Diagnostic aspects of human alphaherpesvirus infections in dermato-venerology
Folkers, E.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Summary

Herpes simplex virus and Varicella-zoster virus infections usually present a characteristic clinical picture. Tzanck smear and viral culture are just two of several laboratory tests to confirm the clinical diagnosis. The Tzanck smear especially is useful for office diagnosis in dermatovenerological practice. The results of our investigations on this subject are described in this chapter. In clinical diagnostic work, sensitivity and specificity are key assay features. A systematic diagnostic approach of pustular eruptions in the neonate is proposed.
3. Tanzania's Sour and Milk Culture

In discussions of Africa's simplex virus and varicella-zoster virus

Introduction

Sudan

Phenotypic differences and serologic cross-reactivity among VZV vaccine strains. Studies in mice and nonhuman primates have revealed a correlation between mouse-adapted VZV strains and clinical disease in immunologically naive mice. In addition, studies in mice have shown that VZV vaccine strains that induce a type-specific immune response are more likely to be effective in humans. The development of an effective vaccine for VZV remains a major goal for future research. A vaccine would be expected to induce a type-specific immune response in humans and provide lifelong protection against VZV infection. Recent advances in the development of VZV vaccines and the clinical evaluation of candidate vaccines have contributed to the progress in the development of an effective VZV vaccine.
2.1 A rapid diagnostic test (Tzanck test) to exclude herpesvirus infections in vesicles, blisters and pustules

In 1948 Tzanck described a microscopic test for the identification of skin disorders. He used skin scrapings of the affected skin. Over the years several modifications of the Tzanck test have been described, which allow cytologic differentiation between a herpetic infection and other skin disorders which involve vesicles, blisters and pustules. When there is suspicion of herpetic infections, for example neonatal herpes, eczema herpeticum or generalized herpes zoster, a rapid diagnosis can be of utmost importance to differentiate between bacterial and yeast infections, especially in newborns, in immunocompromised or immunosuppressed patients, or in patients with severe malignancies. Referring to a few case histories of children the speed, efficacy and simplicity of this method will be demonstrated. It can be stated that, in a large number of cases, not performing the Tzanck test can be considered a grave omission.

Folkers E\textsuperscript{1}, Oranje AP\textsuperscript{2}

\textsuperscript{1} Department of Dermatology, Hospital ‘de Heel’, Zaandam, The Netherlands
\textsuperscript{2} Department of Dermatology and Venereology, Erasmus University, Rotterdam, The Netherlands

(Published in: Ned Tijdschr Geneesk 1985; 129: 241-243. This article was originally written in Dutch.)
Case A was born at night after a normal pregnancy. Skin lesions were noticed at birth, consisting of erythema, vesicles, pustules and extensive scaling. A herpes infection was suspected and aciclovir medication was given intravenously. The next morning doubt rose about the diagnosis that was made. Skin scrapings for cytologic examination and skin cultures were taken. The Tzanck test proved negative, which means that no abnormal epidermal cells were observed. Spores and pseudomycelia were seen in the Gram-preparation, which led to the clinical diagnosis of candidiasis. The aciclovir medication was stopped and local antimycotic treatment with miconazol cream was initiated. The culture for Candida albicans proved later to be positive, confirming the clinical diagnosis. The skin lesions healed quickly with the topical antimycotic treatment.

Case B was a 2 year old boy suffering from atopic eczema. He was admitted with a generalized vesiculo-pustular eruption and based on the clinical picture eczema herpeticum was suspected. Giant cells, characteristic for herpetic infection, were observed in the Tzanck smear. He was treated intravenously with aciclovir. The virus culture from the pustules proved later to be positive for herpes simplex virus type I. The patient continued to be extremely ill and developed a sepsis, probably of bacterial origin. Blood cultures proved positive only for Staphylococcus epidermidis. Supplementary treatment was given intravenously with dicloxacillin and gentamicin. His condition slowly improved; the fever subsided, but rose again sharply, attended by diarrhoea. Gastroenteritis was diagnosed and Salmonella typhimurium was isolated from faeces. By that time the skin lesions were healing, the vesicles were dried and no recurrence was noted.

Case C was an eight day old male baby. The first days after birth there were no medical problems; he drank good and grew at a normal rate. On the sixth day after birth he developed diarrhoea, he vomited and became pale. A sepsis was supposed, he was therefore transferred to our hospital. On the seventh day he developed several pustules with underlying redness scattered over the body; some had spontaneously disappeared leaving behind a small ulcer. On the left wing of the nose was a group of three pustules. Material for cytologic smears and viral culture was taken. The Tzanck test demonstrated besides numerous granulocytes, macrophages and a few lymphocytes, numerous epithelial cells with monstrously swollen, often multilobed nuclei, and frequently with multiple nuclei. In the Gram-preparation some Gram-positive cocci were found, but no yeast. Herpetic infection was diagnosed. The child died on the ninth day after birth. (At that time treatment with aciclovir was not possible.) Afterwards herpes simplex virus type I was isolated from the pustules. Post mortem viral culture and immunohistochemical examination from the heart muscle and liver tissue revealed herpes simplex virus type I as well.

With these three case histories we have demonstrated the value of the Tzanck test. We consider it therefore important to discuss with you how to carry out the test.

It is very important to obtain the material for cytologic examination in the correct manner. The vesicle, blister or pustule in question must be opened using a vaccinostyle or small lancet. The content is used for cultures and microscopic examination for the presence of
bacteria, yeasts or fungi. Using the curved side of the vaccinostyle or lancet, material is obtained from the surface of the bottom of the lesion, smeared on a glass slide and air dried. The preparation is then coloured and examined under the microscope using a 25x and 40x magnification. A herpes positive preparation demonstrates a specific cytological picture: epithelial cells with monstrously swollen nucleus, often multilobated or multinucleated, surrounded by a perinuclear halo (fig.1). Cytologic differentiation between herpes simplex, herpes zoster and varicella is not possible.

Fig. 1. Tzanck smears coloured with Hemacolor®. Epithelial cells with monstrously swollen, lobated nuclei and perinuclear halo. (magnification 400x)

Several colouring methods have been used for the Tzanck test. Most commonly used are the methods according to Giemsa, Wright, and Papanicolaou, and colouring with haematoxylin-eosin and methyleneblue. The Giemsa and Papanicolaou methods and haematoxylin-eosin colouring are very suitable for the Tzanck test, but time consuming. They are therefore less suitable for consulting-room diagnostics. The Wright and methyleneblue colouring methods consume little time, but give for the Tzanck test less clear results. The Giemsa colouring method was modified to give faster results, but the smears obtained are less preservable. The Paragon colouring method\(^2\) gives fast results and is therefore suitable for the Tzanck test, but is not available in our country. Therefore we have searched for another rapid
colouring method for the Tzanck. We now use a rapid haematologic colouring method (Hemacolor; fig. 2), making it possible to examine the smear within a few minutes. This method makes use of a fixing solution (methanol pro analyse), a red colouring agent (eosin solution) and a blue colouring agent (thiazine solution). Buffered, distilled water at pH 7.2 is used as a rinsing agent. Approximately half a minute is all that's needed for this colouring.

![Diagram of the Hemacolor® rapid blood smear staining process](image)

**Fig. 2.** Hemacolor® rapid blood smear staining suitable for the Tzanck test. Immerse the air-dried smear as many times as specified for about 1 second in the 3 solutions. Drain off, rinse with buffer solution pH 7.2 and allow to dry.

If the epitheloid shapes are difficult to discern as a result of overcolouring, in most cases the swollen nuclei often with several lobes or multinucleation can still be observed with stronger light (see fig. 1). If only one smear is available as a result of scarcity of lesions, the already coloured preparation can be decoloured using hydrochlorate alcoholic solution (R/acid. hydrochloricum 25%; alcohol ketonatus 95% ad 100 ml). The decoloured preparation can then be reused with other colouring methods.
Because of its simplicity, the Tzanck test can easily be performed in the consulting-room of the general practitioner and dermatovenereologist. Its sensitivity has been found to be high enough in comparison with the sensitivity of the virus culture. The results shall depend on the experience of the investigator with cytologic examination, for example the obtaining of material, preparing the slide and interpreting it, and depend on the duration of the lesion. We have observed that in cases of herpetic infection treated with aciclovir, the Tzanck test will be positive for herpetic infection for a certain amount of time. In cases which were previously diagnosed as herpetic infection based on the clinical picture, and later proven incorrectly diagnosed based on the Tzanck test, we deemed it responsible to stop the aciclovir medication.

For optimal diagnostic results, both cytologic examination and virus culture should be performed; false negative results are still possible if the material obtained is of insufficient quality.

Beside the aforementioned diseases is the Tzanck test also valuable for the diagnosis of other skin disorders accompanied by acantholysis, for example discerning between pemphigus vulgaris and pemphigoid. Namely in newborns with vesicles, blisters and pustules, as in bacterial, viral and yeast infections, and in case of erythema toxicum neonatorum, incontinentia pigmenti, staphylococcal scalded skin syndrome, toxic epidermal necrolysis or miliaria, is an extensive differential diagnostic examination required; a rapid cytological diagnostic test is in these cases of utmost importance. This was reason enough for us to bring this, for many of you, unfamiliar diagnostic test to your attention.
REFERENCES

2.2 Diagnostic value of Tzanck smear in herpetic and non-herpetic vesicular and bullous skin disorders in pediatric practice

Abstract

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.

Oranje AP 1, Folkers E 2, Choufoer-Habova J 3, Duivenvoorden JN 4

1 Department of Dermatology and Venereology (Pediatric Dermatology), Erasmus University, Rotterdam, The Netherlands
2 Department of Virology, Erasmus University, Rotterdam and Academic Hospital Rotterdam/Sophia Children's Hospital, Rotterdam, The Netherlands
3 Department of Dermatology, Hospital 'de Heel', Zaandam, The Netherlands
4 Department of Pathological Anatomy (Cytology), Hospital 'de Heel', Zaandam, The Netherlands
(Published in: Acta Derm Venereol (Stockh) 1986; 66: 127-133)
Introduction

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 personnel) 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 7 hospital personnel. 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. 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 \((0,2 \text{ ml/tube, 2 tubes/specimens})\) 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\(^x\), objective 10\(^x\) and 40\(^x\)).

