# ACTA DERMATOVENEROLOGICA

ALPINA, PANNONICA ET ADRIATICA

Volume 25

Issue 4

Ljubljana, December 2016

ISSN 1318-4458

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica


Volume 25, Issue 4, December 2016



**Editorial Office and Administration**
Department of Dermatovenerology, Zaloška 2, SI-1525 Ljubljana, Slovenia. Tel: +386 1 522 41 58, Fax: +386 1 522 43 33; E-mail: office@acta-apa.org

The Journal is published quarterly. The yearly subscription is 55 EUR for individuals and 65 EUR for institutions plus postage.
Indexed and abstracted by: BIOMEDICINA SLOVENICA, EMBASE/Excerpta Medica and Index Medicus/MEDLINE, Thomson Reuters' Web of Science Core Collection Emerging Sources Citation Index (ESCI)

**Submission guidelines**
The submission guidelines, updated on 1 January 2012, can be accessed at the following URL address:
http://www.acta-apa.org/submission-guidelines.pdf

**Orders and Payments**
**Orders:** Association of Slovenian Dermatovenerologists, Zaloška 2, SI-1525 Ljubljana, Slovenia
**Payments:** Nova Ljubljanska banka, d.d., Zaloška 7, Ljubljana
IBAN: SI 56020140089341717, SWIFT: LJBA2X, VAT: SI 32325029

**Published by**
Association of Slovenian Dermatovenerologists, Zaloška 2, SI-1525 Ljubljana, Slovenia

**Founded by**
A. Kansky in 1992, Ljubljana

**Design and print**
**Design:** Modra Jagoda (www.modrajagoda.si)
**Copy Editor for articles:** Donald F. Reindl
**Typesetting:** Milan Števanec
Printed on acid free paper ISO 9706 by: Nonparel d.o.o.

Journal Acta Dermatovenerologica Alpina, Pannonica et Adriatica is enlisted in Razvid medijev.

Journal Acta Dermatovenerologica Alpina, Pannonica et Adriatica is financially supported by the Slovenian Research Agency (Javna agencija za raziskovalno dejavnost Republike Slovenije).
Printed in 250 copies.

Acta Dermatovenerol APA

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica

2016;25(4)

# Table of Contents

LA ROCHE-POSAY
LABORATOIRE DERMATOLOGIQUE

# LIPIKAR AP+

**ZA PACIENTE Z ZELO SUHO, RAZDRAŽENO IN K ATOPIJI NAGNJENO KOŽO**

Rutina, ki obnavlja lipide z *AQUA POSAE FILIFORMIS*

**NOVO**

1


## **LIPIKAR** SYNDET AP+

Ocena po prhanju
Nežno čisti: 96 %*
Se zlahka spere: 91 %*
Kožo pusti navlaženo: 96 %*

BREZ MILA
NEVTRALEN PH
BREZ DIŠAV
BREZ PARABENA

* Na 54 odraslih osebah

2

## **LIPIKAR** BAUME AP+

Zmanjšuje srbenje
Obnavlja kožno pregrado
Pripomore k manj pogostim izbruhom*

BREZ DIŠAV
BREZ PARABENA

* Zelo močna suhost


LA ROCHE-POSAY. PREDAN DERMATOLOGIJI.

Acta **Dermatovenerol** APA

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica

2016;25:63
**doi: 10.15570/actaapa.2016.19**

# Editorial

## The first 25 years of *Acta Dermatovenerologica Alpina, Pannonica et Adriatica*

Jovan Miljković, Editor in Chief

The publication of the first issue of *Acta Dermatovenerologica Alpina, Pannonica et Adriatica* (*Acta Dermatovenerol APA*) 25 years ago was the result of the vision of a single exceptional man: Aleksej Kansky, one of the leading figures in the history of dermatology in Slovenia and central Europe. The primary intention was for the journal to function as forum for research and discussion articles, sharing the ideas and experience of professionals in the region. The journal was also to serve as a platform to assist young dermatologists in the region in publishing their first peer-reviewed manuscripts.

The first issue appeared in spring 1992, with the support of prominent dermatologists including H. Peter Soyer, Stefan Hödl, Helmut Kerl (Graz), Carmelo Scarpa, Giusto Trevisan (Trieste), Janez Fettich, Marko Potočnik (Ljubljana), Marija Berčič (Maribor), Günter Burg (Zurich), and Michael Lomuto (San Giovanni Rotondo). In the very first volume of the journal, its contributors already hailed from a variety of European countries (Germany, Austria, Italy, and Bosnia and Herzegovina), signaling its international character.

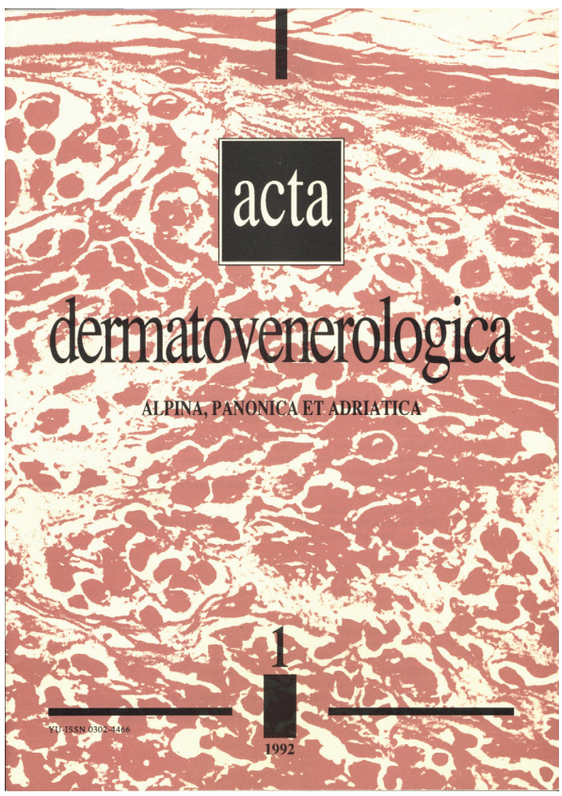
For the first 15 years, Aleksej Kansky worked hard to establish and maintain quality of the journal. In 2009, Aleksej Kansky stepped down as editor-in-chief and became editor emeritus, and Jovan Miljković took over as acting editor-in-chief assisted by Mario Poljak as associate editor.

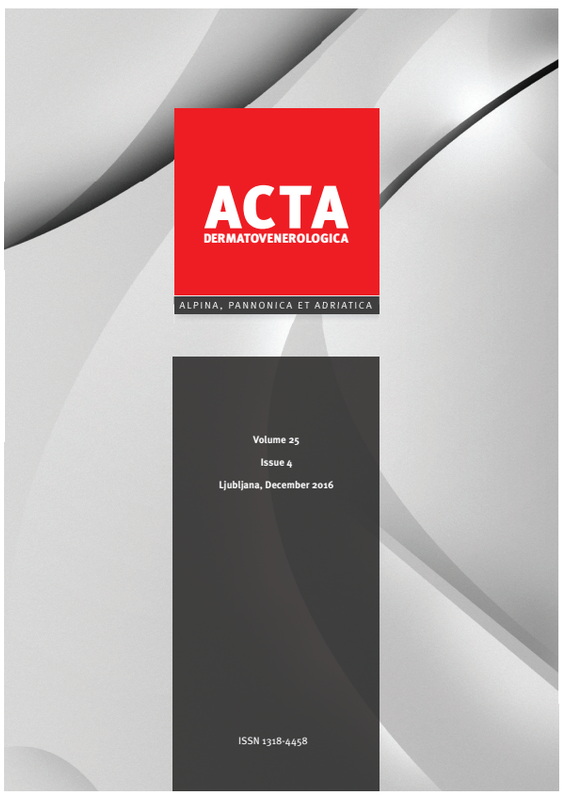
Now we celebrate the 25th anniversary of the journal and can look back over these 25 years of hard work and achievements.

The Slovenian Dermatology Society was founded in 1999, and it became the official publisher of the journal. Since 2000, *Acta Dermatovenerol APA* has been an open-access journal with the entire content of the journal freely available at the journal's website: http://www.acta-apa.org/.

In 2005, the journal achieved full indexing status in Index Medicus/MEDLINE, EMBASE/Excerpta Medica, and Biomedicina Slovenica. The entire content of the journal has been included in Pubmed, the most important database for medical journals.

Although *Acta Dermatovenerol APA* is a "small journal from a small country," it has significantly improved its quality and international profile over the last 25 years. When celebrating the 25th anniversary of *Acta Dermatovenerol APA*, we return once again to the vision of its founding editor, Aleksej Kanksy, and to the people that translated his vision into reality. The best recognition of their efforts is certainly the news that Thomson Reuters accepted *Acta Dermatovenerol APA* this year for coverage in the new Thomson Reuters Web of Science Core Collection index called the Emerging Sources Citation Index (ESCI).




## NATIONAL SUCCINT STATEMENT

▼This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. See section 4.8 for how to report adverse reactions.

