



Revaluating the Tzanck Test: A Comparative Study with Direct Immunofluorescence for Herpes Virus

A. Rocher^{1*}, A. Angeleri¹, J. Rofrano¹, R. Absi², L. Sardi Segovia¹
and L. Palaoro¹

¹Department of Clinical Biochemistry, Cytology Laboratory, UBA, Buenos Aires, Argentina.

²Department of Clinical Biochemistry, Immunology Laboratory, UBA, Buenos Aires, Argentina.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AR and AA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JR managed the literature searches and the statistics. Authors RA and LSS developed the immunofluorescence analysis. Author LP made diagnoses and analyzed the results. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/22473

Editor(s):

(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.

Reviewers:

(1) Maria Cristina de Melo Pessanha Carvalho, Federal University of Rio de Janeiro, Brazil.

(2) Anonymous, University of Minnesota, USA.

(3) Anonymous, Pontifical Catholic University of Chile, Chile.

(4) Mihai Mares, Brad University of Agricultural Sciences and Veterinary Medicine, Romania.

Complete Peer review History: <http://sciencedomain.org/review-history/12607>

Original Research Article

Received 2nd October 2015
Accepted 28th November 2015
Published 9th December 2015

ABSTRACT

Introduction: Tzanck Test (TT) is a sensitive method applicable to mucocutaneous lesions. Direct immunofluorescence (DIF) for Simplex Herpes Virus (HSV) is now a simple, no routine clinical laboratory practice, allows to differentiate the types of virus.

Objectives: To evaluate the diagnostic sensitivity of Tzanck test vs DIF for herpetic lesions, and to know its usefulness as a complementary diagnostic tool to clinical skin lesions in patients with erosive, vesicular, bullous and pustular lesions.

Materials and Methods: The TT was carried out in 157 lesion patient's samples admitted to the Laboratory of Cytology of our Hospital from 1 January 2010 until 30 December 2014. Smears were stained with Giemsa and in parallel we performed DIF for HSV-1, HSV-2 and Varicella Zoster over 112 samples.

Results: Of the total samples, 40 (25%) were unsatisfactory for cytodagnosis. The TT was positive in 60 samples (38%), of which 55 (35%) showed cytopathic effect for Herpes virus, 2 (1.6%)

*Corresponding author: E-mail: adrianarocher@yahoo.com.ar;

samples showed characteristic inclusion bodies of *Molluscum contagiosum* infection (Henderson-Paterson bodies), 1 (0.6%) sample showed acantholytic cells typical of *Pemphigus Vulgaris*, 1 (0.6%) showed morphological characteristics of *Incontinentia Pigmenti* and 1 (0.6%) presented immature cells of lymphoid appearance. 64 samples were positive by DIF and 58 by TT for HSV. Specificity and sensitivity for TT were 100% and 88% respectively.

Conclusion: The TT should be appreciated as a useful tool in the diagnosis of skin lesions by the rapid implementation and their ease interpretation, as well as being affordable and accessible for most cytology laboratories.

Keywords: Tzanck test; skin lesions; herpes virus; direct immunofluorescence.

1. INTRODUCTION

Exfoliative cytology is a discipline that deals with the morphological study of cells that shed spontaneously.

Arnault Tzanck, in 1947, proposed a simple method applicable to the diagnosis of mucocutaneous lesions [1]. Although it is a simple technique, which is applied to different kinds of diseases, their diagnosis value is not only effective in herpes infections, but also in other diseases, such as skin infections caused by other microorganisms, neoplastic and autoimmune pathologies [2-4].

Infection with herpes simplex virus (HSV) is common in daily practice; there are two serotypes that can cause infection in humans: type 1 (HSV-1) in primarily extragenital location with predilection for ectodermal tissues and type 2 (HSV-2) that infects the genital and perianal mucosa preferably [5,6]. Another type of herpes virus is varicella-zoster virus (VZV), also called Human Herpesvirus 3 (HHV-3) causes typical rash illness of childhood. During the reactivation in older people, it manifests as herpes zoster, developing chickenpox lesions located in a dermatome [7].