<table>
<thead>
<tr>
<th>Table I. Clinical diagnoses in investigated population (n=76) of pediatric practice supported by viral and bacterial cultures or/and Tzanck smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with clinical herpetic infections</td>
</tr>
<tr>
<td>Neonatal herpes simplex</td>
</tr>
<tr>
<td>Labial herpes simplex</td>
</tr>
<tr>
<td>Cutaneous herpes simplex</td>
</tr>
<tr>
<td>Eczema herpeticum</td>
</tr>
<tr>
<td>Varicella</td>
</tr>
<tr>
<td>Herpes zoster</td>
</tr>
<tr>
<td>Patients without clinical herpetic infections</td>
</tr>
<tr>
<td>Hand, foot and mouth disease</td>
</tr>
<tr>
<td>Impetigo</td>
</tr>
<tr>
<td>Staphylococcal scalded skin syndrome</td>
</tr>
<tr>
<td>Infected eczema</td>
</tr>
<tr>
<td>Toxic erythema of newborn</td>
</tr>
<tr>
<td>Miliaria</td>
</tr>
<tr>
<td>Other diseases</td>
</tr>
</tbody>
</table>

**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.

**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).
Diagnostic aspects of human alphaherpesvirus infections in dermatovenereology

Fig. 1. Early stage of nuclear enlargement. Note coarse nuclear content (x330)

Fig. 2. Multinucleated cells typical of herpetic infections (x330)
**Definitions**

**Sensitivity**
\[
\text{Sensitivity} = \frac{\text{diseased persons with a positive test}}{\text{all diseased persons tested}} \times 100\%
\]

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

**Predictive value of a positive test** = \( PV^+ \):
\[
\text{PV}^+ = \frac{\text{number of diseased persons with a positive test}}{\text{total number of persons with a positive tested}}
\]

**Predictive value of a negative test** = \( PV^- \):
\[
\text{PV}^- = \frac{\text{number of non-diseased persons with a negative test}}{\text{total number of persons with a negative tested}}
\]

**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 I); 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 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).

Summarized Tzanck smear sensitivity versus clinical picture is >80%, versus culture proven herpetic infection >85%.
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

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

<table>
<thead>
<tr>
<th>Investigators:</th>
<th>AB</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Patients ((n=41)) with herpetic infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (%) versus clinical picture</td>
<td>81</td>
<td>88</td>
<td>83\textsuperscript{a}</td>
</tr>
<tr>
<td>Sensitivity (%) versus culture proven herpetic infection</td>
<td>86</td>
<td>92</td>
<td>86\textsuperscript{a}</td>
</tr>
<tr>
<td>B. Patients ((n=35)) without clinical herpetic infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>97</td>
<td>91\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Using McNemar's test no significant differences were obtained between the investigators \((p>0.1)\)

Patients without herpetic infections \((n=35)\)
Table II\textsubscript{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, described in this article, is about 50\%.
For this study predictive values can be calculated as followed: In this kind of population (fictive \(n = 1000\)) 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:

\[ (PV+) = 0.88 \left( \frac{400}{400+50} \right) \]
and of a negative \((PV-) = 0.82 \left( \frac{400}{400+50} \right) \).
Table III. Results of Tzanck smears in patients with clinical herpetic infection without positive cultures

<table>
<thead>
<tr>
<th>Diagnosis (culture negative)</th>
<th>Diagnosis</th>
<th>Tzanck smear result obtained by investigators</th>
<th>AB</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Varicella, early stage</td>
<td>AB</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2. Varicella, early stage</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. Varicella</td>
<td>D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Varicella</td>
<td>E</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5. Labial Herpes simplex, late stage</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. Labial Herpes simplex, late stage</td>
<td>G</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. Labial Herpes simplex</td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

-= Negative, + = positive

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 already 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.

Acknowledgement

The authors are indebted to H.J.A. Schouten of the Department of Biostatistics for statistical advices.
REFERENCES

2.3 Tzanck smear in diagnosing genital herpes

Summary

In 126 patients with anogenital lesions, in which herpes simplex virus (HSV) infection was suspected or included in the differential diagnosis, the results of cytodiagnosis of herpetic infection (Tzanck smear) were compared with virus culture. Cervical lesions were excluded from this study.

HSV infection was proved by culture in 78 patients and was absent or non-active in 41 patients. Excluded from this study were seven patients who did not yield the virus on culture but had positive Tzanck smear results from three investigators. The characteristic cytopathic effect of herpetic infection was found in 56 patients who yielded HSV on culture. Tzanck smear sensitivity for skin lesions was 79% and for mucous membrane lesions was 81% in men and 52% in women. Tzanck smear specificity for the 41 patients without herpetic infection proved by virus culture was 93%. Differences in sensitivity and specificity between the results found by three investigators (double blind screening) were not significant. The Tzanck smear is reliable, inexpensive, and easy and quick to perform; it is suitable for office diagnosis because it does not require a specialised laboratory.

Folkers E, Oranje AP, Duivenvoorden JN, van der Veen JPW, Rijlaarsdam JU, Emsbroek JA

1 Department of Dermatology, Hospital 'de Heel', Zaandam, The Netherlands
2 Department of Dermatology and Venereology (Pediatric Dermatology), Erasmus University, Rotterdam, The Netherlands
3 Department of Pathological Anatomy (Cytology), Hospital 'de Heel', Zaandam, The Netherlands
4 Department of Dermatology, Free University, Amsterdam
5 Clinic for Sexually Transmitted Diseases of the Municipal Health Service (GG&GD), Amsterdam, The Netherlands

(Published in: Genitourin Med 1988; 64: 249-254)
Introduction

Herpetic infections of the skin and adjacent mucous membranes can be diagnosed by clinical features in most cases. In patients with an inconclusive clinical diagnosis, in whom herpetic infection is suspected, confirmation by laboratory tests will be necessary. Virus culture remains the standard method, but direct and quick confirmation of these herpetic infections can be obtained by the Tzanck smear.

In previous studies, we reported our experience with a cytopathological test based on the Tzanck smear in diagnosing herpetic and non-herpetic vesicular and bullous skin disorders in paediatric practice and in varicella-zoster virus infections (Folkers E et al unpublished observation). We obtained a sensitivity of the Tzanck smear in herpetic infection of more than 80% and a specificity of at least 90%. We confirmed previous reports, that the Tzanck smear is a useful tool in diagnosing cutaneous herpetic infections.

In this study we describe the application of the Tzanck smear for anogenital lesions suspected of being caused by herpes simplex virus (HSV) infection, and we compare the results with those of virus culture.

Patients, materials, and methods

Patients
From May 20 to December 31 1986 at one of the outpatient clinics for sexually transmitted diseases (STDs) of the Municipal Health Service in Amsterdam we collected Tzanck smears from 126 patients with anogenital lesions, in which HSV infection was suspected or included in the differential diagnosis. Lesions of the skin (n = 46) and mucous membranes (n = 80) were classified as being vesicular, pustular, or ulcerous. Cervical specimens were excluded as cervical cytology is hard for inexperienced observers to judge.

Tzanck smear
A Tzanck smear was routinely taken first, followed by a swab for virus culture. Scrapings from the edge and base of each vesicle, pustule, or erosion were smeared on to a glass slide, air dried, stained with Diff-Quik (Merz-Dade AG, 3186 Düdingen, Switzerland) according to the manufacturer's instructions, after which the smear was examined by light microscopy (ocular 10x, objectives 10x, 25x, and 40x magnification).

All Tzanck preparations were examined first by one investigator (EF, dermatovenereologist) and then double blind by two others (APO, dermatovenereologist, and JND, cytotechnologist).

Criteria for microscopic diagnosis of herpetic infection
Epithelial cells showing characteristic and typical herpetic changes were classified as positive. These changes include enlargement, multinucleation and crowding of the nuclei with nuclear "moulding", different stages of peripheral margination of the nuclear chromatin, and alteration of the ground substance, which can be more coarse or have an opaque ("ground glass") appearance. As well as the nuclear changes described above, mononucleate, nongiant cell virocytes can also be seen (figs 1-4). Sometimes intranuclear inclusions surrounded by a prominent halo are recognisable.
**Virus culture**

After a sample had been taken for Tzanck preparation, the base and edge of each lesion were swabbed vigorously, and the specimen was transferred immediately into a viral transport medium. Virus culture and HSV subtyping were carried out according to standard virological procedures for HSV diagnosis.

---

**Fig. 1.** Tzanck smear of genital mucosal ulcer showing multiple different multinucleated epithelial giant cells, abundant erythrocytes, and sporadic leucocytes (Diff-Quik stain)
Definitions
Definitions of sensitivity, specificity, and the predictive value of a positive or negative result of Tzanck smear, were as described previously.1

\[
\text{Sensitivity} = \frac{\text{No culture and Tzanck test positive}}{\text{No culture positive people tested}} \times 100\%
\]

\[
\text{Specificity} = \frac{\text{No culture and Tzanck test negative}}{\text{No culture negative people tested}} \times 100\%
\]

Predictive value of a positive test (PV+): \[\text{PV}^+ = \frac{\text{No culture and Tzanck test positive}}{\text{No Tzanck test positive}}\]

Predictive value of a negative test (PV−): \[\text{PV}^- = \frac{\text{No culture and Tzanck test negative}}{\text{No Tzanck test negative}}\]

Statistical analysis
McNemar's test was used to compare the percentages of positive and negative results obtained by different investigators (EF, APO, and JND) of Tzanck smears from the same patients.8

Fig.2. Mononucleated epithelial giant cell (Diff-Duik stain)
Results

Clinical features

Of 126 patients with clinically overt genital herpetic infection or genital herpes in the differential diagnosis, 78 yielded the virus on culture. HSV could not be cultured from 48 specimens: in two men syphilis was diagnosed, in seven patients the diagnosis of herpetic infection was maintained because of positive Tzanck smear results by all investigators, and in 39 patients lesions were finally classified as “ulcers of unknown cause”.