**Cosentyx 150 mg solution for injection in pre-filled pen**
**Composition:** Each-pre filled pen contains 150 mg secukinumab in 1 ml. Secukinumab is a recombinant fully human monoclonal antibody selective for interleukin-17A. Secukinumab is of the IgG1/κ class produced in Chinese Hamster Ovary (CHO) cells. **Therapeutic indications:** Plaque psoriasis: moderate to severe plaque psoriasis in adults who are candidates for systemic therapy. Psoriatic arthritis: alone or in combination with methotrexate (MTX) in adult patients when the response to previous disease modifying anti rheumatic drug (DMARD) therapy has been inadequate. Ankylosing spondylitis: in adults who have responded inadequately to conventional therapy. **Posology:** Cosentyx is intended for use under the guidance and supervision of a physician experienced in the diagnosis and treatment of conditions for which Cosentyx is indicated. *Dosage:* For all indications initial dosing is at Weeks 0, 1, 2 and 3, followed by monthly maintenance dosing starting at Week 4. Consideration should be given to discontinuing treatment in patients who have shown no response by 16 weeks of treatment. Some patients with an initial partial response may subsequently improve with continued treatment beyond 16 weeks. *Recommended dose:* Plaque psoriasis: 300 mg of secukinumab. Each 300 mg dose is given as two subcutaneous injections of 150 mg. Psoriatic arthritis: 150 mg or 300 mg of secukinumab. For patients with concomitant moderate to severe plaque psoriasis or who are anti-TNFα inadequate responders (IR), the recommended dose is 300 mg by subcutaneous injection. Each 300 mg dose is given as two subcutaneous injections of 150 mg. For other patients, the recommended dose is 150 mg by subcutaneous injection. Ankylosing spondylitis: 150 mg of secukinumab. *Elderly patients (aged 65 years and over):* No dose adjustment is required. *Renal impairment/hepatic impairment:* Cosentyx has not been studied in these patient populations. No dose recommendations can be made. *Paediatric population:* The safety and efficacy of Cosentyx in children below the age of 18 years have not yet been established. No data are available. **Method of administration:** Cosentyx is to be administered by subcutaneous injection. If possible, areas of the skin that show psoriasis should be avoided as injection sites. After proper training in subcutaneous injection technique, patients may self-inject Cosentyx if a physician determines that this is appropriate. However, the physician should ensure appropriate follow-up of patients. Patients should be instructed to inject the full amount of Cosentyx according to the instructions provided in the package leaflet. **Contraindications:** Severe hypersensitivity reactions to the active substance or to any of the excipients listed. Clinically important, active infection (e.g. active tuberculosis). **Special warnings and precautions for use:** Infections: Cosentyx has the potential to increase the risk of infections. In clinical studies infections have been observed most of these were mild or moderate upper respiratory tract infections such as nasopharyngitis and did not require treatment discontinuation. Related to the mechanism of action of Cosentyx, non serious mucocutaneous candida infections were more frequently reported for secukinumab than placebo in the psoriasis clinical studies (3.55 per 100 patient years for secukinumab 300 mg versus 1.00 per 100 patient years for placebo). Caution should be exercised when considering the use of Cosentyx in patients with a chronic infection or a history of recurrent infection. Patients should be instructed to seek medical advice if signs or symptoms suggestive of an infection occur. If a patient develops a serious infection, the patient should be closely monitored and Cosentyx should not be administered until the infection resolves. No increased susceptibility to tuberculosis was reported from clinical studies. However, Cosentyx should not be given to patients with active tuberculosis. Anti-tuberculosis therapy should be considered prior to initiation of Cosentyx in patients with latent tuberculosis. Crohn's disease: Caution should be exercised when prescribing Cosentyx to patients with Crohn's disease as exacerbations of Crohn's disease, in some cases serious, were observed in clinical studies in both Cosentyx and placebo groups. Patients who are treated with Cosentyx and have Crohn's disease should be followed closely. Hypersensitivity reactions: In clinical studies, rare cases of anaphylactic reactions have been observed in patients receiving Cosentyx. If an anaphylactic or other serious allergic reactions occur, administration of Cosentyx should be discontinued immediately and appropriate therapy initiated. Latex sensitive individuals: The removable cap of the Cosentyx pre filled pen contains a derivative of natural rubber latex. No natural rubber latex has to date been detected in the removable cap. Nevertheless, the use of Cosentyx pre filled pens in latex sensitive individuals has not been studied and there is therefore a potential risk for hypersensitivity reactions which cannot be completely ruled out. Vaccinations: Live vaccines should not be given concurrently with Cosentyx. Patients receiving Cosentyx may receive concurrent inactivated or non live vaccinations. In a study, after meningococcal and inactivated influenza vaccinations, a similar proportion of healthy volunteers treated with 150 mg of secukinumab and those treated with placebo were able to mount an adequate immune response of at least a 4 fold increase in antibody titres to meningococcal and influenza vaccines. The data suggest that Cosentyx does not suppress the humoral immune response to the meningococcal or influenza vaccines. Concomitant immunosuppressive therapy: In psoriasis studies, the safety and efficacy of Cosentyx in combination with immunosuppressants, including biologics, or phototherapy have not been evaluated. Women of childbearing potential: Women of childbearing potential should use an effective method of contraception during treatment and for at least 20 weeks after treatment. Pregnancy: There are no adequate data from the use of secukinumab in pregnant women. As a precautionary measure, it is preferable to avoid the use of Cosentyx in pregnancy. Breast-feeding: It is not known whether secukinumab is excreted in human milk. Because of the potential for adverse reactions in nursing infants from secukinumab, a decision on whether to discontinue breast feeding during treatment and up to 20 weeks after treatment or to discontinue therapy with Cosentyx must be made taking into account the benefit of breast feeding to the child and the benefit of Cosentyx therapy to the woman. Fertility: The effect of secukinumab on human fertility has not been evaluated. Effects on ability to drive and use machines: Cosentyx has no or negligible influence on the ability to drive and use machines. **Interaction with other medicinal products and other forms of interaction:** Live vaccines should not be given concurrently with Cosentyx. No interaction studies have been performed in humans. There is no direct evidence for the role of IL 17A in the expression of CYP450 enzymes. The formation of some CYP450 enzymes is suppressed by increased levels of cytokines during chronic inflammation. Thus, anti inflammatory treatments, such as with the IL 17A inhibitor secukinumab, may result in normalisation of CYP450 levels with accompanying lower exposure of CYP450 metabolised co medications. Therefore, a clinically relevant effect on CYP450 substrates with a narrow therapeutic index, where the dose is individually adjusted (e.g. warfarin) cannot be excluded. On initiation of secukinumab therapy in patients being treated with these types of medicinal products, therapeutic monitoring should be considered. No interaction was seen when Cosentyx was administered concomitantly with methotrexate (MTX) and/or corticosteroids in arthritis studies (including in patients with psoriatic arthritis and ankylosing spondylitis). **Undesirable effects:** ***Very common:*** Upper respiratory tract infections. ***Common:*** Oral herpes, Rhinorrhoea, Diarrhea. ***Uncommon:*** Oral candidiasis, Tinea pedis, Otitis externa, Neutropenia, Conjunctivitis, Urticaria. ***Rare:*** Anaphylactic reactions.

**Marketing authorisation holder:** Novartis Europharm Limited, Frimley Business Park, Camberley GU16 7SR,United Kingdom. **Additional information and literature:** Novartis Pharma Services Inc., Podružnica v Sloveniji, Verovškova ulica 57, 1000 Ljubljana. **General classification for supply:** Rp/Spec. **Please read the summary of product characteristics before prescribing. This text was last revised in** December 2015.

Only for expert public.

COS-AD04-03/16-SI

NOVARTIS

Novartis Pharma Services Inc., Podružnica v Sloveniji
Verovškova 57, 1000 Ljubljana, telefon: 01/300-75-50

Cosentyx®
secukinumab

Acta Dermatovenerol APA

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica

2016;25:65-71
**doi: 10.15570/actaapa.2016.20**

# Development of a novel multiplex type-specific quantitative real-time PCR for detection and differentiation of infections with human papillomavirus types HPV2, HPV27, and HPV57

Lea Hošnjak[1], Kristina Fujs Komloš[1], Boštjan J. Kocjan[1], Katja Seme[1], Mario Poljak[1] ✉

## Abstract

**Introduction:** The present study describes the development and evaluation of the first multiplex type-specific quantitative real-time PCR (RT-PCR), enabling simple, rapid, sensitive, and specific concurrent detection and differentiation of human papillomavirus (HPV) types HPV2, 27, and 57 in a single PCR reaction.
**Results:** The HPV2/27/57 multiplex RT-PCR with a dynamic range of seven orders of magnitude (discriminating 10 to $10^8$ viral genome equivalents/reaction) has an analytical sensitivity of at least 10 viral copies of each targeted HPV type/reaction, and no cross-reactivities were observed among the included targets. All three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of $10^8$, $10^7$, 500, 100, and 10 copies of non-targeted viral DNA/reaction, and the performance of the HPV2/27/57 multiplex RT-PCR was additionally not affected by the presence of background human genomic DNA. When testing DNA isolates obtained from fresh-frozen tissue specimens of various children's warts, the results of the HPV2/27/57 multiplex RT-PCR were completely in line with the results of the conventional Low-risk *Alpha*-PV PCR.
**Conclusion:** The newly developed HPV2/27/57 multiplex RT-PCR is an appropriate test for use in routine clinical laboratory settings and for studies focusing on the molecular epidemiology, pathogenesis, and natural history of HPV2/27/57-related lesions.