HSV infection is generally diagnosed clinically, but there are cases in which the presentation of the disease is atypical, especially in immunocompromised patients. In these patients, extensive skin involvement may be accompanied by symptoms such as fatigue and fever and in some cases there may be disseminated HSV infection, with involvement of internal organs (especially liver), associated with increased morbidity and mortality [8,9].

Pemphigus is an autoimmune bullous disease of the skin and mucous membranes, and is characterized by painful erosions in the oral mucosa and / or flaccid papules and extensive skin lesions. Histopathological studies show

intraepidermal bullae produced by loss of keratinocyte intercellular adhesion, as well as localized and circulating IgG antibodies that are directed against the surface of these cells. It is classified as *pemphigus vulgaris*, *foliaceus*, *vegetans*, *erythematosus* and *paraneoplastic* [10,11].

Molluscum contagiosum is caused by a member of the poxvirus family. The infection affects both children and adults, although the peak incidence occurs in children under 5 years, where lesions are preferentially located in trunk and extremities. These are firm papules, spherical, raised, translucent or yellowish, 3-5 mm, centrally umbilicated which may vary in number from one to hundreds, and usually asymptomatic. Diagnosis generally is determined by the typical clinical appearance of umbilicated lesions. The diagnostic confirmation is sometimes necessary and is performed by staining with Giemsa of the cytologic samples or by biopsy of the papules. [12-14].

Incontinentia pigmenti (IP) is a hereditary skin condition that causes ampules and unusual changes in the skin color, caused by a genetic abnormality detected on the X chromosome [15,16].

Although most of the skin lesions can be diagnosed visually, the laboratory can provide appropriate diagnostic procedures for this purpose. These can be simple as Tzanck (TT) [17] or immunofluorescence methods, or more complex and costly, such as cell culture and molecular biology techniques.

PCR has by far the highest sensitivity and is recommended as the test of choice for symptomatic cases, but its high cost for developing countries difficult to use routinely [18,19].

Direct immunofluorescence (DIF) for HSV is now a simple analysis of clinical laboratory, but their

use is not routine to differentiate between HSV vs VZV (Varicela Zoster virus) which produce skin infections [20]. DIF specificity approaches 100%. The best use of DIF is in combination with culture, which can increase the overall sensitivity for HSV detection from about 50. [21] 20”

The objectives of this paper are to evaluate the diagnostic sensitivity of TT vs DIF for herpetic lesions, and to know its usefulness as a complementary diagnostic tool to clinical skin lesions in patients with erosive, vesicular, bullous and pustular lesions.

2. MATERIALS AND METHODS

TT was performed on 157 samples from patients admitted to the Laboratory of Cytology of our Hospital of Clínica “José de San Martín” from 1 January 2010 until 30 December 2014. The samples were extracted by physicians of the different hospital services and corresponded mostly to immunocompromised patients by the following conditions: HIV (+), leukemias (AML, acute lymphocytic lymphoma (Mantle, Hodgkin, Burkitt, anaplastic), autoimmune diseases (rheumatoid arthritis, Wegener granulomatosis, systemic lupus erythematosus), transplants (kidney, bone marrow) and cancer patients in treatment by chemotherapy, etc. Skin and mucosal lesions were taken in duplicate and sent to the cytology laboratory. The smears were fixed with methanol for 3 minutes, stained with Giemsa solution 10% for 10 minutes and observed under an optical microscope at 100 and 400x. In some cases, skin biopsy was performed with hematoxylin-eosin to confirm the diagnosis of TT. In 112 samples was performed DIF for HSV 1, 2 and VZV using monoclonal antibodies using FITC (Fluorescein isothiocyanate) filter (Simulfluor HSV / VZV, Chemicon International). Then the samples were incubated with fluorescein conjugated antibody for 30 minutes in a moist chamber, rinsed with PBS and observed in the fluorescence microscope at 100 and 400x.