The 46 skin lesions were found on the penis (30), scrotum (1), vulva (1), perineum (2), anorectal region (5), buttocks (2), pubic region (3), and groins (2). In men, Tzanck smears were taken from mucous membrane lesions on the glans penis, coronal sulcus, or subpreputial sac (35), and from the anorectal mucous membrane (6). In women, Tzanck smears were taken from mucous membrane lesions in the vestibule or vagina (39). More lesions were ulcerous (99) than vesicular (25) or pustular (2). The average duration of the vesicular lesions of the skin and mucous membranes of men and women at the time of
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

Sampling was about two days, and of ulcerous skin lesions was about five days; the average duration of ulcerous mucous membrane lesions at the time of sampling was about four days in men and six days in women.

Fig. 4. Multinucleated epithelial giant cells, one with an inclusion body (arrowed) (Diff-Quik stain)

No laboratory investigations were undertaken to classify the basic type of the herpetic episodes in the population investigated. Based on each patient's history, 14 were experiencing an initial episode and 28 a recurrence of genital herpes. Insufficient data were available to classify the basic type of herpetic episode experienced by the remaining 84 patients (including 41 finally regarded as having no or non-active herpetic infection).
Virus culture

HSV 2 serotype was found in 76 and HSV 1 in two isolates. Table 1 shows that HSV was isolated from 12 out of 13 vesicular, 1 out of 2 pustular, and 16 out of 26 ulcerous skin lesions; 8 out of 9 vesicular mucous membrane lesions; and 25 out of 39 ulcerous mucous membrane lesions in men and 16 out of 30 ulcerous mucous membrane lesions in women.

Table 1. Tzanck smear sensitivity in 119* patients with culture proved genital infection with herpes simplex virus (HSV)

<table>
<thead>
<tr>
<th>Location of lesions</th>
<th>Sex of patients</th>
<th>Stage of lesions</th>
<th>Culture (n=78)</th>
<th>Tzanck smear (n=56)**</th>
<th>Sensitivity of %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Men and women</td>
<td>Vesicular</td>
<td>13</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ulcerous</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ulcerous</td>
<td>26</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Mucous membrane</td>
<td>Men and women</td>
<td>Vesicular</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Ulcerous</td>
<td>39</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>Ulcerous</td>
<td>30</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>

* Excluding seven patients not yielding virus on culture but with unanimously positive Tzanck smear results.
** Results obtained by APO.

Tzanck smear

The most important factors in obtaining a positive Tzanck smear were the stage of lesion development at the time of sampling and whether it was located on skin or mucous membrane. These data are also listed in table 1. Tzanck smear sensitivity was measured against positive HSV culture results, and was 72% with APO, 77% with EF, and 81% with JND (data not given). Using McNemar’s test these differences were not significant (p>0.1). Tzanck smear sensitivity for all skin lesions (in men and women together) that yielded HSV on culture was 79% (23/29). Mucous membrane lesions of men (one vesicular, 25 ulcerous) and women (seven vesicular, 16 ulcerous) yielding HSV showed Tzanck smear sensitivities of 81% (21/26) in men and 52% (12/23) in women.

Table 2 shows a discrepancy between unanimously positive Tzanck smear results and negative virus culture in seven patients. These patients were diagnosed as having genital herpes, despite their negative culture results, but were not included in calculations of the sensitivity and specificity of the Tzanck smear.

All 41 patients with no (or late non-active) herpetic infection yielded negative results to the Tzanck smear when tested by EF and APO (specificity 100%); positive results were
obtained only by JND (cytotechnologist) in two men with penile skin ulcers and in one woman with a mucous membrane ulcer (combined specificity 93% (38/41)).

Table 2. Discrepancies between Tzanck smear results in 10 patients with genital lesions in which genital herpes was suspected or included in differential diagnosis but yielding negative culture results

<table>
<thead>
<tr>
<th>Location</th>
<th>Stage of lesion</th>
<th>Duration of clinical symptoms (days)</th>
<th>Tzanck smear by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penile skin</td>
<td>Vesicular</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Vesicular</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Ulcerous</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Ulcerous</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Ulcerous</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Ulcerous</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Penile skin</td>
<td>Ulcerous</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Penile mucous membrane</td>
<td>Vesicular</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Vulval mucous membrane</td>
<td>Ulcerous</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>Vulval mucous membrane</td>
<td>Ulcerous</td>
<td>?</td>
<td>-</td>
</tr>
</tbody>
</table>

? = data not known.

Results + (positive), - (negative)

Table 3 lists the Tzanck smear sensitivities and specificities and the predictive values of positive (PV+) and negative (PV-) results in active herpetic lesions at different clinical stages and locations. We calculated predictive values of positive and negative Tzanck smears for the total study group on the basis of 72% sensitivity and 93% specificity. The prevalence of active genital herpes (proved by virus culture) in the study population was about 65% (78/119). The predictive value of a positive Tzanck smear (PV+) was 0.95 and of a negative Tzanck smear (PV-) was 0.64.
Table 3. Sensitivity, specificity, and predictive values of positive (PV+) and negative (PV-) results of Tzanck smears obtained from lesions at different stages and locations in patients with herpetic infection proved by virus culture

<table>
<thead>
<tr>
<th>Location</th>
<th>Stage of lesion</th>
<th>Sex of patients</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Prevalence PV+</th>
<th>PV-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin Ulcerous</td>
<td>Men and women</td>
<td>81</td>
<td>100</td>
<td>88</td>
<td>1</td>
<td>0.42</td>
</tr>
<tr>
<td>Mucous membrane Ulcerous or pustular</td>
<td>Men and women</td>
<td>75</td>
<td>92†</td>
<td>62</td>
<td>0.94</td>
<td>0.69</td>
</tr>
<tr>
<td>Skin Ulcerous</td>
<td>Men and women</td>
<td>80</td>
<td>92†</td>
<td>64</td>
<td>0.95</td>
<td>0.72</td>
</tr>
<tr>
<td>Mucous membrane Ulcerous</td>
<td>Men</td>
<td>44</td>
<td>92†</td>
<td>53</td>
<td>0.86</td>
<td>0.59</td>
</tr>
<tr>
<td>Mucous membrane Ulcerous</td>
<td>Women</td>
<td>72</td>
<td>93</td>
<td>65*</td>
<td>0.95</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* Prevalence of culture proved herpetic infection in lesions of patients suspected of having genital herpes or in whose differential diagnosis it was included.
† Summarized specificity obtained in all ulcerous lesions (no clear differences obtained between men and women).

Discussion

In treating genital herpes, confirmation of the clinical diagnosis of HSV infection is desirable. Acyclovir, the preferred drug for antiviral treatment, is more effective the earlier it is given. As swift diagnosis can therefore lead to more effective treatment, the advantage of a rapid diagnostic test is obvious.

Several rapid direct diagnostic tests using monoclonal antibody immunofluorescence or immunoperoxidase techniques have been developed recently, but these do not yet show acceptable sensitivity and specificity compared with virus culture. Culture is still the standard verification method for HSV infections, and it can confirm the clinical diagnosis within 24 hours. It does not, however, reach 100% sensitivity even in clinically typical cases. HSV culture commonly gives positive results in the vesicular and pustular stages of the infection, but its sensitivity decreases considerably when lesions are old and crusted or in the ulcerous stage.

Direct and rapid confirmation of herpetic infection, though not specific for HSV 1, HSV 2, or varicellazoster virus, is possible with the Tzanck smear, using rapid staining techniques. Our previous study, which was conducted in a paediatric clinic and focused on herpetic and non-herpetic vesicular and bullous skin disorders, indicated the high sensitivity (80%) and specificity (90%) of the Tzanck smear. The Tzanck smear as a diagnostic tool can reliably support a clinical diagnosis of herpetic skin infections.

Clinicians can easily obtain experience in using the Tzanck smear effectively in office practice with the help of supplemental screening by a cytopathologist or cytotechnologist. We do not consider routine Tzanck smear examination to be suitable for office diagnosis of cervical lesions, because examining cervical smears is complex, takes time, and demands cytopathological experience. Cervical lesions were therefore excluded from this study. Nevertheless a positive Tzanck smear of cervical specimens can support the diagnosis of HSV infection.
The two most important factors in obtaining positive Tzanck smear results were the stage of the infection at the time of sampling and the location of the lesion. Moseley et al. reported a Tzanck smear sensitivity of 38% in genital herpes. Our study, however, indicated a higher sensitivity of the Tzanck smear. The Tzanck smear sensitivity in skin lesions of our patients yielding virus on culture was 79%. In vesicular lesions of skin and mucous membranes, the Tzanck smear reached a sensitivity of 81% in patients with culture proved genital herpes infection. Tzanck smear sensitivity was 80% in men and 44% in women with HSV positive ulcerous mucous membrane lesions. In the total group of patients with culture proved genital herpes the summarised Tzanck smear sensitivity compared with virus culture was 72%.

A discrepancy between a unanimously positive Tzanck smear result and negative culture results was found in seven patients. These can probably be considered as culture failures. These patients were finally assigned a clinical diagnosis of genital herpes, but they were not included in the calculations of this study. As already mentioned, the sensitivity of HSV culture is not 100%, but is presumably more than 90%.

The results showed reduced Tzanck smear sensitivities with differing sites of infection and stages of lesions. The average duration of ulcerous skin lesions was about five days and of ulcerous mucous membrane lesions about four days in men and six days in women. Viral shedding decreases with the duration of herpetic lesions. A decrease in viral shedding from herpetic lesions of longer duration may correlate with the lower sensitivity of the Tzanck smear. The speed with which cell cultures develop a cytopathological effect (CPE) can be used to estimate roughly the amount of herpes virus antigen present in the lesions investigated. We found no appreciable difference, however, in average time to developing CPE in herpetic mucous membrane lesions between men (7.7 days) and women (7.4 days). Necrotic syncytial giant cells can be found in herpetic lesions that are more than 72 hours old. The characteristic cytomorphological features of herpetic infection are obscured by the rapid loss of nuclear details in cells infected for longer. The longer average duration of ulcerous mucous membrane lesions in women in this study compared with the other herpetic lesions investigated might be a cytomorphological explanation for the lower Tzanck smear sensitivity.

