Diagnostic Value of Tzanck Smear in Herpetic and Non-Herpetic Vesicular and Bullous Skin Disorders in Pediatric Practice

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The diagnostic value of the Tzanck smear was investigated in 76 patients of a pediatric hospital population suffering from vesicular, erosive or bullous skin disorders. Examination took place by two investigators together (AB), besides the smears were examined by two others (C and D) double blind. Sensitivity for patients with clinical herpetic infections was > 80%, specificity for those without herpetic infections was > 90%. These figures are higher than expected from literature. Reliability was also high; between the three investigators no significant differences were found. The Tzanck smear is simple, inexpensive, easy to perform and rapid; it does not require a specialized laboratory, but experience and correct technique of sampling is required. (Received August 23, 1985.)

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In 1948 Tzanck (1) introduced a test as a diagnostic aid in order to identify vesicular, bullous and erosive dermatoses using scrapings from diseased skin lesions. During the next decades several modifications of this microscopic test, known as the Tzanck smear, have been described (2, 3). The Tzanck smear is used above all in the diagnosis of herpetic infections (4, 5). It is of value for the diagnosis of eczema herpeticum, neonatal herpes, but also for varicella or herpes zoster. It is of greatest importance in the newborn, in pregnant women and immune compromised hosts and it is also applicable in other skin diseases as pemphigus vulgaris, pemphigoid, staphylococcal scalded skin syndrome, toxic epidermal necrolysis and other vesicular, bullous and erosive skin diseases (6). The test is simple, inexpensive, easy to perform and rapid.

In this paper we present the results of a study performed in a pediatric hospital population (including children, parents and hospital personnal) thus illustrating the sensitivity, specificity and reliability of this test.

MATERIAL AND METHODS

Patients

From July 22, 1983 to March 31, 1985 samples were obtained from vesicular, bullous and erosive skin diseases from totally 76 patients (66 children aged 0–18 years, 3 parents and hospital personnal (n^*=7)). The children (of whom 15 infants) were hospitalized in the Sophia Children's Hospital (Rotterdam, The Netherlands) or attended the Outpatients department of Pediatric Dermatology.

* n=number of patients or examinations

Table I. *Clinical diagnoses in investigated population (n=76) of pediatric practice supported by viral and bacterial cultures or/and Tzanck smear*

	n
Patients with clinical herpetic infections	41
Neonatal herpes simplex	2
Labial herpes simplex	8 (3 culture negative)
Cutaneous herpes simplex	11
Eczema herpeticum	4
Varicella	10 (4 culture negative)
Herpes zoster	6
Patients without clinical herpetic infections	35
Hand, foot and mouth disease	4
Impetigo	6
Staphylococcal scalded skin syndrome	3
Infected eczema	4
Toxic erythema of newborn	4
Miliaria	4
Other diseases	11

The investigated population was assigned to the following groups:

Patients with herpetic infection (n=41)
- suffering from herpes simplex infection (n=25)
- suffering from herpes zoster infection (n=6)
- suffering from varicella (n=10)

Patients without herpetic infection (n=35)

Detailed diagnoses are listed in Table I. From each patient single specimen for culture and smears were taken.

Viral cultures

From vesicular or bullous diseases a lesion was opened using a vaccinostyle, the content was taken on a swab, that was placed and shaken into 3 ml transport medium (Dulbecco's modification of Eagles medium with 10% fetal bovine serum and antibiotics). From erosive lesions a swab was taken and treated as described above. Each specimen was inoculated into tube cultures of HEL (Human Embryonal Lung) fibroblasts (0,2 ml/tube, 2 tubes/specimens) within 1/2 hour after collection. Virus isolation was attempted on these HEL cells at 37°C for maximal 2 weeks stationary and daily scored for cytopathic effect. Identification of isolated viruses was performed in immunofluorescence tests with monoclonal antisera to herpes simplex viruses and human antiserum to varicella/zostervirus. In the case of negative results a blind passage was made for another 2 weeks.