The antigen-antibody complex to identify HSV exhibits an apple green fluorescence, whereas

fluorescence to identify VZV is yellow-gold. Uninfected cells stain a dull red.

All samples were collected for examination and diagnostic purposes and were thoroughly anonymized for the use in this study. Thus no informed consent was needed. (National Law on Protection of Personal Data, No. 25326 - Argentina), this study was approved by the Institutional Review Board at the Clinical Hospital-University of Buenos Aires. Researchers respect the Helsinki Declaration in its latest version (World Medical Association Declaration of Helsinki 2013) [22].

3. RESULTS

TT was performed on 157 samples. 40 (25%) were unsatisfactory for cytodagnosis, 31 due to the small number of cells and 9 samples due to their large inflammatory changes. Of the samples that were satisfactory for the study, the TT was positive in 60 samples (51%), of which 55 (47%) showed cytopathic effect for Herpes virus (Giant cell syncytios with molded nuclei showing the typical appearance of ground glass) (Fig. 1a), 2 (1.6%) had inclusion bodies characteristic of infection by Molluscum contagiosum (Henderson-Paterson bodies) (Fig. 2a), which were confirmed by biopsy (Fig. 2b), 1 (0.8%) presented rounded acantholytic cells characteristics of Pemphigus Vulgaris, which also was confirmed by biopsy (Figs. 2c-d), 1 (0.8%) presented morphological characteristics of Incontinentia Pigmenti (eosinophilic infiltrate) (Fig. 3) and 1 (0.8%), immature lymphoid cells. 3 samples were suspicious for HSV because the images were not conclusive for cytopathic effect (Table 1).

DIF was performed on samples that were positive, negative and suspicious for HSV by TT. The 55 samples that showed cytopathic effect for HSV by Tzanck method were positive by DIF. Of 54 samples that were TT negative, 8 were positive by DIF (Fig. 1b). Of the three suspicious samples by TT, 2 were positive and 1 negative by DIF (Table 2). The sensitivity and specificity of TT were 100% and 87% respectively.

Table 1. Pathologies of the patients

F:87 M:70	Immunocompromised patients					
	Leukemias	Lymphomas	Autoimmune diseases	HIV positive	Transplants	Chemotherapy
N: 157	44	35	19	27	16	16

F: Female, M: Male

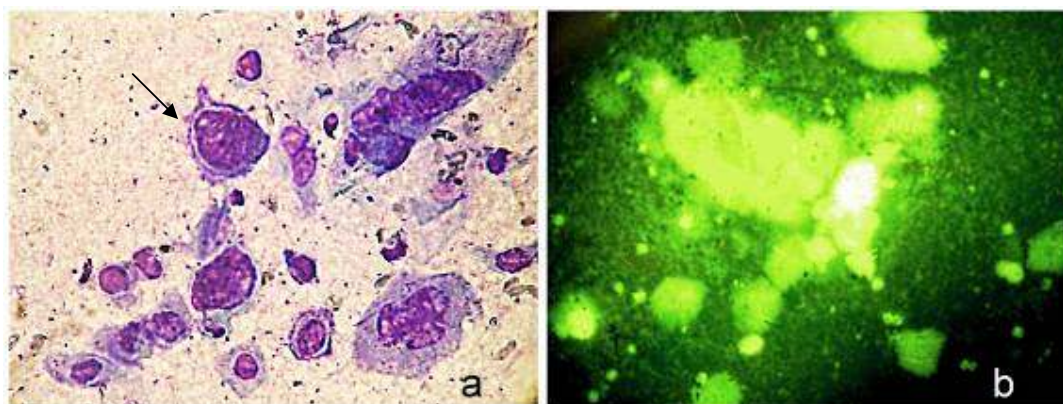


Fig. 1. a) Skin scraping: Herpes infection. Multinucleated giant cells, with molded nuclei of ground glass aspect (Arrow)- Giemsa stain 400x. b) Skin scraping: Herpes infection – DIF 400x