In vesicular lesions, a Tzanck smear specificity of 100% was obtained by all investigators. This study showed a summarised specificity of at least 93% for the Tzanck smear in genital herpes with lesions of skin and adjacent mucous membranes. By using McNemar's test, statistical analysis showed no significant differences between the Tzanck smear sensitivity and specificity obtained by EF, APO, and JND. With the aid of sensitivity, specificity, and prevalence figures, the predictive values for positive (PV+) and negative (PV-) results were calculated. The best PV+ was attained in vesicular lesions, the best PV- in mucous membrane ulcers of men.

Positive and negative predictive values depend on the prevalence of the disease in the population investigated. With lower prevalence figures the PV+ decreases, but the PV- increases. For example, a prevalence of 50% would give a PV+ of 0.91 and a PV- of 0.77, whereas a prevalence of 10% would give a PV+ of 0.5 and a PV- of 0.97. In the population attending this STD clinic the prevalence of herpetic infections proved by virus culture of ulcerous genital lesions is about 50%.

The usefulness of a test with a given sensitivity and specificity depends on the purpose it
has to be used for. For screening in high risk populations, such as prostitutes, tests with a high sensitivity giving minimum false negative test results are desirable, in which case a high PV– will be obtained. Cytodiagnosis of herpetic infection cannot respond to criteria of a routine screening test excluding herpetic infection; the Tzanck test is therefore not suitable for antenatal screening for HSV infection. On the other hand positive Tzanck smear results are very reliable in confirming the clinical diagnosis of herpetic infection. In cytodiagnosis PV+ and PV– values depend on the skill and experience of the observer. Application of the Tzanck smear routinely in herpetic infections has the advantage of helping observers to gain and maintain experience with cytodiagnosis, so that they can rely on observation and interpretation of the test in cases with inconclusive clinical diagnosis. A positive Tzanck smear result in patients with equivocal clinical features may even obviate the need for virus culture, thus saving cost. Immunofluorescence cytology using monoclonal antibodies potentially increases the specificity and sensitivity of cytological preparations, but this method is time consuming and therefore not particularly suitable for office diagnosis. Further investigation of this technique is required.

This study, conducted in an STD clinic, shows Tzanck smear results of comparable sensitivity and specificity to those of our previous investigation (with the exception of the results of Tzanck smear sensitivity obtained in women with ulcerous mucous membrane lesions). Our results show highly reliable positive Tzanck smear results in venereological practice. Moreover this test is inexpensive, quick, and easy to perform, which makes it suitable for office diagnosis.

In dermatological and venereological practice, positive Tzanck smear result can lead to rapid confirmation of the diagnosis of herpetic infection, prompt treatment with acyclovir, if necessary, and greater assurance of the drug’s therapeutic effect. Confirmation of clinically diagnosed herpetic infection is possible with the Tzanck smear, even when virus culture fails.

Acknowledgements

We thank H.J.A. Schouten, Department of Medical Informatics and Statistics, University of Limburg, Maastricht, for statistical advice, and the staff of this clinic for their part in this investigation, particularly Sylvia Bos, Adrienne Flipse and Joyce Noordhoek Hegt-de Graaf for technical help, and Wies van Bolderik and Yvonne Creusen for supplying data on patients.
REFERENCEs


2.4 Diagnosis and treatment of pustular disorders in the neonate

Abstract

The diagnosis of a pustular dermatosis occurring during the first months of life is usually based on clinical findings. However, some cases may require simple investigations including microscopic examination of pustular content, cultures, and skin biopsies. The main benign transient neonatal types of pustulosis include erythema toxicum neonatorum, infantile acropustulosis, transient neonatal pustular melanosis, and neonatal acne. The most common causes of infectious pustular skin lesions include bacterial infections, which may be initially localized (Staphylococcus aureus) or septicemic (with Listeria monocytogenes as the leading causitive agent); viral infections (herpes simplex, varicella zoster, and cytomegalovirus infections); fungal infections (candidiasis); or parasitic disorders (scabies). The main objective of this review article is to propose a systematic approach of pustular eruptions in the neonate. The need for investigating every neonate with pustules for an infectious disease, is emphasized. The Tzanck smear, the Gram stain and a potassium hydroxide preparation are the most important quick diagnostic tests. The Tzanck smear is a very easy, rapid and sensitive test for detection of a herpetic infection (multinucleated giant cells) as well as noninfectious pustular eruptions (eosinophils, neutrophils). Therefore, the Tzanck smear should be the first test to be performed. Moreover, a Gram stain and potassium hydroxide preparation should be performed in cases of neonatal pustular disorders to detect bacterial and fungal infections. The goal of this diagnostic approach is to spare a healthy neonate, with a benign transient condition, an invasive evaluation for sepsis, potentially harmful antibiotic therapy, and prolonged hospitalization with its own inherent morbidity.

van Praag MCG 1, van Rooij RWG 1, Folkers E 2, Spritzer R 3, Menke HE 1, Oranje AP 4

1 Department of Dermatology, Sint Franciscus Hospital, Rotterdam, The Netherlands
2 Department of Dermatology, Hospital 'de Heel', Zaandam, The Netherlands
3 Department of Pediatrics, Sint Franciscus Hospital, Rotterdam, The Netherlands
4 Department of Dermatology and Venereology (Pediatric Dermatology), Erasmus University and University Hospital Dijkzigt/Sophia, Rotterdam, The Netherlands
(Published in: Pediatr Dermatol 1997; 14: 131-143)
Introduction

In the first 4 weeks of life (defined as the neonatal period), the infant is extremely vulnerable to bacterial, viral and fungal infections. Therefore, the presence of pustular lesions in the neonatal period evokes justifiable concern in the clinician caring for these infants. Pustular eruptions in neonates present a diagnostic challenge to the skilled dermatologist and pediatrician (Table 1). Simple diagnostic techniques can differentiate transient benign pustular eruptions from serious and life-threatening conditions that require immediate therapy. In this way a healthy neonate, with a benign transient condition, can be spared an invasive evaluation for sepsis, a potentially harmful antibiotic therapy, and prolonged hospitalization with its own inherent morbidity. We are aware of the fact, that a strict distinction between pustular disorders and vesiculobullous dermatoses in the neonate is rather artificial, because vesicles are frequently seen as precursor lesions in pustular dermatoses. However, primary vesiculobullous diseases in the neonate, such as milia cristallina, acrodermatitis enteropathica, epidermolysis bullosa, epidermolytic hyperkeratosis, herpes gestationis, pemphigus vulgaris, and urticaria pigmentosa, fall beyond the scope of this article.

The discussion below will provide information on history, physical and laboratory findings that help to distinguish between transient benign disorders, mild infections, and serious infectious conditions that all can occur during the neonatal period with pustular eruptions. The histopathology of the pustular dermatoses will be discussed, if relevant for establishing a diagnosis. Very rare neonatal pustular eruptions will be only briefly discussed here. Further information on issues not covered in detail here may be found in more exhaustive sources, such as Hurwitz1 and Schachner and Hansen2. Information about therapeutic intervention will be included where appropriate. The main objective of this review article is to propose a practical approach of neonatal pustular dermatoses in clinical practice.

Noninfectious neonatal pustular eruptions

Erythema toxicum neonatorum

Erythema toxicum neonatorum is a benign, self-limited neonatal eruption seen in approximately one-third of all full-term newborns3. Black and white infants are affected equally. The condition usually appears after 24 to 72 hours of life although it has been reported at birth as well4,5. The etiology of erythema toxicum is unknown. The lesions evolve from poorly defined erythematous macules to red, white, or yellow papules to a vesicular and, more rarely, pustular eruption on an erythematous base. They are asymptomatic and evanescent, and may disappear within hours after eruption. The sites of predilection are the forehead, face, chest, trunk, and extremities (Fig. 1). It is unusual to see lesions on the palms or soles.
Chapter 2 / Tzanck Smear and Viral Culture

Table 1. Neonatal pustular eruptions

<table>
<thead>
<tr>
<th>NONINFECTIOUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema toxicum neonatorum</td>
</tr>
<tr>
<td>Infantile acropustulosis</td>
</tr>
<tr>
<td>Transient neonatal pustular melanosis</td>
</tr>
<tr>
<td>Neonatal acne</td>
</tr>
<tr>
<td>Pustular miliaria</td>
</tr>
<tr>
<td>Eosinophilic pustular folliculitis of infancy</td>
</tr>
<tr>
<td>Incontinentia pigmenti</td>
</tr>
<tr>
<td>Congenital self-healing Langerhans cell histiocty</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INFECTIOUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
</tr>
<tr>
<td>Staphylococcus aureus (bullous impetigo)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>Streptococcus (group B β-hemolytic)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Hemophilus influenzae</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes virus infections</td>
</tr>
<tr>
<td>Herpes simplex</td>
</tr>
<tr>
<td>Varicella zoster</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidiasis</td>
</tr>
<tr>
<td>Congenital</td>
</tr>
<tr>
<td>Neonatal</td>
</tr>
<tr>
<td>Pityrosporum folliculitis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasitic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scabies</td>
</tr>
</tbody>
</table>

Laboratory findings in erythema toxicum may include eosinophilia up to 18% in as many as 15% of the cases. Otherwise there are no abnormal hematologic findings. Microscopic examination of pustular content is essential to confirm the diagnosis. A Tzanck smear (Giemsa, Wright, or Hemacolor® stain) or a Gram’s stain of lesional content demonstrates numerous eosinophils. Histopathologic examination reveals intrafollicular, subcorneal pustules with a dense accumulations of eosinophils. Eosinophils also infiltrate the outer root sheath of the pilosebaceous unit proximal to the sebaceous duct. More macular lesions may present with sparse perivascular accumulations of eosinophils in the dermis. Erythema toxicum will resolve spontaneously without residua within days to weeks, the usual duration being less than 1 week. Therefore, the diagnosis is based on the clinical
Diagnostic aspects of human alphaherpesvirus infections in dermatovenerology

appearance and evanescent nature of the lesions, as well as their eosinophil content. No treatment is necessary other than reassurance to the parents.