**Keywords:** human papillomavirus types HPV2, HPV27, and HPV57, detection, differentiation, multiplex type-specific quantitative real-time PCR, development

**Received:** 3 October 2016 | **Returned for modification:** 19 October 2016 | **Accepted:** 3 November 2016

## Introduction

Human papillomavirus (HPV) types 2, 27, and 57, clustering within the species *Alphapapillomavirus* (*Alpha*-PV) 4, are etiologically associated with more than 65% of verrucae vulgares or common warts, the most frequent HPV-associated benign lesions of the skin, with the highest prevalence in children and immunosuppressed patients (1–9, 11, 12). Two other *Alpha*-PV types, HPV6 and HPV11, are in contrast the main etiological agents of condylomata acuminata or anogenital warts, the most frequent HPV-related benign lesion in the anogenital region of both sexes. However, common warts caused by HPV2, HPV27, and HPV57 can also frequently be found in the anogenital region, especially in children, as a result of autoinoculation from common warts from other parts of the body or infection transmitted from common warts of their parents or household members, and could be clinically misdiagnosed as condylomata acuminata (11–19). Such a misdiagnosis could have potential serious consequences because the appearance of new wart(s) in a child's anal or genital region can be considered an indicator of sexual abuse and can potentially trigger legal action against the parents or household members. Thus, although routine detection of HPV types present in tissue specimens or swabs of both condylomata acuminata (anogenital warts) and verrucae vulgares (common warts) is not generally recommended, it could be very helpful in some clinical circumstances and/or for legal purposes, especially in children. However, to be used for such purposes, diagnostic test(s) for detecting and distinguishing HPV types causing condylomata acuminata versus verrucae vulgares should be highly reliable and accurate.

In addition to *in situ* hybridization methods (20–22), several conventional broad-spectrum polymerase chain reactions (PCR)—which enable detection and differentiation of HPV types that are etiologically associated with condylomata acuminata and verrucae vulgares by subsequent laborious and time-consuming typing of PCR products using agarose gel electrophoresis, hybridization on strips/microtiter wells, and direct Sanger sequencing—have been described previously (23–31). Because *in situ* hybridization and conventional PCRs are suboptimal methods, de Koning et al. (32) and Schmitt et al. (33) developed broad-spectrum HPV typing bead-based xMAP Luminex suspension arrays, which are able to detect and differentiate 23 and 19 HPV types, respectively, that are most frequently found in common warts, including HPV2, HPV27, and HPV57. In addition, Köhler et al. (7) developed a multiplex type-specific quantitative real-time PCR (RT-PCR), which enables detection and differentiation of infections with HPV27 and HPV57. However, to the best of our knowledge, no quantitative real-time PCR allowing simultaneous amplification and differentiation of HPV2, HPV27, and HPV57 has been developed so far.

This study describes the development and analytical and clinical evaluation of a novel multiplex type-specific quantitative RT-PCR, allowing rapid, sensitive, and specific concurrent detection and differentiation of infections with HPV2, HPV27, and HPV57 in a single PCR reaction. The HPV2/27/57 multiplex RT-PCR was evaluated on a collection of fresh-frozen tissue specimens of condylomata acuminata and verrucae vulgares, obtained from children in a routine clinical laboratory setting.

## Materials and methods

To determine the most suitable viral genomic region(s) for designing a multiplex RT-PCR, enabling reliable detection and differentiation of infections with HPV2, HPV27, and HPV57, ten complete

[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. ✉ **Corresponding author: mario.poljak@mf.uni-lj.si**

genome sequences of targeted HPV types retrieved from the GenBank database (accession nos. X55964, EF117890, EF117891, EF362754, EF362755, X74473, AB211993, X55965, U37537, and AB361563) were aligned using the MAFFT v6.846 algorithm (34), as described previously (35). After evaluating the multiple alignment of complete HPV2, HPV27, and HPV57 genome sequences, the HPV L2 gene was selected as the most appropriate target region. Type-specific RT-PCR primers and hydrolysis probes (Table 1), allowing amplification of 144-, 145-, and 157-bp fragments of the respective L2 genes, were designed using Vector NTI Advance v11 software (Thermo Fisher Scientific, Carlsbad, CA) and subsequently revised for thermodynamic features of primer/probe and the potential of binding to non-targeted DNA sequences using the web-based applications NetPrimer (PREMIER Biosoft International, Palo Alto, CA), Primer3Plus (36), BLAST (National Center for Biotechnology Information, US National Library of Bethesda, MD), and MFEprimer-2.0 (37). As shown in Table 1, primer combinations 2–27F(59.8)/2R(59.2), 2–27F(59.8)/27R(57.6), and 57F(57.8)/57R(57.9) were used to amplify targeted regions of HPV2, HPV27, and HPV57, respectively. Type-specific hydrolysis probes—HPV2-P0(68.25), HPV27-P0(68.55), and HPV57-P0(65.34) (Table 1)—hybridized completely (100%) only with targeted HPV types and presented several (up to seven) nucleotide mismatches with non-targeted nucleotide sequences, enabling reliable discrimination between infections with HPV2, HPV27, and HPV57 (Fig. 1).

In order to optimize the amplification conditions and to evaluate the sensitivity, specificity, and efficiency of the HPV2/27/57 multiplex RT-PCR, plasmid standards containing viral sequences with binding sites of type-specific primers and probes were generated as follows. Three respective sense primers—HPV2-L2-FW (5'-CCCATTGTGTGATATTTGC-3'), HPV27-L2-FW (5'-CACCCTCATTGGCTTATTA-3'), and HPV57-L2-FW (5'-CGTCTGCTGCAGTAGTGTAC-3')—were used in combination with the consensus antisense primer HPV2,27,57-L2-RW (5'-TGACATAGACATCCGTACTGA-3') to amplify 1,730- , 1,599- , and 1,580-bp fragments of HPV2, 27, and 57, respectively. The obtained PCR amplicons were puri-

**Table 1** | **Nucleotide sequences of primers and hydrolysis probes designed for amplification of partial L2 genes of HPV2, HPV27, and HPV57.**

| Primer/probe | Nucleotide sequence (5'–3') | Nucleotide position[a] | Amplicon size |
|---|---|---|---|
| 2–27F(59.8)[b] | TACCTGCCCCCCAGACATT | HPV2 (4,386–4,404), HPV27 (4,359–4,377) | HPV2 (144-bp) |
| 2R(59.2) | GGAATGTACCCAGTACGCCC | HPV2 (4,529–4,510) | HPV27 (145-bp) |
| 27R(57.6) | AGGAATATACCCGGTACGTCC | HPV27 (4,503–4,483) | |
| 57F(57.8) | GCAAGCAGGCTGGAACG | HPV57 (4,327–4,343) | HPV57 (157-bp) |
| 57R(57.9) | GGTATGTAGCCTGTGCGTCC | HPV57 (4,483–4,464) | |
| HPV2-P0(68.25) | TEX-CCCAAGAGTGGAACAGAACACTTTAGCA-BBQ | HPV2 (4,407–4,434) | |
| HPV27-P0(68.55) | YAK-CTAGGGGTCTTCTTTGGCGGTCTTG-BBQ | HPV27 (4,432–4,456) | |
| HPV57-P0(65.34) | FAM-TTCGGTGGCCTCGGTATAGGTACT-BBQ | HPV57 (4,425–4,448) | |

Legend/abbreviations: [a]Nucleotide positions of primers and probes were compared to HPV2, HPV27, and HPV57 reference sequences (GenBank accession nos. X55964, X74473, and X55965, respectively), which were adjusted to start with a first nucleotide of respective E6 genes. [b]A single sense primer was used to amplify targeted regions of two HPV types (HPV2 and HPV27).

