[25] Histopathology, while more definitive, depends on biopsy quality and lesion selection.^[26] DIF, despite its precision, remains impractical for routine use in many clinical scenarios.^[27] The study's strengths include its prospective design, uniform inclusion criteria, and simultaneous application of all three diagnostic modalities. However, limitations include the modest sample size and single-institution setting, which may affect generalizability. Future studies with larger cohorts and multi-center data could enhance validation and offer subgroup-specific insights.

CONCLUSION

Intraepidermal autoimmune blistering diseases, particularly pemphigus, demand accurate and timely diagnosis to avoid morbidity and mortality associated with delayed treatment. Direct immunofluorescence remains the definitive diagnostic modality due to its high sensitivity and specificity. However, in settings where DIF is not readily accessible, histopathology offers an excellent alternative, providing detailed morphologic and diagnostic clarity. Tzanck smear, despite its lower specificity, serves as an effective, low-cost screening tool that can be readily applied at the bedside or outpatient clinic. When used in conjunction, Tzanck smear and histopathology significantly enhance diagnostic confidence and can reduce dependency on DIF, especially in high-volume or low-resource dermatology practices. Thus, an integrated diagnostic approach

using clinical evaluation, cytology, histopathology, and immunofluorescence, where feasible, is advocated. Our findings suggest that with proper training and morphological correlation, significant diagnostic accuracy can be achieved using conventional tools. This not only ensures better resource utilization but also faster initiation of treatment for patients with potentially debilitating autoimmune skin diseases.

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