Tzanck smear

From the base of the vesicles, bullae or erosions scrapings for the Tzanck test were smeared on a slide and air dried. After drying the material was fixed in methanol and stained within 1/2 minute with Hemacolor® (Merck).

Briefly this method includes dipping 5 times in methanol, 3 times in a red fluid (eosine) and again 3 times in a blue reagent (thiazine). After this procedure the slide was washed in buffered distilled water (pH=7,2) and was ready for microscopic examination (ocular 10×, objective 10× and 40×). (7).

Criteria for microscopic diagnosis of herpetic infection

Epidermal cells with characteristic and typical herpetic changes were scored as positive (Figs 1 and 2).

These nuclear changes include enlargement, multinucleation and crowding resulting in moulding of adjacent nuclei. In the nuclei chromatinic margination beneath the nuclear membrane is typical. If the nuclei are enlarged, the content can be more coarse or show an opaque homogenization (ground-glass aspect). Also inclusion bodies, surrounded by a halo, can be visible in most or some of the nuclei.

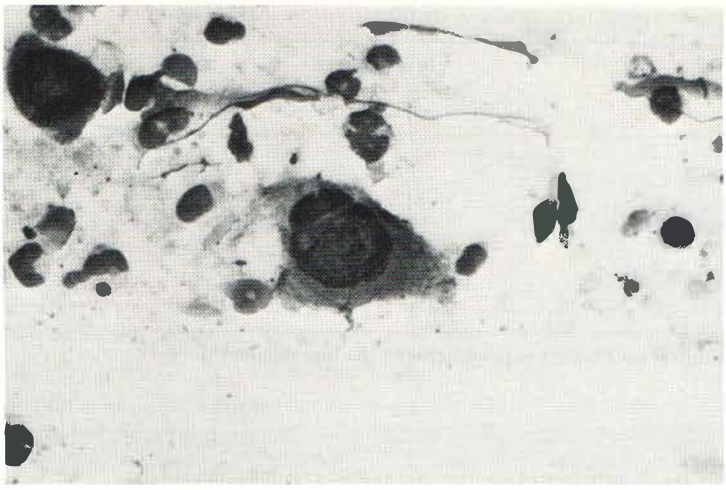

Fig. 1. Early stage of nuclear enlargement. Note coarse nuclear content (×330).

Investigation of Tzanck Smears

Slides were examined by investigators AB (A.P.O. dermatologist and J.C. virologist) and later on double blind by investigators C (E.F. dermatologist) and D (J.N.D. cytotechnologist).

Definitions (8)

$$\text{Sensitivity} = \frac{\text{diseased persons with a positive test}}{\text{all diseased persons tested}} \times 100\,\%$$

$$\text{Specificity} = \frac{\text{non-diseased persons with a negative test}}{\text{all non diseased persons tested}} \times 100\,\%$$

Predictive value of a positive test = PV+

$$\text{PV+} = \frac{\text{number of diseased persons with a positive test}}{\text{total number of persons with a positive test}}$$

Predictive value of a negative test = PV−

$$\text{PV−} = \frac{\text{number of non-diseased persons with a negative test}}{\text{total number of persons with a negative test}}$$

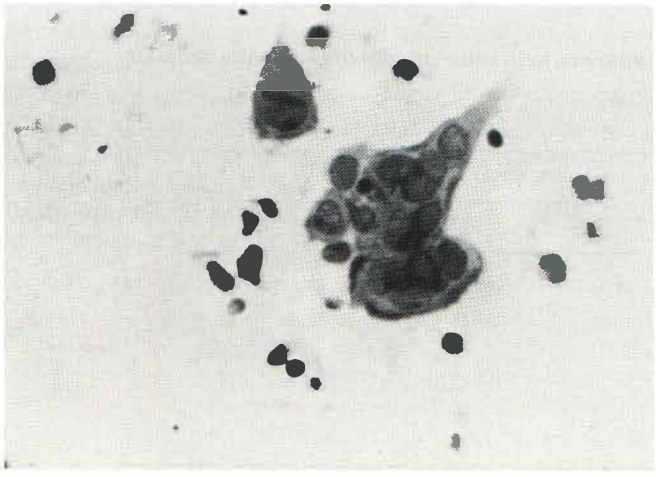

Fig. 2. Multinucleated cells typical of herpetic infections (×330).