Infantile acropustulosis

Infantile acropustulosis is a relatively uncommon disorder first reported by Kahn and Rywlin and Jarratt and Ramsdell in 1979. It occurs primarily in black infants and in boys. Outside the United States, the preponderance in blacks has not been observed. The condition may begin during the neonatal period and continue throughout infancy and early childhood.

Clinical manifestations are limited to the skin, and affected neonates are healthy otherwise. The appearance of lesions is accompanied by pruritus, making the neonate irritable and restless. The lesions begin as small red papules, which evolve within 24 hours into vesicles and pustules several millimeters in diameter. The pustules are intensely pruritic, last for 7 to 10 days, and appear in crops every 2 to 3 weeks. They are found on the hands and feet predominantly and occasionally on the scalp, face and trunk. Mucosal surfaces are not reported to be affected, and sites of involvement can heal with slight residual pigmentation and scaling.

Laboratory studies are usually normal, but peripheral blood eosinophilia has been reported. A Tzanck smear (Giemsa, Wright, or Hemacolor® stain) or Gram's stain of pustular content reveals numerous neutrophils, occasionally eosinophils, and no bacteria. On histopathologic examination well-circumscribed, subcorneal pustules with polymorphic neutrophils and occasional eosinophils are seen. The underlying dermis is edematous and has a perivascular, mainly lymphocytic infiltrate. No correlation can be found between blood eosinophilia, composition of cutaneous infiltrate, age of the infant, and course of eruption.

Therefore, diagnosis is based on distribution of lesions, their pattern of recurrence, and appropriate stains of the pustules. The etiology of infantile acropustulosis is unknown. It is a self-limited disease, with exacerbations and remissions over 2 to 3 months and afterwards complete resolution. It is frequently worse in the summer months. Topical corticosteroids may be valuable. Antihistamines may provide relief of itching in older infants but are contraindicated in neonates because of the undesirable side effect of sedation. In severe cases dapsone at a dose of 1 to 2 mg/kg/day may be effective.

Transient neonatal pustular melanosis

Transient neonatal pustular melanosis (TNPM) was first described by Ramamurthy et al. in 1976. This disorder is present at birth in 4% to 5% of black infants and in 0.1% to 0.3% of white infants. Males and females are affected equally. The etiology of TNPM is unknown, although an increased incidence of placental squamous metaplasia has been reported in mothers whose infants develop this dermatosis.

Cutaneous lesions consist of vesiculopustules without surrounding erythema (Fig. 2). These are noted at birth or during the first day of life. They rupture easily, with the formation of pigmented macules that are surrounded by a characteristic collarette of scales. The macules may persist for several months but usually fade spontaneously within 3 to 4 weeks. Most commonly affected areas include the chin, neck, upper chest, lower back, buttocks, abdomen, and thighs, but all areas may be affected. Palms and soles are often
involved (Fig. 2). No systemic symptoms are associated with the lesions\textsuperscript{16}.

A Tzanck smear (Giemsa, Wright, or Hemacolor\textsuperscript{\textregistered} stain) or Gram’s stain of pustular content in TNPM demonstrates polymorphic neutrophils and occasional eosinophils. On histopathologic examination, intracorneal and subcorneal pustules with collections of polymorphic neutrophils and a few eosinophils are seen under a thickened stratum corneum\textsuperscript{7,13}. The dermis is generally uninvolved although in a later stage postinflammatory changes and melanophages may be seen\textsuperscript{17}.

Thus, the diagnosis of TNPM is suggested by vesiculopustular neutrophilic lesions in association with hyperpigmented macules that are present at birth. No specific therapy is recommended for TNPM since it resolves spontaneously. Parents should be informed that the hyperpigmented macules will disappear within several weeks to months.

**Neonatal acne**

Mild acne is fairly common in the newborn\textsuperscript{18}. The typical eruption consists of closed comedones on the nose, forehead, and cheeks. Open comedones, inflammatory papules and, pustules, may also occur. Although the etiology is not clearly defined, neonatal acne appears to result from stimulation of sebaceous glands by maternal and infant androgens. Lesions spontaneously resolve within 1 to 3 months as the sebaceous glands involute, and scarring is absent\textsuperscript{18}. Most cases of neonatal acne do not require treatment. If necessary, neonates can be treated with a 2.5 % benzoyl peroxide lotion\textsuperscript{19}. As therapy of choice, we would suggest 1 % salicylic acid, 1 % resorcin in a cream or 2% erythromycin in an alcoholic solution.

**Miliaria**

Miliaria is often seen in the first weeks of life. It is a manifestation of sweat retention due to occlusion of the immature eccrine sweat ducts resulting in rupture of the ducts with escape of sweat into the surrounding epidermis. There are three types of miliaria: crystallina, rubra, and profunda. These disorders may be distinguished by the level of obstruction within the ducts\textsuperscript{20}.

Miliaria crystallina is the most superficial, and is caused by subcorneal obstruction of the eccrine ducts, whereas miliaria rubra is intermediate in depth and results from intraepidermal duct obstruction, with outpouring of sweat into the lower epidermis. The lesions of miliaria crystallina consist of asymptomatic, clear, thin-walled, easily ruptured vesicles that appear in a generalized distribution, with an increase in intertriginous areas. Miliaria rubra consists of itchy, small, red papules or papulovesicles (“prickly heat”). The lesions are frequently grouped and extrafollicular in location, making the distinction between acneiform lesions possible\textsuperscript{1}. In neonates the face, neck, and trunk are most commonly involved\textsuperscript{21}. Miliaria rubra may progress to pustular lesions (pustular miliaria or miliaria profunda), particularly in climates with high temperature and humidity or during treatment with occlusive dressings or ointments. Miliaria profunda is rare in neonates. Microscopic examination of miliaria profunda shows a mononuclear infiltrate and eccrine obstruction at the dermo-epidermal junction, with disruption of the dermal eccrine system\textsuperscript{7}.

The diagnosis of miliaria is usually made on clinical observation. The treatment of choice is to reduce the infant’s environmental temperature and clothing. Cool soaks or shake lotions are helpful. Appropriate medication should be used to treat any suspected bacterial or candidal infection superimposed on miliaria.
Eosinophilic pustular folliculitis of infancy

Eosinophilic pustular folliculitis was first described in adults by Ofuji et al. Additional cases have been described recently. This rare disorder shows male predominance and can be present at birth. It is characterized by eosinophilic infiltration of hair follicles, resulting in pruritic grouped papules and pustules. In infants, unlike adults with eosinophilic pustular folliculitis, the lesions appear in a perifollicular pattern on the scalp, hands, and feet. They occur as recurrent crops of 1 to 3 mm white to yellow pruritic pustules on an erythematous base. They can also be present on the trunk. Many lesions have secondary crusting. There are no signs of systemic disease.

A Tzanck smear (Giemsa, Wright, or Hemacolor® stain) or Gram stain of pustular content demonstrates numerous eosinophils. Some patients have eosinophilia as well as leucocytosis on blood counts obtained during outbreaks. Biopsy of the pustules show eosinophils and eosinophilic spongiosis in the epidermis, with a dense dermal perifollicular infiltrate of eosinophils, histiocytes, and lymphocytes.

Diagnosis is based on the distribution of the pustules, appropriate stains of pustular content or biopsy revealing an eosinophilic infiltrate, and the presence of peripheral blood eosinophilia. The etiology is unknown although the possibility of an immunologic abnormality or an exaggerated allergic response to insects, mites, or parasites has been raised. More recently an association with human immunodeficiency virus has been reported.

Many other diagnoses can be considered in the differential diagnosis of eosinophilic pustulosis of the scalp, in particular Langerhans cell histiocytosis. In adults, the course is variable with exacerbations and remissions. In infants the total duration varies from 3 months to 5 years. Topical treatment with corticosteroids may reduce the pruritus and may attenuate the recurrences. Systemic antibiotics, antihistamines, and other topical agents are ineffective. Sulfonamides, sulfones, and systemic steroids have been used in adults with severe eosinophilic pustular folliculitis with variable success. Similar therapeutic experience is not available in infants.

Incontinentia pigmenti

Incontinentia pigmenti (Bloch-Sulzberger syndrome) is an uncommon and unusual genodermatosis that is believed to be transmitted as an X-linked dominant trait, lethal in most males; primarily female infants are affected. This disease is a multisystem syndrome having dermatologic, neurologic, skeletal, ocular, and dental manifestations. Classically, the infant manifests characteristic linear vesicular lesions that evolve into verrucous lesions within a few weeks, to be followed by a peculiar, swirled pigmentation that lasts for several weeks. In the early vesicular stage, which is rarely pustular, one can find microscopically intraepidermal, spongiotic blisters containing mainly eosinophils.

Congenital self-healing Langerhans cell histiocytosis

Congenital self-healing Langerhans cell histiocytosis (CSHLCH) is a rare condition initially seen at birth or in the neonatal period with generalized papules, vesicles, pustules, or nodules. The characteristic features of CSHLCH appear to be an otherwise healthy infant with no or mild systemic symptoms, histopathology demonstrating a Langerhans cell infiltrate, and spontaneous involution of skin lesions. Immunohistochemically Langerhans cells can be identified by demonstration of important markers, such as CD1a.
(OKT6), and the S-100 protein, and electronmicroscopically by the presence of Birbeck granules. No histopathologic features and immunohistochemical characteristics separate definitively CSHLCH from malignant forms of Langerhans cell histiocytosis (LCH). The ultrastructural findings of CSHLCH differ only in degree from those other forms of LCH by fewer cells with Birbeck granules and more with dense bodies, multivesicular bodies, and “comma-like” or “worm-like” bodies. At the present time, the clearing of skin lesions and failure to develop other organ involvement over the ensuing months is the only definitive way to be sure that an infant has congenital self-healing type of LCH rather than a malignant form of LCH. Although CSHLCH is usually a benign, self-limited condition, careful evaluation for systemic disease must be performed. Long-term follow-up for evidence of relapse or progression of disease is essential.