| **HPV2-P0(68.25)** | C | C | C | A | A | G | A | G | T | G | G | A | A | C | A | G | A | A | C | A | C | T | T | T | A | G | C | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HPV2a-X55964 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| HPV2-EF117890 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| HPV2-EF117891 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| HPV2-EF362754 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| HPV2-EF362755 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| HPV27-X74473 | T | • | • | • | • | • | G | C | • | A | • | • | • | • | • | A | • | • | • | • | • | C | • | • | G | • | • | • |
| HPV27b-AB211993 | T | • | • | • | • | • | G | C | • | • | • | • | • | • | • | A | • | • | • | • | • | C | • | • | G | • | • | • |
| HPV57-X55965 | A | • | • | T | • | • | G | • | • | • | • | • | • | • | • | • | G | • | • | • | • | A | • | • | • | • | • | T |
| HPV57b-U37537 | A | • | • | T | • | • | G | • | • | • | • | • | • | • | • | • | G | • | • | • | • | A | • | • | • | • | • | T |
| HPV57c-AB361563 | A | • | • | T | • | • | G | • | • | • | • | • | • | • | • | • | G | • | • | • | • | A | • | • | • | • | • | T |
| **HPV27-P0(68.55)** | C | T | A | G | G | G | G | T | C | T | T | C | T | T | T | G | G | C | G | G | T | C | T | T | G | | | |
| HPV2a-X55964 | T | • | • | • | • | T | • | • | G | • | • | T | • | • | • | • | • | G | • | • | • | • | • | A | • | | | |
| HPV2-EF117890 | T | • | • | • | • | T | • | • | G | • | • | T | • | • | • | • | • | G | • | • | • | • | • | A | • | | | |
| HPV2-EF117891 | T | • | • | • | • | T | • | • | G | • | • | T | • | • | • | • | • | G | • | • | • | • | • | A | • | | | |
| HPV2-EF362754 | T | • | • | • | • | T | • | • | G | • | • | T | • | • | • | • | • | G | • | • | • | • | • | A | • | | | |
| HPV2-EF362755 | T | • | • | • | • | T | • | • | G | • | • | T | • | • | • | • | • | G | • | • | • | • | • | A | • | | | |
| HPV27-X74473 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | |
| HPV27b-AB211993 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | |
| HPV57-X55965 | • | • | G | • | • | • | • | • | • | • | • | T | • | • | C | • | • | T | • | • | C | • | • | C | • | | | |
| HPV57b-U37537 | • | • | • | • | • | • | • | • | • | • | • | T | • | • | C | • | • | T | • | • | C | • | • | C | • | | | |
| HPV57c-AB361563 | • | • | • | • | • | • | • | • | • | • | • | T | • | • | C | • | • | T | • | • | C | • | • | C | • | | | |
| **HPV57-P0(65.34)** | T | T | C | G | G | T | G | G | C | C | T | C | G | G | T | A | T | A | G | G | T | A | C | T | | | | |
| HPV2a-X55964 | • | • | T | • | • | G | • | • | T | • | • | A | • | • | • | • | • | • | • | • | C | • | • | C | | | | |
| HPV2-EF117890 | • | • | T | • | • | G | • | • | T | • | • | A | • | • | • | • | • | • | • | • | C | • | • | C | | | | |
| HPV2-EF117891 | • | • | T | • | • | G | • | • | T | • | • | A | • | • | • | • | • | • | • | • | C | • | • | • | | | | |
| HPV2-EF362754 | • | • | T | • | • | G | • | • | T | • | • | A | • | • | • | • | • | • | • | • | C | • | • | • | | | | |
| HPV2-EF362755 | • | • | T | • | • | G | • | • | T | • | • | A | • | • | • | • | • | • | • | • | C | • | • | C | | | | |
| HPV27-X74473 | • | • | T | • | • | C | • | • | T | • | • | T | • | • | • | • | • | • | • | • | C | • | • | • | | | | |
| HPV27b-AB211993 | • | • | T | • | • | C | • | • | T | • | • | T | • | • | • | • | • | • | • | • | C | • | • | • | | | | |
| HPV57-X55965 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | | |
| HPV57b-U37537 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | | |
| HPV57c-AB361563 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | | |

Figure 1 | Schematic diagram showing hybridization of HPV2, HPV27, and HPV57 type-specific hydrolysis probes to respective L2 gene sequences. The figure was obtained from a multiple sequence alignment of type-specific hydrolysis probes and complete genome sequences of respective HPV types that were retrieved from the GenBank database (GenBank accession numbers are provided next to all full genome sequences included). Dots show the nucleotide positions of hydrolysis probes identical to the targeted regions of HPV2, HPV27, and HPV57.

fied using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and cloned into plasmid vectors with the TOPO XL PCR Cloning Kit (Thermo Fisher Scientific). Plasmid HPV2/27/57 DNA was purified using the QIAprep Spin Miniprep Kit (Qiagen), verified by direct Sanger sequencing and quantified at 260 nm using a NanoDrop ND-2000c spectrophotometer (NanoDrop Technologies, Oxfordshire, UK). The quantified plasmids contained $1.31 \times 10^{10}$, $3.30 \times 10^{10}$, and $2.59 \times 10^{10}$ copies of HPV2, 27, and 57 DNA per µl, respectively, and were subsequently serially diluted, as described previously (38). All commercially available reagents were used according to the manufacturers' instructions.

The HPV2/27/57 multiplex RT-PCR was performed in a 96-well plate on a LightCycler 480 Instrument II using a LightCycler 480 Probes Master kit (Roche Diagnostics, Mannheim, Germany). The RT-PCR protocol was designed following the manufacturer's instructions and adjusted to (i) characteristics of targeted nucleotide sequences and synthesized primers/probes and (ii) estimated length of RT-PCR amplicons. The thoroughly optimized reaction mixture consisted of 10 µl of 2 × LightCycler 480 Probes Master (Roche Diagnostics), 0.5 µM of each RT-PCR primer (Table 1), with the exception of the 2–27F(59.8) primer, which was used in a concentration of 1 µM, 0.1 µM of each probe, 5 µl of template DNA (50–100 ng of DNA extracted from clinical samples and $1 \times 10 - 1 \times 10^8$ DNA copies/reaction of plasmid standards), and PCR-grade water up to the final reaction volume of 20 µl. The amplification of targeted nucleotide sequences was performed as follows: (i) initial denaturation of template DNA at 95 °C for 10 min (temperature transition rate of 4.4 °C/s), (ii) followed by 40 amplification cycles consisting of three incubation steps: 95 °C for 10 s (4.4 °C/s), 60 °C for 30 s (2.2 °C/s), and 72 °C for 1 s (4.4 °C/s; fluorescent signal acquisition), and (iii) a final cooling step at 2.2 °C/s to 40 °C with a 30 s hold. Since type-specific hydrolysis probes were labeled with three different 5' fluorophores (TEX, YAK, and FAM; Table 1), real-time monitoring of the fluorescent signal was performed on 610, 560, and 530 nm channels, indicating amplification of HPV2, HPV27, and HPV57, respectively. In addition, due to the slight overlap of the emission spectra of the dyes, the software's color compensation function was applied during the analysis of all RT-PCR experiments. Moreover, the specificity of all HPV2/27/57 RT-PCR amplicons was further confirmed by direct Sanger sequencing with the same primers as used for the RT-PCR, as described previously (39).

The performance of the HPV2/27/57 multiplex RT-PCR in the routine clinical laboratory setting was evaluated on 35 fresh-frozen tissue samples, obtained from the same number of children, 2 to 18 years old, with common warts (10 samples) and anogenital warts (25 samples) that were referred to the Laboratory for Molecular Microbiology and Slovenian HIV/AIDS Reference Centre, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, in the last 10 years.

The tissue samples were first processed for total DNA extraction with a QIAamp DNA Mini Kit (Qiagen) and spectrophotometric analysis of eluted DNA, as described previously (40). Up to 100 ng of extracted DNA was used for downstream PCR analyses. The integrity of the extracted DNA was determined by the quantitative RT-PCR, enabling amplification of the 150-bp fragment of human beta-globin gene. Briefly, the beta-globin RT-PCR was performed on a LightCycler 2.0 Instrument (Roche Diagnostics) using a QuantiTect SYBR Green PCR + UNG Kit (Qiagen). The reaction mixture consisted of 12.5 µl of 2 × QuantiTect SYBR Green PCR Master mix, 0.5 µM of each primer (41), 5 µl of extracted DNA, and PCR-grade water up to the final reaction volume of 25 µl. The amplification of human DNA was performed as follows: (i) initial denaturation of template DNA at 95 °C for 15 min (temperature transition rate of 20 °C/s), (ii) followed by 45 amplification cycles consisting of three incubation steps: 94 °C for 15 s (20 °C/s), 60 °C for 20 s (20 °C/s), and 72 °C for 20 s (2 °C/s; fluorescent signal acquisition at 530 nm), (iii) a melting curve analysis, consisting of three incubation steps: 95 °C for 0 s (20 °C/s), 50 °C for 30 s (20 °C/s), and 95 °C for 0 s (0.1 °C/s), and (iv) a final cooling step at 20 °C/s to 40 °C with a 30 s hold. Testing triplicates of 10-fold serially diluted standards of commercially available human DNA (Human Genomic DNA; Promega, Madison, WI), spanning from 100 ng to 1 pg of DNA per reaction, showed that the beta-globin RT-PCR had a sensitivity of at least 10 pg of human DNA per reaction. The correlation coefficient ($R^2$) of the standard curve estimated from amplification of human DNA standards over six orders of magnitude and the efficiency of human DNA amplification (E) were estimated at 0.996 and 91.4%, respectively. Only beta-globin-positive DNA isolates (melting peaks between 80.5 and 81.5 °C) were considered adequate for further analyses.