Table II. *Comparison of Tzanck smear sensitivity obtained by investigators AB, C and D*

	Investigators		
	AB	C	D
A. *Patients (n=41) with herpetic infection*			
Sensitivity (%) versus clinical picture	81	88	83[a]
Sensitivity (%) versus culture proven herpetic infection	86	92	86[a]
B. *Patients (n=35) without clinical herpetic infection*			
Specificity (%)	100	97	91[a]

[a] Using McNemar's test no significant differences were obtained between the investigators ($p>0.1$).

Statistical analysis

Mc Nemar's test (9) was used to compare the percentages of positive and negative results obtained by different investigators (AB, C, D) from the same patients.

RESULTS

Patients with herpetic infections (n=41)

Out of 25 patients with clinical herpes simplex virus infection, culture was positive for herpes simplex virus type 1 in 22 cases (sensitivity culture=88%). In three cases of recurrent labial herpes simplex the cultures were negative (Table 1); two of them were in a late disease stage (crusts).

Out of 16 patients with clinical herpes zoster/varicella infections, culture was positive in 12 cases (sensitivity=75%). In four cases of varicella (two in early stage) the culture was negative. Table II_A lists the percentages of positive results by microscopic examination in patients with herpetic infections obtained by investigators AB, C and D. Based on clinical picture and culture separately sensitivity is calculated. Primary screening done by investigators AB achieved a sensitivity of 81% (clinical picture) and 86% (culture proven). Investigator C reached a sensitivity of 88% (clinical picture) and 92% (culture proven), D 83% and 86% respectively.

The differences in sensitivity obtained by the different investigators AB, C and D were not significant (Mc Nemar's test).

Table III. *Results of Tzanck smears in patients with clinical herpetic infection without positive cultures*

−=Negative, +=positive.

Diagnosis (culture negative)	Tzanck smear result obtained by investigators		
	AB	C	D
1. Varicella, early stage	−	−	+
2. Varicella, early stage	+	+	+
3. Varicella	−	−	−
4. Varicella	+	+	+
5. Labial Herpes simplex, late stage	−	−	−
6. Labial Herpes simplex, late stage	−	+	−
7. Labial Herpes simplex	−	−	−

Summarized Tzanck smear sensitivity versus clinical picture is >80%, versus culture proven herpetic infection >85%.

Patients without herpetic infections (n=35)

Table II_B lists the percentages of negative results in patients without herpetic infections obtained by investigators AB, C and D. Investigators AB achieved a specificity of 100%, B 97% and C 91% respectively.

The differences in specificity obtained by the different investigators AB, C and D were not significant. Summarized specificity is >90%.

Discrepancies between clinical diagnosis and cultures in comparison with Tzanck smears

Table III lists results of Tzanck smears in patients with clinical herpetic infection without positive cultures.

In two cases of culture-negative varicella the Tzanck smear is considered positive by all three investigators. In three cases (varicella -early stage-, herpes simplex -late stage- and labial herpes simplex) all three investigators found no herpetic changed cells in the smears. In the two other cases one of the three investigators considered the smear as positive. An early or late stage of disease represented the majority of the cases in which a discrepancy was found.

Predictive value of Tzanck smear in investigated population

Predictive values of a positive and negative smear are calculated, when sensitivity is considered as >80% and specificity as >90%. The prevalence of herpetic infections in the investigated population, decribed in this article, is about 50%.

For this study predictive values can be calculated as followed: In this kind of population (fictive n = 1 000) 500 persons will have a herpetic infection; the Tzanck smear will be positive in >400 and false negative in <100 persons.