Infectious neonatal pustular eruptions

Bacterial infections

Impetigo bullosa

Certain strains of *Staphylococcus aureus* (e.g., phage group II, commonly lysotypes 3A, 3C, 55 or 71) have the ability to produce an exfoliative exotoxin. When the infection and toxin remain localized, focal *S. aureus* infection results (impetigo bullosa). When the toxins enter systemic circulation, there is potential involvement of the entire cutaneous surface. This disorder is called the staphylococcal scalded skin syndrome.

Impetigo bullosa may appear as early as the second or third day of life. It consists of vesicles, pustules or bullae on erythematous bases. Typical bullae are flacid, filled with clear yellow or turbid fluid and rupture easily, leaving a narrow rim of scale at the edge of a shallow, moist erosion (Figure 3). These lesions reepithelialize rapidly and do not result in scars. The diaper region and intertriginous areas of the body, such as the axilla and the neck, are commonly involved. Polymorphic neutrophils and gram-positive cocci in clusters are seen on a Gram’s stain of lesional contents. Cultures will grow *Staphylococcus aureus*. Histopathology demonstrates subcorneal pustules with gram-positive cocci and polymorphic neutrophils. The infection is chiefly limited to the epidermis and does not usually produce systemic manifestations. Localized infections can be treated with a topical antibiotic such as mupirocin or fucidic acid. More widespread lesions require a systemically administered antibiotic for seven days: a penicillinase-resistant penicillin (i.e., flucloxacillin, cloxacillin), macrolides (i.e., erythromycin, clarithromycin), or cephalosporins (i.e., cephalaxin, cefprozil). If fever, irritability, or other signs of systemic illness are present, the neonate requires admission to the hospital for a complete evaluation for sepsis and intravenous antibiotics. The hospital at which the infant was born should be notified, as the infection may indicate nursery contamination.
Bacterial infections can be acquired prenatally, during delivery, or after birth. Group B β-hemolytic streptococcus, *Listeria monocytogenes*, *Hemophilus influenza*, and *Pseudomonas aeruginosa*, are bacterial pathogens that may produce pustules and sepsis in the neonate\(^47\). The diagnosis of these bacterial infections requires the isolation of the organism by cultures.

Neonatal β-hemolytic streptococcus group B infection was uncommon, until the 1960s. For unknown reasons the incidence of the disease has increased, and β-hemolytic streptococcus group B now being the most common agent responsible for bacteremia or meningitis in the first month of life\(^48\). The optimal antimicrobial therapy is benzylpenicillin.

Listeriosis is a septicemic or meningitic disease, caused by a gram-positive bacteria, *Listeria monocytogenes*. The organism is isolated from soil, animals, and transmission is mainly foodborne. Two clinical forms of neonatal listeriosis have been described, an early and a late onset form. Early onset neonatal listeriosis occurs in infants infected in utero, most often infants of mothers who experienced the bacteremic, flulike prodrome before onset of labor. Widely disseminated granulomas are characteristic of severe disease in the newborn who is infected in utero, which is apparent either at birth, within a few hours after birth, or within a few days of life. In this disorder, lesions are most common in the liver and placenta, but can also be found in the skin, brain, adrenal glands, spleen, kidney, lungs and the gastrointestinal tract. There may be little clinical evidence to distinguish early-onset neonatal listeriosis from other forms of neonatal sepsis, but placental and posterior pharyngeal granulomas, as well as multiple small granulomas on the skin, can be early clues to the diagnosis\(^49\).

Late-onset neonatal listeriosis usually affects full-term infants of mothers who have experienced uncomplicated pregnancies. These infants are usually healthy at birth, and manifestations of the infection are apparent several days to several weeks after birth. The clinical manifestations of infection in this group are more likely to be related to meningitis than to sepsis. The characteristic skin eruption, which can occur in the early onset as well as in the late onset listeriosis, consists of a grayish-white maculopapulovesicular or pustular rash\(^36\). The treatment of choice is the combination of amoxicillin and tobramycin.

*Hemophilus influenza* is a small gram-negative coccobacillus and the main cause of meningitis in young children\(^2\). Cutaneous manifestations can be variable: patients with fulminant sepsis may have a petechial rash and occasionally bright pink tender macules, papules or pustules on the extremities and the trunk\(^2\)-\(^36\). As treatment amoxicillin (β-lactamase negative H. influenza) and cefuroxim (β-lactamase positive H. influenza) are recommended.

Sepsis caused by *Pseudomonas aeruginosa* occurs most commonly in the child with an underlying illness. It is seen in children with immune deficiency due to cancer chemotherapy, in those with malnutrition, and in those with extensive burns. Premature infants exposed to excessive humidity are also at risk for its development. The cutaneous manifestations of *Pseudomonas* sepsis are erythematous macules and petechiae, but the disease may also present with small, discrete nodules, vesicles and bullae. The classic skin lesion of *Pseudomonas* sepsis is termed ecthyma gangrenosum\(^50\). Although these lesions were once thought to occur in patients with bacteremia, ecthyma gangrenosum may
appear without evidence of hematogenous spread\textsuperscript{51,52}. The lesions typically begin as erythematous or purpuric macules that become indurated and progress to hemorrhagic blue-black bullae. The bullae rupture, leaving a central area of necrosis. The progression of the lesions is rapid, evolving in 12 to 24 hours. Moist areas of the body are most frequently the sites of these infections, especially the groin, axillae, and perianal areas. The lesions rarely occur on the mucous membranes, palms, or soles\textsuperscript{50}. Therapy with the combination of cefazidim and tobramycin should be initiated immediately. Supportive therapy for the cause of the predisposing illness must also be started.

Viral infections

\textit{Herpes simplex virus infection}

Most (70%) neonatal herpes simplex virus (HSV) infections are due to herpes simplex type 2 (HSV-2)\textsuperscript{53}. HSV-2 may be acquired by the neonate transplacentally by viremia during gestation, intranatally by passage through an infected birth canal, or postnatally by direct contact with infected humans. It is becoming clear that 16-30\% of woman in the USA are HSV-2 seropositive, and 0.3-2.0\% of woman shed HSV from the vagina at the time of delivery\textsuperscript{54}. The incidence of neonatal HSV-2 infection is dependent upon the type of maternal infection. In the case of primary HSV infection approximately 30-60\% of neonates will be infected, compared to only 1-3\% if the genital infection is a recurrence\textsuperscript{55}. Approximately 5\% of neonatal HSV is a truly intrauterine infection, resulting in a baby infected at birth\textsuperscript{56}. Since the primary period of viral inoculation is intrapartum, and given the variable incubation time, neonatal HSV may be present any time in the first 4-6 weeks of life. However, the majority of cases will present in the first week of life\textsuperscript{57}. Up to one-quarter of infected neonates have signs of infection on the first day of life\textsuperscript{58}.

The initial symptoms of disseminated HSV infection are lethargy, hypo- or hyperthermia, irritability, and poor feeding. Cutaneous findings are the first visible sign in about two-third of neonates infected with HSV\textsuperscript{54}. Grouped or single vesicles or pustules on erythematous bases appear in crops on the skin and mucous membranes (Fig. 4). The eyes may also be affected. Vesicles can coalesce to form bullae. Mucosal vesicles may quickly erode or may become pustular and crusted. Neonatal herpes may spread rapidly to involve the central nervous system and/or multiple internal organs. This may progress to rapid deterioration of the neonate's condition\textsuperscript{17,54}.

A Tzanck smear (Wright, Giemsa or Hemacolor\textsuperscript{5}) of vesicle bases reveals ballooned and multinucleated giant epithelial cells, indicating a herpes infection (Fig. 5). The virus itself can be demonstrated by culture, direct immunofluorescent testing and immunoelectron microscopy\textsuperscript{59} of material obtained from a herpetic lesion or a conjunctival swab. In the future the polymerase chain reaction may facilitate early diagnosis. Histopathologically an intraepidermal vesicle produced by ballooning and reticular degeneration of epidermal cells is seen. Marked acantholysis is present. Multinucleated cells and eosinophilic inclusion bodies can be seen. In the dermis an inflammatory infiltrate is present\textsuperscript{7}.

If left untreated, disseminated HSV infection is fatal in many cases\textsuperscript{17}. Early antiviral therapy may reduce overall mortality from 65 to 25\% \textsuperscript{60}. Survivors often have severe developmental and neurologic deficits.
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

Although it has been demonstrated that vidarabine is as effective as acyclovir in the HSV infected neonate\textsuperscript{56}, most experts utilize acyclovir in these patients because of relative ease of administration\textsuperscript{54}.

If active genital herpetic lesions are present in a pregnant woman at the time of labor, nearly all experts recommend cesarean section if the fetal membranes have been ruptured for less than 6 hours\textsuperscript{54}. Prevention is the best treatment, and newborns should be protected from exposure to HSV whenever possible.

\textbf{Varicella}

Varicella-zoster (VZ) virus is the agent responsible for varicella (chickenpox) and herpes zoster (shingles). Neonates can only develop varicella, as the development of herpes zoster would require prior exposure to the virus. Neonatal varicella occurs when a pregnant woman develops chickenpox during the last 2 or 3 weeks of pregnancy or the first few days post partum. In such instances, the timing of the onset of disease in the mother and her newborn are critical. If the disease onset in the mother is 5 or more days before delivery or in the newborn during the first 4 days of life, the infection usually results in no major sequelae for mother, fetus, or newborn. In contrast, however, congenital VZ infection acquired from 5 days prior to delivery until 3 days postdelivery may result in a neonatal varicella of increased morbidity and mortality due to insufficient passive transfer of maternal antibody and insufficient active development of neonatal antibody to varicella. Those neonates acquiring chickenpox after the third day of life tend to have a benign course\textsuperscript{47,48-57}.