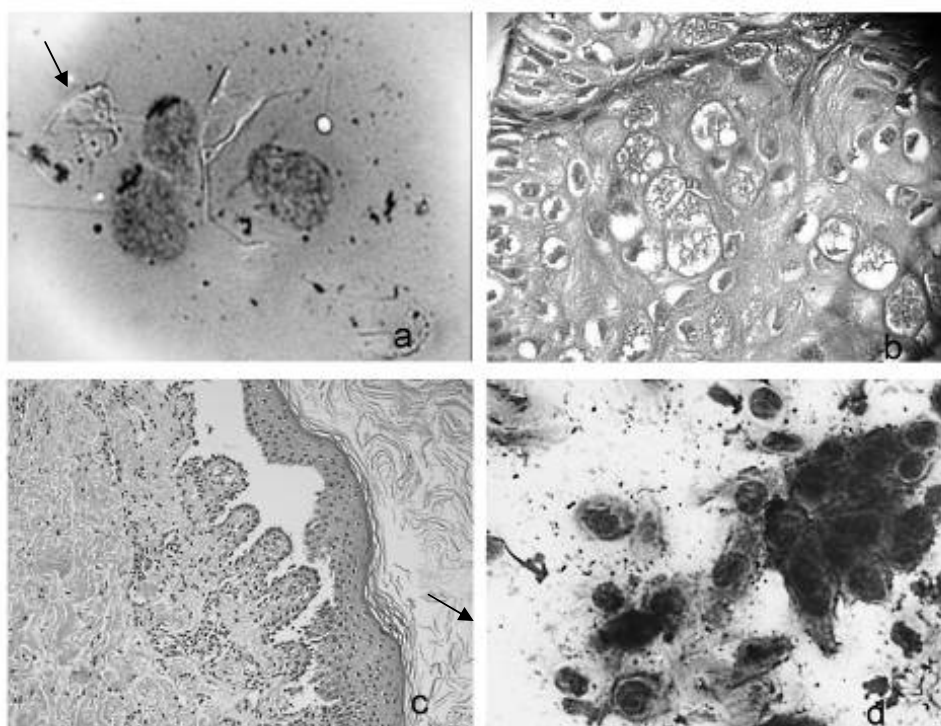


Fig. 2. a) Skin scraping: Molluscum contagiosum- Nuclei displaced by cytoplasmic vacuoles (Arrow)– Giemsa stain 400x-b) Histology of Molluscum contagiosum showing Henderson-Paterson bodies- H&E – 400x-c) Biopsy of Pemphigus- H&E – 100x. d) - Skin scraping: Pemphigus – acantholytic cells (Arrow) – Giemsa stain 400x

Table 2. Tzanck test in skin and mucosal lesions

N	NR	TT positive					TT negative	TT suspect
		HSV	MC	PV	IP	L		
157	40	55	2	1	1	1	54	3

N: Total Cases, NR: Non Representative Smear; TT: Tzanck Test; HSV: Simplex Herpes Virus, MC: Molluscum Contagiosum; PV: Pemphigus Vulgaris; IP: Incontinentia Pigmente L: Immature lymphoid cells