Also 500 persons will not have a herpetic infection; the Tzanck smear will be negative in >450 and false positive in <50 persons. The predictive value of a positive scored Tzanck smear

(PV+) is $>0{,}88 \left(\frac{400}{400+50}\right)$ and of a negative (PV−) $>0{,}82 \left(\frac{450}{450+100}\right)$.

DISCUSSION

Herpes simplex, herpes zoster or varicella will be diagnosed easy on clinical aspects in most of the cases. In difficult diagnostic instances confirmation by laboratory test, e.g. culture, will be necessary.

Several (most quick) tests have been developed recently. Herpes enzyme (commercially ELISA test), Micro trak (commercially immunofluorescence test) both for herpes simplex virus type 1 and 2. Monoclonal antibody assays in immunofluorescence tests are available for herpes simplex virus 1, 2 and varicella. Those tests are however expensive and need sometimes a specialized laboratory.

Direct and quick confirmation of herpetic infection, though not specific for herpes simplex type 1 or 2, or varicella, is possible by the Tzanck smear and by direct electron microscopy (negative staining). Especially the Tzanck smear, as allready stated, is simple, inexpensive, easy to perform and rapid; this test is suitable (in experienced hands) for usage in the office practices, but second screening is an important supplementary diagnostic procedure.

Solomon et al (10) studied the results of Tzanck smears and viral cultures in 30 patients (32 examinations) with clinical cutaneous herpes simplex. Cultures were positive in 78% and Tzanck smears in 53%. The sensitivity of the culture was 78% and of the Tzanck smear 53%. They concluded that the Tzanck smear looses its sensitivity as the herpetic lesions age.

Veien and Vestergaard (4) compared viral cultures, indirect immunofluorescent staining and Tzanck smears from 32 patient with clinical cutaneous herpes simplex. The three tests were almost equally sensitive (>63%). It was of great interest to observe that the results of viral culture and Tzanck smear both were negative in herpetic infections of longer duration (about 9 days).

Our study indicates a higher sensitivity of the Tzanck smear (>80%) and specificity (>90%) than described in these previous studies (4, 10). In our studied population, the predictive values of Tzanck smear are satisfactory high, PV+>0,88 and PV−>0,82. Besides these results were achieved after rescreening the smears twice; no significant differences between investigations AB, C and D were obtained. This indicates a high reliability of the Tzanck smear if performed by experienced specialists. Rescreening of smears is considered as an important quality control procedure in cytopathology in some or other form (12).

Next to 25 herpes simplex virus infections we also studied 16 herpes zoster/varicella cases. Almost all herpes simplex lesions were cutaneous, only 8 were located on the lips. From these labial herpes simplex infections three were culture negative (two of them were in a late stage). It is well known, that varicella cultures are prone to failure; in our material four cultures were negative of whom two were in a very early stage of disease.

The cytologic features of herpes zoster/varicella and herpes simplex are basically and morphologically the same (11). It is not possible to distinguish different types of herpes simplex virus 1 and 2, or varicella-zoster virus infection from each other by cytopathology. Probably the lesions of herpes zoster/varicella show less cell destruction and inflammation in early stages than those of (primary) herpes simplex, although this is doubtful and needs further confirmation.

The Tzanck smear is probably more sensitive in cutaneous herpetic infections than in infections of the mucous membranes. In genital herpes Moseley (13) achieved with the Tzanck smear a sensitivity of 38%. Further study is needed to evaluate the value of this test in these types of infection. Preliminary results (Folkers et al, unpublished data) also indicate a high sensitivity and specificity in herpetic infections of the mucous membranes.

In summary our findings suggest a high sensitivity and specificity of the Tzanck smear in herpetic infections. Diagnosis of herpetic infections is not always confirmed in early and late disease stage by both culture and Tzanck smear. The Tzanck smear results, obtained by us, show a higher sensitivity and specificity than expected from literature (4, 10). Our results indicate, that the Tzanck smear is a quick and reliable test for the diagnosis of herpetic infections. It is easy to perform and does not require specialized laboratory equipments. It does however, require experience.

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