The incubation time for chickenpox is approximately 2 weeks (range 10 to 23 days). Mild prodromal symptoms like fever, malaise, and upper respiratory symptoms may precede the onset of rash by 1 to 2 days but are generally absent in neonates. The skin lesions erupt in crops over 1 week and evolve within 12 to 24 hours of eruption from small red macules to papules to vesicles and pustules on erythematous bases. The lesions are typically described as umbilicated and appear to sit on top of the skin. Crusting occurs 1 to 3 days following eruption. Viral transmission is possible from 1 day before the rash erupts until all lesions are crusted over\textsuperscript{47}.

Diagnosis may be aided by the finding of multinucleated giant cells on a Tzanck smear of the base of an intact vesicle. Histopathology is identical to that seen in herpes simplex infections. Final prove is established by a positive VZ culture or immunoelectron microscopy\textsuperscript{61,62}.

Treatment of varicella in uncomplicated cases is supportive and symptomatic. Immunocompromised exposed newborns or neonates who were infected within 5 days prior to or several days after delivery should receive 125 to 250 units of zoster immune globulin (ZIG) from convalescing zoster patients or varicella-zoster immune globulin (VZIG) from high-titered normal adults intramuscularly as soon as possible. If ZIG or VZIG is not available, 1.3 ml/kg\textsuperscript{*} of regular immune serum globulin may be used\textsuperscript{58}. In addition, acyclovir needs to be given (preferably within the first 24 hours after delivery) in severe neonatal varicella infection and when zoster immune globulin is not available or is too late to use effectively.

\textbf{Cytomegalovirus infection}

Although Cytomegalovirus (CMV) infection in neonates is generally transmitted from a pregnant mother with inapparent infection across the placenta to the fetus late in gestation, it can also be transmitted by passage through an infected maternal genital tract.
at the time of delivery or postnatal CMV-seropositive bloodtransfusion. Approximately 90% of congenital CMV infections are asymptomatic. The remaining 10% may have mild to severe, and occasionally result in fatal, cytomegalic inclusion disease. CMV may directly invade fetal organs and result in defects in organogenesis. Skin findings include petechiae and purpura, a generalized maculopapular eruption, and in some instances, a generalized papulonodular eruption with blueberry muffin lesions similar to those seen in infants with congenital rubella and neonatal toxoplasmosis. Although vesicles and pustules are unusual in congenital CMV infection, the disorder must be considered in the differential diagnosis of cutaneous vesiculopustular lesions in the neonate. Diagnosis is often based on the finding of intranuclear inclusions in epithelial cells of urinesediment, which accounts for the name cytomegalic inclusion disease. CMV may also be isolated from placental tissues, amniotic fluid, blood, and cerebrospinal fluid (CSF). There is no effective therapy, and prognosis for the infant with severe involvement is poor.

Fungal infections

Candidiasis
Candidiasis exists in two forms, congenital and neonatal. Congenital candidiasis is an intrauterine infection, while neonatal candidiasis is acquired as the infant passes through a contaminated vagina. In both forms the causative organism is Candida albicans, a pathogen found in the vaginal canal of 20% to 25% of pregnant women. A possible way in which intrauterine infection may occur include Candida organisms ascending via the vagina and crossing ruptured or intact fetal membranes. In congenital candidiasis lesions are present at birth or usually within 12 hours following delivery. The rash is usually diffusely scattered over the whole body, including the face, chest, back, and extremities. Oral and diaper area involvement is generally absent. The congenital form usually starts as erythematous macules and papulovesicles. Over the next 4 to 7 days the lesions become pustular. A pronounced desquamation follows the acute phase with exfoliated crusted lesions. Signs of systemic disease and hematological abnormalities are generally absent. Stool cultures at birth are normally sterile.

Neonatal candidiasis is usually seen after the seventh day of life by oral thrush and lesions confined to the diaper area. Pustules and vesicles arising from the perianal area erode and spread peripherally with satellite lesions. The intergluteal fold, perineum, genitalia, suprapubic area, buttocks, and inner thighs are frequently involved. In these areas candidiasis evolves into scaling confluent plaques of a beefy red color, with distinct pustular and vesicular satellite lesions at the periphery of the plaques. Constitutional symptoms are absent. In neonatal candidiasis, C. albicans can often be isolated from the feces. Diagnosis of candidiasis is made by finding pseudohyphae and spores of a potassium hydroxide preparation of a pustule or scale. Candida albicans may be cultured from the vesicles and pustules. Candidiasis is treated topically with imidazole derivatives, such as miconazole, clotrimazole or ketoconazole cream. Lesions last approximately 2 weeks, desquamate, and resolve without residua. Thrush is treated by oral nystatine. Disseminated systemic candidiasis may occur rarely and is primarily an infection of...
Diagnostic aspects of human alphaherpesvirus infections in dermatovenereology

preterm, low birth weight infants, immunologically compromised patients and neonates requiring intensive care, with invasive procedures\textsuperscript{67}. It may affect the lungs, bronchial tree, meninges, kidneys, bladder, joints, and, less commonly, the liver, myocardium, endocardium, and eyes\textsuperscript{1}. Disseminated candidiasis is associated with significant morbidity and mortality. The spreading of a candidal diaper rash to the trunk and extremities is indicative of a localized infection beginning to disseminate. Intermittent, spiking, therapy resistant fever, with cutaneous candidal lesions or cellulitis at the site of an intravenous catheter, and persistent candidemia or candiduria, even in the absence of skin findings or systemic symptoms, indicate the presence of disseminated disease\textsuperscript{47,67}. Confirming the diagnosis of suspected disseminated candidiasis is difficult. Widespread infection despite negative cultures is common\textsuperscript{67}. The diagnosis is confirmed by isolating \textit{Candida albicans} from blood, abcesses, urine, or other body fluids, or by demonstration of the organism in a cutaneous biopsy or other surgical specimens. The early institution of treatment is the critical prognostic factor. Amphotericin B or 5-flucytosine intravenously are the drugs of choice\textsuperscript{68,69}. The use of these medications requires careful monitoring as their side effects are considerable\textsuperscript{67}.

\textit{Pityrosporum folliculitis}

\textit{Pityrosporum} yeasts (\textit{Malassezia furfur}) are the cause of pityriasis versicolor, which is usually seen as a disorder of adolescents and young adults, but may be a very rare cause of folliculitis in neonates\textsuperscript{36,70}. Cutaneous lesions consist of follicular papules and sparse pustules on the face and scalp. The diagnosis is based on direct microscopy (potassium hydroxide preparation) and culture of pustular contents. \textit{Pityrosporum folliculitis} can be successfully treated topically with imidazole derivatives, such as miconazole, clotrimazole or ketoconazole cream\textsuperscript{71}.

\textbf{Parasitic infections}

\textbf{Scabies}

Scabies is a contagious disorder caused by \textit{Sarcoptes scabiei}, a parasitic mite, which invades the stratum corneum. After an incubation period of 3-6 weeks, an extremely pruritic dermatitis develops\textsuperscript{72,73}. If infestation occurs soon after delivery, the disorder may be seen in the neonate\textsuperscript{73}. Scabies is a distinct clinical eruption characterized by pruritic papules, vesicles, and linear burrows mixed with excoriations, eczematization, crusting, or secondary infection. The clinical pattern of scabies in newborns differs from that seen in older infants, children, and adults. In older children and adults most of the lesions are concentrated on the finger webs, wrist, axillae, arm flexures, beltline, perineum and genitals. In infants and young children, the infestation rapidly becomes more generalized, usually involving the palms, soles, head, neck, and face\textsuperscript{73}. Vesicles are common in neonates, and there is tendency to pustule formation early in the course of the infestation (Fig. 6). Irritability, poor feeding, and failure to gain weight are also quite characteristic. A careful history and examination of the baby's caretakers will frequently disclose a history of pruritus and/or typical scabies lesions. Frequent maternal sites of lesions are periareolar regions of the breasts, as well as the wrists and fingers.
Definitive diagnosis is made by microscopic examination of scrapings from unexcoriated lesions in a potassium hydroxide or mineral oil preparation. The presence of the adult mite, ova, and/or larva confirms the diagnosis. The treatment of choice appears to be permethrin 5% cream, which must be applied from scalp to toes for six hours before rinsing with soap and water\textsuperscript{72}. When permethrin 5% cream is not available, neonates can also be treated with 5% precipitated sulfur in petrolatum\textsuperscript{73}. Sulfur-containing preparations are messy, staining, and odoriferous, and must be applied for three nights\textsuperscript{74}. All family members need to be treated at the same time. Clothing and bedding should be washed in high temperature water\textsuperscript{75}.

**Direct preparations for establishing a diagnosis**

Definitive diagnosis is usually obtained with smears and stains. Pustular eruptions of infectious etiology, caused by herpes simplex and varicella zoster virus, or cytomegalovirus must be ruled out with a Tzanck smear; bacterial infections, such as impetigo with routine Gram stain; candidiasis with routine potassium hydroxide (KOH) preparation, and scabies with appropriate mineral oil or KOH preparations. A Giemsa, Wright, or Hemacolor\textsuperscript{®} stained Tzanck smear is useful in identifying cell types in noninfectious pustular eruptions, especially eosinophils\textsuperscript{76}. For the Tzanck smear, a scalpel is used to carefully scrape material from the base of a fresh vesicle or pustule and the scraping is smeared on a slide and air dried. After drying, the material is fixed in methanol and stained preferentially by us with Hemacolor\textsuperscript{®} (Merck), because the staining can be done within one minute. Briefly this method includes dipping 5 times in methanol, 3 times in eosine and again 3 times in thiazine. After this procedure the slide is washed in buffered distilled water and ready for light microscopic examination\textsuperscript{77}. A herpes positive preparation demonstrates a specific cytological picture: epithelial cells with monstrously swollen nucleus, often multilobated or multinucleated, surrounded by a perinuclear halo. Cytologic differentiation between herpes simplex, herpes zoster and varicella is not possible. If the epitheloid shapes are difficult to discern as a result of overcolouring, in most cases the swollen nuclei often with several lobes or multinucleation can still be observed with stronger light (Fig. 5). If only one smear is available as a result of scarcity of lesions, the already coloured preparation can be decoloured using acid hydrochloride 25% in ethyl alcohol 95%. The decoloured preparation can then be restained according to other colouring methods, for example Gram’s stain.