To detect low-risk *Alpha*-PVs associated with various mucosal and cutaneous warts, a PCR protocol targeting an approximately 190-bp fragment of the E1 gene of HPV2, HPV3, HPV6, HPV7, HPV10, HPV11, HPV13, HPV27, HPV28, HPV29, HPV32, HPV40, HPV42, HPV43, HPV44, HPV57, HPV74, HPV77, HPV78, HPV91, HPV94, HPV117, and HPV125 was performed, as described elsewhere (31), and HPV types were subsequently determined by direct Sanger sequencing of all eligible PCR products, as described previously (39). Furthermore, a FRET-based HPV6/11 RT-PCR (40), enabling reliable detection and differentiation of 25.3, 42.9, and 43.4 DNA copies of HPV11 and prototypic and non-prototypic HPV6 genomic variants, respectively, was additionally used to determine the causal agents of condylomata acuminata.

## Results

Testing replicates of 10-fold serially diluted plasmids containing targeted fragments of HPV2, HPV27, and HPV57 in concentrations spanning from $1 \times 10^8$ to $1 \times 10$ DNA copies per reaction, in a background of 100 ng of Human Genomic DNA, showed that the HPV2/27/57 multiplex RT-PCR is able to detect at least 10 viral copies of each targeted HPV type per a single reaction (Fig. 2; A1, B1, C1). The dynamic range of HPV2/27/57 multiplex RT-PCR was seven orders of magnitude for all targeted HPV types, enabling reliable discrimination of 10 to $10^8$ viral genome equivalents per a single reaction. The correlation coefficients ($R^2$) of standard curves estimated from amplification of plasmid standards containing fragments of HPV2, HPV27, and HPV57 were 0.999, 0.999, and 0.998, respectively. The amplification efficiencies (E) were estimated at 95.2, 92.0, and 92.2% for HPV2, HPV27, and HPV57, respectively, and the performance of the HPV2/27/57 multiplex RT-PCR was not affected by the presence of background human genomic DNA. In addition, as shown in Fig. 2 (A2, B2, and C2), no cross-reactivities of HPV27/HPV57, HPV2/HPV57, and HPV2/HPV27 were observed when using primer/probe combinations targeting HPV2, HPV27, and HPV57, respectively. Moreover, all three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of $1 \times 10^8$, $1 \times 10^7$, 500, 100, and 10 copies of non-targeted viral DNA per reaction (Fig. 2; A3, B3, C3).

As shown in Table 2, the targeted fragment of human beta-globin gene was successfully amplified from all 35 DNA isolates obtained from fresh-frozen tissue specimens of condylomata acuminata and verrucae vulgares. HPV2, HPV27, and HPV57 were

detected in 7/10 (70.0%) tested verrucae vulgares using both Low-risk *Alpha*-PV PCR and HPV2/27/57 multiplex RT-PCR; and in all seven HPV-positive cases both PCRs identified the same HPV type (Table 2; samples nos. 1–7). The results of both PCRs were additionally completely concordant when testing different warts from the anogenital region, since HPV2, HPV27, and HPV57 were detected in 13/25 (52.0%) tested samples, irrespective of the method used. Furthermore, in seven condylomata acuminata that were previously HPV6-positive using Low-risk *Alpha*-PV PCR, the presence of HPV6 was confirmed with the HPV6/11 RT-PCR and all seven samples tested HPV2/27/57-negative using the HPV2/27/57 multiplex RT-PCR (Table 2, samples nos. 24–30). Using the PCR protocols mentioned above, *Alpha*-PV DNA was absent in three and five samples of tested verrucae vulgares and condylomata acuminata, respectively (Table 2, samples nos. 8–10 and nos. 31–35, respectively).

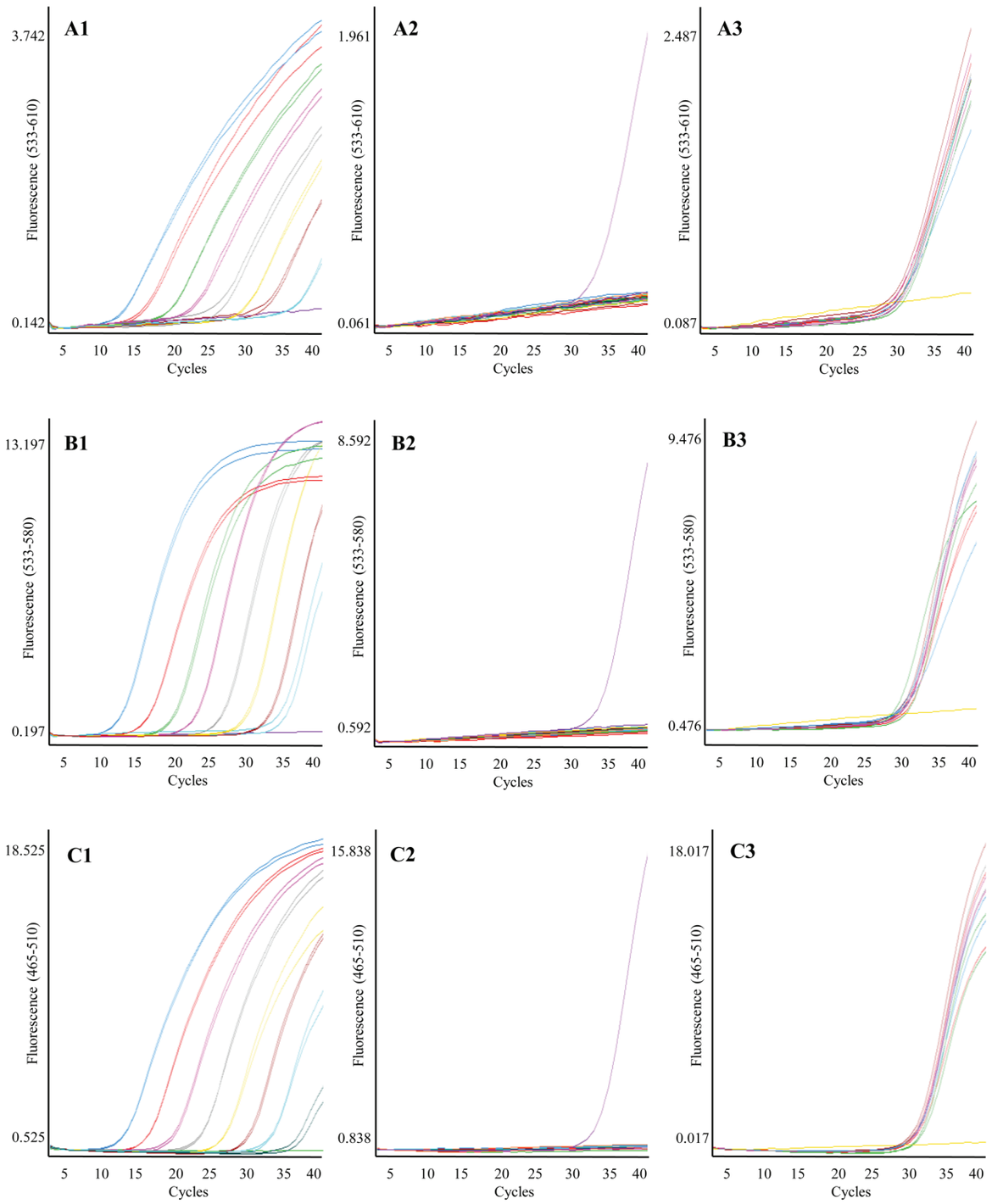



Figure 2 | Evaluation of the performance of HPV2/27/57 multiplex RT-PCR based on the amplification of plasmid standards containing targeted nucleotide sequences of HPV2, HPV27, and HPV57. (A1, B1, and C1) RT-PCR amplification plots of replicates of 10-fold serially diluted plasmids containing targeted fragments of HPV2 (A1), 27 (B1), and 57 (C1) in concentrations spanning from $1 \times 10^8$ to $1 \times 10$ DNA copies per reaction, in a background of 100 ng of commercially available human DNA (Human Genomic DNA; Promega, Madison, WI), showing that the HPV2/27/57 multiplex RT-PCR is able to detect at least 10 viral copies of each targeted HPV type per a single reaction. (A2, B2, and C2) No amplification of HPV27/HPV57 (A2), HPV2/HPV57 (B2), and HPV2/HPV27 (C2) was observed when using primer/probe combinations targeting HPV2, HPV27, and HPV57, respectively. (A3, B3, and C3) RT-PCR amplification plots showing that all three primer/probe combinations are efficient in amplifying 500 copies of HPV2 (A3), HPV27 (B3), and HPV57 (C3) in a background of $1 \times 10^8$, $1 \times 10^7$, 500, 100, and 10 viral copies of HPV27/HPV57, HPV2/HPV57, and HPV2/HPV27 per reaction, respectively.