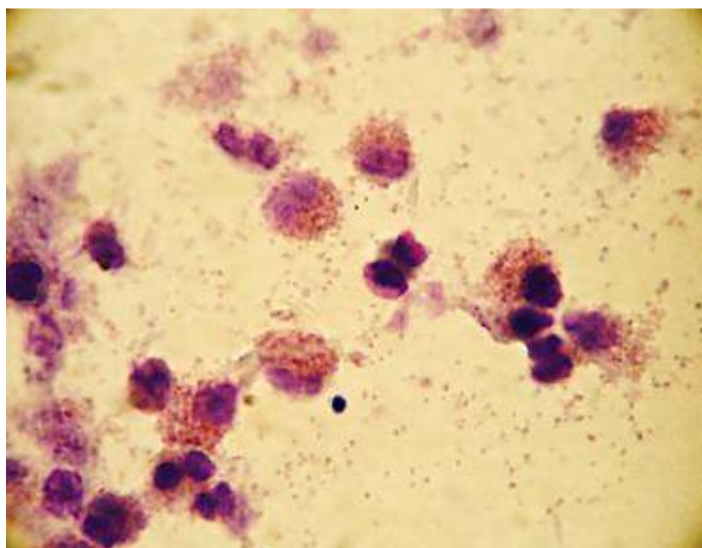


Fig. 3. Skin scraping -Incontinentia pigmenti – Giemsa stain 400x

4. DISCUSSION

Although the diagnosis of lesions of the skin is often only clinical without further evidence to confirm this, there are situations in which the characteristic of lesion is atypical, such as in immunosuppressed patients, in these cases a rapid and efficiently diagnosis is essential to start treatment [23].

Table 3. Tzanck test vs DIF for HSV

	TT	DIF
Positive	55	65*
Negative	54	47
Suspect	3	0

TT: Tzanck Test; DIF: Direct immunofluorescence
 * (+) HSV: Simplex Herpes virus, (-) VZV: varicella-zoster virus

Twenty five of samples were unsatisfactory for evaluation by presenting very little material or inflammatory.

Fifty percent of our samples were positive for TT, where the most frequent pathology was Herpes virus infection (47%) as other authors refer [24]; the remainder consists of Molluscum infections, pemphigus, Incontinentia Pigmenti and blood disorders.

TT is mostly used in the study of infection by herpes simplex or varicella-zoster, being also useful in the diagnosis of other diseases of skin [2]. The TT has the disadvantage of not

distinguishing between HSV serotypes and not differentiating between varicella-zoster virus and HSV [25].

The sensitivity of cytodagnosis (80%) is significantly smaller than the culture techniques or detection of specific DNA by PCR, and its specificity was 90% [2,6]. This is due to the duration of the lesion and its structure: the fresh and intact papules of 1-3 days duration are optimal for diagnosis [8-25]. In our study, the cytodagnosis of Tzanck with DIF, where all samples were positive for the TT, were confirmed by DIF, raising a specificity of 100% was compared. The sensitivity was 87%.

DIF has the advantage over TT to differentiate between HSV and VZV; however, in our study we did not register any positive case for the latter.

In pemphigus, the history and physical examination of the patient approaching a correct diagnosis through TT, but diagnostic confirmation is given by the biopsy and the immunohistochemistry. The presumptive diagnosis of pemphigus with TT is through epidermal acantholytic cells compatible with this pathology. The TT would serve as guiding the diagnosis; the type of pemphigus should be confirmed by other more complex methods of detection of autoimmune antibodies [26]. The diagnosis of Molluscum contagiosum is usually clinical; however, it is possible to obtain a definitive diagnosis by biopsy or cytology. These

techniques are useful in cases clinically difficult [14].

Incontinentia pigmenti is a rare multisystem disease that requires a multidisciplinary study. In a first phase, the diagnosis is based on dermatological disorders characterized by large eosinophilic infiltrate that can be revealed by the TT [16].

The only case that showed immature cells of lymphoid appearance could not be defined due to lack of complementary studies.

Although most Tzanck test applications are used for the diagnosis of HSV, there are other infectious diseases like molluscum contagiosum, histoplasmosis, cryptococcosis, cutaneous immune disorders (pemphigus vulgaris, Stevens-Johnson syndrome, erosive lichen planus, and others), cutaneous tumor lesions, where the test can be useful, so its use should not be dismissed or neglected by the clinician [27].

5. CONCLUSION

Although DIF is more sensitive for the diagnosis of HSV, TT is a good method, providing rapid results with acceptable sensitivity and specificity. The TT should be appreciated as a useful tool in the diagnosis of skin lesions by the speed of execution and simplicity of their interpretation, as well as being affordable and accessible for most cytology laboratories.

This method is underutilized by ignorance of its benefits and the belief that other more complex and innovative diagnostic methodologies, as the molecular type, are more efficient, regardless of the cost / benefit. TT is particularly relevant in the immunocompromised patient where the skin manifestations may be atypical.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical

standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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