**Discussion**

It is essential to investigate every neonate presenting with pustules for an infectious disease. A rapid differentiation between benign transient noninfectious pustular eruptions and such diseases as sepsis and herpes infections can be life saving. In few other conditions maternal history is important, since maternal infection should immediately suggest the possibility of transmission to the neonate. Features of the history of the disease and physical examination of skin lesions will often yield the correct diagnosis. For example, a
Figure 1
Erythema toxicum neonatorum in a caucasian neonate. (Reproduced with permission from Dr. A.P. Oranje. Aspecten van de kinderdermatologie. De Tijdstroom, Lochem, 1990; page 198)

Figure 2
Transient neonatal pustular melanosis in a negroid neonate from Suriname.

Figure 3
Impetigo bullosa in a caucasian neonate.
(Reproduced with permission from Dr. A.P. Oranje. Aspecten van de kinderdermatologie. De Tijdstroom, Lochem, 1990; page 42)
Figure 4
Herpes simplex type 2 in a caucasian neonate.
(Courtesy of Dr. J.H. Sillevis Smitt, dermatologist, AMC, Amsterdam)

Figure 5
A Tzanck smear of vesicle bases reveals ballooned and multinucleated giant epithelial cells.
(Courtesy of F.H.J.M. van de Noort, cytotechnologist, Department of Pathology, Sint Franciscus Gasthuis, Rotterdam)

Figure 6
Scabies in a caucasian neonate.
Table 2. Characteristic data of neonatal pustular disorders

| Disease                                           | Incidence*                                                                 | Age of onset                              | Duration **
|--------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------------|---------------------------------
| Erythema toxicum neonatorum                      | Approximately 1/3 of full-term neonates                                   | 24-72 hours                               | 1 week                         |
| Infantile acropustulosis                         | <1%, possibly increased in blacks and males                               | Hours after birth to 10 months             | 2-3 years                      |
| Transient neonatal pustular melanosis            | 5% of all black neonates; <1% in Caucasians                               | Birth, indicative of intrauterine involvement | Pustules: days                |
| Pustular miliaria                                | Unknown                                                                   | First weeks of life                        | Macules: 3 months              |
| Eosinophilic pustular folliculitis (Ojiuji’s disease) | Unknown reported or deported in adults                              | From birth; most cases                    | Hours to days                  |
| Congenital self-healing Langerhans cell histiocytosis | Rare reported in adults                                                | At birth or in the neonatal period         | Years                          |
| Incontinentia pigmenti                           | Rare                                                                      | Birth or within the first weeks of life   | Usually a benign self-limited condition within 4-24 months |
| Neonatal acne                                    | Unknown                                                                   | Variable                                   | Linear vesiculopustular lesions evolve into verrucous lesions within a few weeks |
| Candidiasis, congenital neonatal                  | <1%, equal among sexes                                                   | Birth-24 hours                             | 1 to 3 months                  |
| Pityrosporum folliculitis                        | Approx. 4-5%                                                              | after the first week of life               | 2 weeks                        |
| Scabies                                          | Very rare reported cases in adults                                       | From birth; most                          | 1 to 2 weeks                   |
| Impetigo bullosa                                 | Common, but rare in neonates                                            | 2-4 weeks                                 | Responds rapidly to treatment  |
| Herpes simplex (HSV)                             | 1/3000 to 1/20,000 births                                               | Majority in the first week life            | Approx. 5-10 days              |

* The incidence of most of the above mentioned disorders is not precisely known and shows a strong geographic variation.
** In case of infectious neonatal pustular disorders, average duration after treatment is reported.
history of recurring crops of pustules distributed on the hands and feet should immediately suggest a diagnosis of acropustulosis of infancy. An organized approach to the diagnosis of pustular eruptions in the neonate is outlined in Tables 2 and 3. The Tzanck smear can be used for detection of an herpetic infection (multinucleated giant cells) as well as noninfectious vesiculopustular eruptions. For example, the Tzanck smear is very useful to identify other cytomorphologic determinants, such as eosinophilic granulocytes in erythema toxicum neonatorum, eosinophilic pustular folliculitis, arthropod bites or incontinentia pigmenti. The Tzanck smear may also be used to detect neutrophilic granulocytes in infantile acropustulosis, or transient neonatal pustular melanosis. Therefore, we advocate the Tzanck smear as the first test to be performed. Secondly, Gram’s stain is indicated for observing bacteria. Good cooperation between the pediatrician and dermatologist is of utmost importance to make a rapid diagnosis and to start appropriate therapy immediately or no treatment at all other than reassurance to the parents in cases of benign transient noninfectious pustular eruptions.

Acknowledgments

The authors are grateful to F.H.J.M. van de Noort (cytotechnologist, Department of Pathology, Sint Franciscus Gasthuis, Rotterdam) for making an excellent colour photograph of a Tzanck smear and Dr. JH Sillevis Smitt (dermatologist, Academic Medical Center, Amsterdam) for providing a beautiful slide of herpes simplex neonatorum. Financial support for the printing of the color photographs in the article was kindly provided by E. Merck Nederland B.V.

*Erratum:* Instead of 0.1 to 0.3 ml/kg (dosis mentioned in the original paper)
Table 3. Morphologic, cytologic and histopathologic features of neonatal pustular disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical picture</th>
<th>Diagnostic test</th>
<th>Characteristic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema toxicum neonatorum</td>
<td>Red macules and papules; white to pink pustules, vesicles on the trunk, extremities and face</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Gram smear</td>
<td>Abundant eosinophils, rare neutrophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biopsy (in general not necessary)</td>
<td>No bacteria; abundant eosinophils</td>
</tr>
<tr>
<td>Infantile acropustulosis</td>
<td>Red papules evolving into pustular and vesicular lesions in one day</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Gram smear</td>
<td>Early: eosinophils predominate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biopsy (in general not necessary)</td>
<td>Late: neutrophils predominate</td>
</tr>
<tr>
<td>Transient neonatal pustular melanosis</td>
<td>Vesicles and pustules desquamate leaving brown macules on the chin, neck, palms, soles</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Gram smear</td>
<td>No bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biopsy (in general not necessary)</td>
<td>Intraepidermal vesicles (early); subcorneal pustules (mostly PMNs); mild perivascular infiltrate</td>
</tr>
<tr>
<td>Pustular millaria</td>
<td>Generalized grouped erythematous papules and pustules with an increase in the intertriginous areas</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Gram smear</td>
<td>Abundant neutrophils; rare eosinophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biopsy (in general not necessary)</td>
<td>No bacteria; neutrophils, rare eosinophils</td>
</tr>
<tr>
<td>Eosinophilic pustular folliculitis</td>
<td>Crops of papules, vesicles, and pustules that crust, primarily on the scalp with some lesions on the trunk and extremities</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Gram smear</td>
<td>Intra- and subcorneal pustule composed almost entirely of neutrophils; rare dermal infiltrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocytes predominate</td>
<td>No bacteria</td>
</tr>
<tr>
<td>Congenital self healing Langerhans cell histiocytosis</td>
<td>Generalized papules, vesicles, pustules or nodules</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) and Gram smear Biopsy</td>
<td>Eosinophils and neutrophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No bacteria</td>
</tr>
</tbody>
</table>

In the upper portion of the dermis an infiltrate composed almost entirely of Langerhans cells identified immunohistochemically by markers such as S-100 protein, and CD1a (OKT6), and electron-microscopically by Birbeck granules.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical picture</th>
<th>Diagnostic test</th>
<th>Characteristic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incontinentia pigmenti (rarely pustular)</td>
<td>Linear irregular vesicular and bullous lesions (rarely pustular) over the trunk and the extremities</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Biopsy</td>
<td>Abundant eosinophils</td>
</tr>
<tr>
<td>Neonatal acne</td>
<td>Closed comedones predominantly; open comedones, papules and pustules in the face</td>
<td>Gram smear Culture</td>
<td>Intraepidermal eosinophilic spongiotic pustules; dermal infiltrate with many eosinophils mixed with lymphocytes in an extralocular location</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>Pink to red macules and papules evolving into pustules and vesicles</td>
<td>KOH preparation Culture</td>
<td>Bacteria and yeast cells Bacterial: <em>Staphylococcus epidermidis, Propionibacterium acnes</em> Yeasts: <em>Pityrosporum ovale</em></td>
</tr>
<tr>
<td><em>Pityrosporum folliculitis</em></td>
<td>Follicular papules and sparse pustules on the face and scalp</td>
<td>KOH preparation Culture</td>
<td>Pseudohyphae and spores <em>Candida albicans</em> isolated</td>
</tr>
<tr>
<td>Scabies</td>
<td>Vesicles, pustules and papules; are burrows on hands, feet, trunk, genitalia</td>
<td>KOH or mineral oil</td>
<td>Yeast cells bud monopolarly with a broad base <em>Pityrosporum ovale</em> isolated</td>
</tr>
<tr>
<td>Impetigo bullosa</td>
<td>Vesicles, pustules, bullae on an erythematous base in the diaper area, neck, groin, axilla</td>
<td>Gram smear Gram of <em>Staphylococcus aureus</em> isolated</td>
<td><em>Sarcoplasma scabiei</em> mites, Preparation eggs, or fecal particles</td>
</tr>
<tr>
<td>Herpes simplex (HSV)</td>
<td>Grouped or single vesicles on erythematous bases in crops on the skin and mucous membranes</td>
<td>Tzanck smear (Giemsa, Wright's, or Hemacolor® stain) Bacterial culture</td>
<td>Positive cocci in clusters and neutrophils Neutrophils and bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tzanck smear Direct immunofluorescence Viral culture</td>
<td><em>Staphylococcus aureus</em> isolated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Multinucleated giant cells Positive for HSV Growth of HSV Identification of HSV DNA</td>
</tr>
</tbody>
</table>

HSV, herpes simplex virus; PMNs, polymorphonuclear leucocytes.
REFERENCES

Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology


Chapter 2 / Tzanck Smear and Viral Culture