**Table 2 | Clinical samples of condylomata acuminata and verrucae vulgares used to evaluate the performance of the HPV2/27/57 multiplex RT-PCR in the routine clinical laboratory setting.**

| Patient no. | Age (years) | Beta-globin RT-PCR[a] | Low-risk *Alpha*-PV PCR[b] | HPV6/11 RT-PCR[c] | HPV2/27/57 multiplex RT-PCR |
|---|---|---|---|---|---|
| 1 | 9 | positive | HPV2 | negative | HPV2 |
| 2 | 5 | positive | HPV2 | negative | HPV2 |
| 3 | 10 | positive | HPV27 | negative | HPV27 |
| 4 | 12 | positive | HPV27 | negative | HPV27 |
| 5 | 7 | positive | HPV57 | negative | HPV57 |
| 6 | 13 | positive | HPV57 | negative | HPV57 |
| 7 | 14 | positive | HPV57 | negative | HPV57 |
| 8 | 18 | positive | negative | negative | negative |
| 9 | 12 | positive | negative | negative | negative |
| 10 | 13 | positive | negative | negative | negative |
| 11 | 4 | positive | HPV2 | negative | HPV2 |
| 12 | 8 | positive | HPV2 | negative | HPV2 |
| 13 | 5 | positive | HPV2 | negative | HPV2 |
| 14 | 6 | positive | HPV2 | negative | HPV2 |
| 15 | 9 | positive | HPV2 | negative | HPV2 |
| 16 | 7 | positive | HPV2 | negative | HPV2 |
| 17 | 6 | positive | HPV57 | negative | HPV57 |
| 18 | 11 | positive | HPV57 | negative | HPV57 |
| 19 | 4 | positive | HPV57 | negative | HPV57 |
| 20 | 5 | positive | HPV57 | negative | HPV57 |
| 21 | 8 | positive | HPV57 | negative | HPV57 |
| 22 | 5 | positive | HPV57 | negative | HPV57 |
| 23 | 12 | positive | HPV57 | negative | HPV57 |
| 24 | 5 | positive | HPV6 | HPV6 | negative |
| 25 | 8 | positive | HPV6 | HPV6 | negative |
| 26 | 6 | positive | HPV6 | HPV6 | negative |
| 27 | 7 | positive | HPV6 | HPV6 | negative |
| 28 | 7 | positive | HPV6 | HPV6 | negative |
| 29 | 4 | positive | HPV6 | HPV6 | negative |
| 30 | 3 | positive | HPV6 | HPV6 | negative |
| 31 | 5 | positive | negative | negative | negative |
| 32 | 7 | positive | negative | negative | negative |
| 33 | 2 | positive | negative | negative | negative |
| 34 | 3 | positive | negative | negative | negative |
| 35 | 4 | positive | negative | negative | negative |

Legend/abbreviations: [a]The integrity of the extracted DNA was determined by the quantitative RT-PCR, enabling amplification of the 150-bp fragment of human beta-globin gene. [b]A previously published Low-risk *Alpha*-PV PCR (31) was used to detect *Alpha*-PVs that are most frequently associated with various mucosal and cutaneous warts. [c]A FRET-based HPV6/11 RT-PCR (40), enabling reliable detection and differentiation of HPV11 and prototypic and non-prototypic HPV6 genomic variants, respectively, was used to determine the causal agents of condylomata acuminata. Tissue samples obtained from patients nos. 8–10 and 31–35 were additionally tested for the presence of *Gamma/Mu*-PVs and/or MCV (data not shown).

## Discussion

Verrucae vulgares or common warts constitute the most frequent benign HPV-associated skin condition, especially in children and immunosuppressed patients (5, 6). Most common warts resolve spontaneously within several months, have a benign nature, and are successfully treated with various regimens or procedures such as cryotherapy, salicylic acid, and topical and intralesional immunotherapy (42). Although they are more prevalent in children, the etiology of common warts does not differ according to patient's age group; common warts are most frequently associated with infections with three HPV genotypes: HPV2, HPV27, and HPV57 (1–12). In contrast, the etiology of warts found in the anogenital region differs between children and adults. Sexually transmitted HPV6 and HPV11 are by far the most common HPV types identified in warts in the anogenital region of adult patients of both genders because the great majority of these warts are indeed condylomata acuminata and only rarely verrucae vulgares (11, 12, 16–18, 43). In contrast, up to two-thirds of warts found in the anogenital region of children are actually verrucae vulgares, which are most frequently etiologically associated with infections with HPV2, HPV27, and HPV57 (13–15, 19). Even though both HPV6 and HPV11 are associated with a small proportion of warts found in the anogenital region of children, the routes of transmission of condylomata acuminata in this population are mostly non-sexual, including vertical transmission and indirect transmission through contaminated objects or surfaces, and are only rarely a result of sexual abuse (13–15, 19).

For years, warts identified in the anogenital region of patients of all ages (including children) referred to our molecular diagnostics laboratory had first been tested for the presence of *Alpha*-PVs using the Low-risk *Alpha*-PV PCR (31), with a turnaround time of at least 370 min, including the analysis of PCR products using direct Sanger sequencing. Although very sensitive and specific, Low-risk *Alpha*-PV PCR is quite laborious, has a long turnaround time, and is therefore inappropriate for use in a routine clinical laboratory setting. The newly developed HPV2/27/57 multiplex RT-PCR is able to specifically detect at least 10 viral copies per a single reaction of each targeted HPV type, irrespective of potentially high concentrations of other HPV types present in a sample (concurrent HPV infection with several HPV types), and its performance is also not affected by the presence of a high background of human genomic DNA. Furthermore, HPV2/27/57 multiplex RT-PCR has a relatively short turnaround time of approximately 70 min, rendering it appropriate for routine diagnostics. Therefore, when testing warts found in the anogenital region of a child, the HPV2/27/57 multiplex RT-PCR recently became the method of choice in our laboratory. HPV2/27/57-negative children's warts are subsequently tested for the presence of HPV6 and HPV11 using the HPV6/11 RT-PCR (40), and when both of these RT-PCRs are negative the conventional Low-risk *Alpha*-PV PCR is used as a supportive method due to its ability to detect several other cu-

taneous and mucosal wart-associated *Alpha*-PV types (31). In contrast, because more than 90% of warts identified in the anogenital region of adult patients are etiologically associated with sexually transmitted HPV6 and HPV11 (11, 12, 16–18, 43), when testing this patient population the method of choice in our laboratory is the HPV6/11 RT-PCR (40), followed in the case of a negative result by HPV2/27/57 multiplex RT-PCR and Low-risk *Alpha*-PV-PCR (31). *Alpha*-PV-negative wart tissue samples are additionally tested in our laboratory for research purposes only for the presence of several *Gamma*- and *Mu*-PVs that cause sporadic cutaneous warts (7, 9, 11, 12, 26, 44, 45). All HPV-negative warts identified in the anogenital region of patients of all ages and both sexes are additionally tested in our laboratory for the presence of molluscum contagiosum virus (MCV) using the MCV FRET RT-PCR (45) because, due to the similar clinical appearance of lesions, up to 10% of molluscum contagiosum lesions can be misdiagnosed as condylomata acuminata or verrucae vulgares and vice versa (45–48).

Because HPV2, HPV27, and HPV57 are associated with a large fraction of verrucae vulgares in immunosuppressed patients, in which they often occur ubiquitously and confluently, Senger et al. provided a basis for the development of virus-like particle-based vaccines against cutaneous *Alpha*-PVs (49, 50). Our HPV2/27/57 multiplex RT-PCR could therefore be additionally applicable for large epidemiological studies of the etiology of common warts in immunosuppressed patients and for potential evaluation of the efficacy of the future vaccine(s) against HPV2, HPV27, and HPV57.

In contrast to previously described conventional PCRs (23–30), which amplify up to 835-bp fragments of HPV DNA, the HPV2/27/57 multiplex RT-PCR targets significantly shorter HPV DNA fragments (144–157-bp), also rendering it very appropriate for HPV typing in archival tissue specimens (51). Furthermore, the majority of conventional broad-spectrum PCRs are not suitable for detecting viral targets present in low concentrations, and Sanger sequencing of PCR products hinders the identification of concurrent HPV infections. Namely, in sporadic cases of common warts concurrent infections with two or more HPV types can be identified, including their well-known etiological agents, such as HPV1, HPV2, HPV4, HPV7, HPV27, HPV57, and HPV65 (3, 7, 10, 26, 32). Because one of the surrogate markers for determining the etiology of common warts is the estimation of the viral load of each HPV type present in the lesion of question (7, 35, 52), HPV2/27/57 multiplex RT-PCR can be used in combination with other quantitative HPV type-specific RT-PCRs to identify the HPV type with the highest HPV viral load and consequently the highest probability of being a "true" etiological agent of the investigated common wart.

In conclusion, the newly developed HPV2/27/57 multiplex RT-PCR, which enables simple, rapid, sensitive, and specific concurrent detection and differentiation of infections with HPV2, HPV27, and HPV57 in a single PCR reaction, is an appropriate test for use in routine clinical laboratory settings and for studies focusing on the molecular epidemiology, pathogenesis, and natural history of HPV2/27/57-related lesions.

## Funding

This study was funded by the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana. The study was partially supported by the European Union's Seventh Framework Program for research, technological development, and demonstration under the CoheaHr project (grant agreement no. HEALTH-F3-2013-603019).

## Conflicts of interest

The authors have no conflicts of interest to declare.

## Acknowledgement

The authors would like to thank our clinical colleagues—Vesna Breznik, Sarah Dobnik, Vlasta Dragoš, Andreja Komac, Petra Meglič, Boštjan Mlakar, Kristina Nikolajević, Katarina Planinc Pustovrh, Ružica Preveden, Marija Rebolj Stare, Mirjam Rogl Butina, Uršula Salobir Gajšek, Mojca Simončič Godnič, Janja Šmid, Mateja Starbek Zorko, Andreja Štolfa, Vesna Tlaker Žunter, Janez Tomažič, and Lina Virnik Kovač—for collecting clinical samples of common warts and anogenital warts. In addition, we would like to thank Robert Krošelj, Blanka Kušar, Petra Markočič, Anja Oštrbenk, Tina Štamol, and Sonja Vuletić for their invaluable technical assistance in detecting infections with HPV and molluscum contagiosum virus.

## References

1. Rübben A, Krones R, Schwetschenau B, Grussendorf-Conen EI. Common warts from immunocompetent patients show the same distribution of human papillomavirus types as common warts from immunocompromised patients. Br J Dermatol. 1993;128:264-70.
2. Jabłońska S, Majewski S, Obalek S, Orth G. Cutaneous warts. Clin Dermatol. 1997;15:309-19.
3. Rübben A, Kalka K, Spelten B, Grussendorf-Conen EI. Clinical features and age distribution of patients with HPV2/27/57-induced common warts. Arch Dermatol Res. 1997;289:337-40.
4. Majewski S, Jablonska S. The role of HPVs in benign and malignant cutaneous proliferations. Papillomavirus Report. 2003;14:1-10.
5. Bouwes Bavinck JN, Euvrard S, Naldi L, Nindl I, Proby CM, Neale R, et al. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in The Netherlands, United Kingdom, Germany, France, and Italy. J Invest Dermatol. 2007;127:1647-56.
6. van Haalen FM, Bruggink SC, Gussekloo J, Assendelft WJ, Eekhof JA. Warts in primary schoolchildren: prevalence and relation with environmental factors. Br J Dermatol. 2009;161:148-52.
7. Köhler A, Meyer T, Stockfleth E, Nindl I. High viral load of human wart-associated papillomaviruses (PV) but not beta-PV in cutaneous warts independent of immunosuppression. Br J Dermatol. 2009;161:528-35.
8. Cardoso JC, Calonje E. Cutaneous manifestations of human papillomaviruses: a review. Acta Dermatovenerol Alp Pannonica Adriat. 2011;20:145-54.
9. de Koning MN, Khoe LV, Eekhof JA, Kamp M, Gussekloo J, ter Schegget J, et al. Lesional HPV types of cutaneous warts can be reliably identified by surface swabs. J Clin Virol. 2011;52:84-7.
10. Bruggink SC, de Koning MN, Gussekloo J, Egberts PF, ter Schegget J, Feltkamp MC, et al. Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. J Clin Virol. 2012;55:250-5.
11. Cubie HA. Diseases associated with human papillomavirus infection. Virology. 2013;445:21-34.
12. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25:2-23.
13. Syrjänen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. Crit Rev Oral Biol Med. 2000;11:259-74.
14. Marcoux D, Nadeau K, McCuaig C, et al. Pediatric anogenital warts: a 7-year review of children referred to a tertiary-care hospital in Montreal, Canada. Pediatr Dermatol. 2006;23:199-207.
15. Aguilera-Barrantes I, Magro C, Nuovo GJ. Verruca vulgaris of the vulva in children and adults: a nonvenereal type of vulvar wart. Am J Surg Pathol. 2007;31:529-35.

16. Aubin F, Prétet JL, Jacquard AC, Saunier M, Carcopino X, Jaroud F, et al. Human papillomavirus genotype distribution in external acuminata condylomata: a large French national study (EDiTH IV). Clin Infect Dis. 2008;47:610-5.
17. Garland SM, Steben M, Sings HL, James M, Lu S, Railkar R, et al. Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent HPV (types 6, 11, 16, 18) vaccine. J Infect Dis. 2009;119:805-14.
18. Komloš KF, Kocjan BJ, Košorok P, Luzar B, Meglič L, Potočnik M, et al. Tumor-specific and gender-specific pre-vaccination distribution of human papillomavirus types 6 and 11 in anogenital warts and laryngeal papillomas: a study on 574 tissue specimens. J Med Virol. 2012;84:1233-41.
19. Varma S, Lathrop E, Haddad LB. Pediatric condylomata acuminata. J Pediatr Adolesc Gynecol. 2013;26:e121-2.
20. Nuovo GJ, Lastarria DA, Smith S, Lerner J, Comité SL, Eliezri YD. Human papillomavirus segregation patterns in genital and nongenital warts in prepubertal children and adults. Am J Clin Pathol. 1991;95:467-74.
21. Anderson KM, Perez-Montiel D, Miles L, Allen CM, Nuovo GJ. The histologic differentiation of oral condylomata acuminatum from its mimics. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96:420-8.
22. Purdie KJ, Surentheran T, Sterling JC, Bell L, McGregor JM, Proby CM, et al. Human papillomavirus gene expression in cutaneous squamous cell carcinomas from immunosuppressed and immunocompetent individuals. J Invest Dermatol. 2005;125:98-107.
23. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells. 1989;7:209-14.
24. Shamanin V, Delius H, de Villiers E. Development of a broad spectrum PCR assay for papillomaviruses and its application in screening lung cancer biopsies. J Gen Virol. 1994;75:1149-56.
25. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J Gen Virol. 1999;80:2437-43.
26. Harwood CA, Spink PJ, Surentheran T, Leigh IM, de Villiers EM, McGregor JM, et al. Degenerate and nested PCR: a highly sensitive and specific method for detection of human papillomavirus infection in cutaneous warts. J Clin Microbiol. 1999;37:3545-55.
27. Lai JY, Doyle RJ, Bluhm JM, Johnson JC. Multiplexed PCR genotyping of HPVs from plantaris verrucae. J Clin Virol. 2006;35:435-41.
28. Jeney C, Takács T, Sebe A, Schaff Z. Detection and typing of 46 genital human papillomaviruses by the L1F/L1R primer system based multiplex PCR and hybridization. J Virol Methods. 2007;140:32-42.
29. Lei YJ, Gao C, An R, Shi Q, Chen JM, Yuan YK, et al. Development of a multiplex PCR method for detecting and typing human papillomaviruses in verrucae vulgaris. J Virol Methods. 2008;147:72-7.
30. Sasagawa T, Mitsuishi T. Novel polymerase chain reaction method for detecting cutaneous human papillomavirus DNA. J Med Virol. 2012;84:138-44.
31. Odar K, Kocjan BJ, Hošnjak L, Gale N, Poljak M, Zidar N. Verrucous carcinoma of the head and neck—not a human papillomavirus-related tumour? J Cell Mol Med. 2014;18:635-45.
32. de Koning MN, ter Schegget J, Eekhof JA, Kamp M, Kleter B, Gussekloo J, et al. Evaluation of a novel broad-spectrum PCR-multiplex genotyping assay for identification of cutaneous wart-associated human papillomavirus types. J Clin Microbiol. 2010;48:1706-11.
33. Schmitt M, de Koning MN, Eekhof JA, Quint WG, Pawlita M. Evaluation of a novel multiplex human papillomavirus (HPV) genotyping assay for HPV types in skin warts. J Clin Microbiol. 2011;49:3262-7.
34. Katoh K, Toh H. Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics. 2010;26:1899-900.
35. Hošnjak L, Kocjan BJ, Pirš B, Seme K, Poljak M. Characterization of two novel gammapapillomaviruses, HPV179 and HPV184, isolated from common warts of a renal-transplant recipient. PLoS One. 2015;10:e0119154.
36. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3—new capabilities and interfaces. Nucleic Acids Res. 2012;40:e115.
37. Qu W, Zhou Y, Zhang Y, Lu Y, Wang X, Zhao D, et al. MFEprimer-2.0: a fast thermodynamics-based program for checking PCR primer specificity. Nucleic Acids Res. 2012;40:W205-8.
38. Kocjan BJ, Poljak M, Seme K. Universal ProbeLibrary based real-time PCR assay for detection and confirmation of human papillomavirus genotype 52 infections. J Virol Methods. 2010;163:492-4.
39. Kocjan BJ, Poljak M, Seme K, Potočnik M, Fujs K, Babic DZ. Distribution of human papillomavirus genotypes in plucked eyebrow hairs from Slovenian males with genital warts. Infect Genet Evol. 2005;5:255-9.
40. Kocjan BJ, Seme K, Poljak M. Detection and differentiation of human papillomavirus genotypes HPV-6 and HPV-11 by FRET-based real-time PCR. J Virol Methods. 2008;153:245-9.
41. van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA, et al. Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int J Cancer. 2002;98:590-5.
42. Sterling J. Treatment of warts and molluscum: what does the evidence show? Curr Opin Pediatr. 2016;28:490-9.
43. Majewski S, Jablonska S. Human papillomavirus-associated tumors of the skin and mucosa. J Am Acad Dermatol. 1997;36:659-85.
44. Ure AE, Forslund O. Characterization of human papillomavirus type 154 and tissue tropism of gammapapillomaviruses. PLoS One. 2014;9:e89342.
45. Hošnjak L, Kocjan BJ, Kušar B, Seme K, Poljak M. Rapid detection and typing of Molluscum contagiosum virus by FRET-based real-time PCR. J Virol Methods. 2013;187:431-4.
46. Hanson D, Diven DG. Molluscum contagiosum. Dermatol Online J. 2003;9:2.
47. Tyring SK. Molluscum contagiosum: the importance of early diagnosis and treatment. Am J Obstet Gynecol. 2003;189:12-6.
48. Trama JP, Adelson ME, Mordechai E. Identification and genotyping of molluscum contagiosum virus from genital swab samples by real-time PCR and pyrosequencing. J Clin Virol. 2007;40:325-9.
49. Senger T, Becker MR, Schädlich L, Waterboer T, Gissmann L. Identification of B-cell epitopes on virus-like particles of cutaneous alpha-human papillomaviruses. J Virol. 2009;83:12692-701.
50. Senger T, Schädlich L, Textor S, Klein C, Michael KM, Buck CB, et al. Virus-like particles and capsomeres are potent vaccines against cutaneous alpha HPVs. Vaccine. 2010;10;28:1583-93.
51. Kocjan BJ, Hošnjak L, Poljak M. Detection of alpha human papillomaviruses in archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens. J Clin Virol. 2016;76 Suppl 1:S88-97.
52. Bzhalava D, Johansson H, Ekström J, Faust H, Möller B, Eklund C, et al. Unbiased approach for virus detection in skin lesions. PLoS One. 2013;8:e65953.


# Enbrel 50 mg raztopina za injiciranje v napolnjenem injekcijskem peresniku

## BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

**ENBREL 25 mg prašek in vehikel za raztopino za injiciranje**[1]
**ENBREL 50 mg raztopina za injiciranje v napolnjeni injekcijski brizgi**[2]
**ENBREL 10 mg prašek in vehikel za raztopino za injiciranje za uporabo pri otrocih**[3]

**ENBREL 50 mg raztopina za injiciranje v napolnjenem injekcijskem peresniku**[4]**Sestava in oblika zdravila:** (1) Ena viala vsebuje 25 mg etanercepta. (2) Ena napolnjena injekcijska brizga vsebuje 50 mg etanercepta. (3) Ena viala vsebuje 10 mg etanercepta. (4) En napolnjen injekcijski peresnik vsebuje 50 mg etanercepta. Etanercept je pridobljen z rekombinantno DNA tehnologijo v ovarijskih celicah kitajskega hrčka. **Indikacije:** (1,2,4) Revmatoidni artritis (RA) - zmeren do hud aktiven RA pri odraslih (v kombinaciji z metotreksatom), kadar odziv na zdravljenje z imunomodulirajočimi zdravili, vključno z metotreksatom (če ta ni kontraindiciran), ni zadosten. Monoterapija, kadar bolnik ne prenese metotreksata ali kadar trajno zdravljenje z njim ni primerno. Hud, aktiven in napredujoč RA pri odraslih, ki še niso dobivali metotreksata. (1,2,3,4) Juvenilni idiopatski artritis (JIA) - poliartritis (pozitiven ali negativen za revmatoidni faktor) in razširjen oligoartritis pri otrocih in mladostnikih, starih 2 leti ali več, ki so se nezadostno odzvali na zdravljenje z metotreksatom ali ga niso prenašali. Psoriatični artritis pri mladostnikih, starih 12 let ali več, ki so se nezadostno odzvali na zdravljenje z metotreksatom ali ga niso prenašali. Artritis, povezan z entezitisom, pri mladostnikih, starih 12 let ali več, ki so se nezadostno odzvali na konvencionalno zdravljenje ali ga niso prenašali. (1,2,4) Psoriatični artritis (PA) - aktiven in progresiven PA pri odraslih, če je bil odziv na zdravljenje z imunomodulirajočimi zdravili nezadosten. (1,2,4) Ankilozirajoči spondilitis (AS) - hud aktiven AS pri odraslih, če je bil odziv na konvencionalno zdravljenje nezadosten. (1,2,4) Radiografsko nezaznavni aksialni spondilartritis - Zdravljenje odraslih s hudim radiografsko nezaznavnim aksialnim spondilartritisom in objektivnimi znaki vnetja, ki imajo nezadostni odziv na NSAID. (1,2,4) Psoriaza v plakih (PP) - zmerna do huda PP pri odraslih, ki se ne odzovejo na drugo sistemsko zdravljenje, vključno s ciklosporinom, metotreksatom ali psoralenom in ultravijolično svetlobo UV-A (PUVA), oziroma je pri njih le-to kontraindicirano ali ga ne prenašajo. (1,2,3,4) Otroška PP - huda kronična PP pri otrocih in mladostnikih od 6. leta starosti naprej, pri katerih se z drugo sistemsko terapijo ali fototerapijo bolezni ne da zadostno obvladati ali jih bolniki ne prenašajo. **Odmerjanje in način uporabe:** Zdravljenje z Enbrelom lahko uvede in nadzoruje le zdravnik specialist, ki ima izkušnje z zdravljenjem navedenih stanj. Bolniki, ki se zdravijo z Enbrelom, naj prejmejo opozorilno kartico za bolnika. Odrasli (vse indikacije): 25 mg dvakrat na teden ali 50 mg enkrat na teden. Klinični odziv pri RA, PA, AS in radiografsko nezaznavnem aksialnem spondilartritisu je običajno dosežen v 12 tednih zdravljenja. Če v tem obdobju ni odziva, je treba o nadaljevanju zdravljenja skrbno razmisliti. *PP:* Če je treba je mogoče uporabljati tudi 50 mg dvakrat na teden do 12 tednov, čemur sledi 25 mg dvakrat na teden ali 50 mg enkrat na teden. Zdravljenje je treba nadaljevati do remisije, vendar največ 24 tednov. Za nekatere bolnike bo morda primerno stalno zdravljenje, daljše od 24 tednov. Če po 12 tednih ni odziva, je treba zdravljenje prekiniti. Če je indicirano ponovno zdravljenje, je odmerek 25 mg dvakrat na teden ali 50 mg enkrat na teden. Pediatrična populacija: *JIA:* Priporočeni odmerek je 0,4 mg/kg telesne mase (do največ 25 mg na odmerek) 2-krat na teden subkutano z razmikom med odmerki 3-4 dni ali 0,8 mg/kg (do največ 50 mg na odmerek) enkrat na teden. *Otroška PP:* 0,8 mg/kg (do največ 50 mg na odmerek) enkrat na teden do največ 24 tednov. Če je indicirano ponovno zdravljenje, je odmerek 0,8 mg/kg (do največ 50 mg na odmerek) enkrat na teden. *Prekinitev zdravljenja:* o prekinitvi je treba razmisliti, če ni odziva po 4 mesecih (JIA) ali 12 tednih (otroška PP) zdravljenja. Način uporabe: subkutana injekcija. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov, sepsa ali možnost nastanka sepse ter aktivne okužbe, vključno s kroničnimi ali lokaliziranimi okužbami. **Posebna opozorila in previdnostni ukrepi:** Okužbe: Pred zdravljenjem, med njim in po njem je treba bolnike pregledati glede okužb in pri tem upoštevati, da je povprečni razpolovni čas izločanja etanercepta iz telesa približno 70 ur (razpon 7-300 ur). Poročali so o primerih resnih okužb. Bolnike, pri katerih se med zdravljenjem pojavi nova okužba, je treba skrbno spremljati. Zdravljenje je treba prekiniti, če pride do resne okužbe. Previdnost je potrebna pri zdravljenju bolnikov s ponavljajočimi se ali kroničnimi okužbami v anamnezi ali z drugimi osnovnimi stanji, ki bi lahko povečala dovzetnost za okužbe. Tuberkuloza: Pred začetkom zdravljenja je treba vse bolnike pregledati glede aktivne kot tudi neaktivne ('latentne') tuberkuloze. Priporočljivo je, da se ti testi vpišejo v bolnikovo opozorilno kartico. Obstaja nevarnost lažno negativnih rezultatov tuberkulinskega kožnega testa, še posebej pri bolnikih, ki so hudo bolni ali imunokompromitirani. Pri aktivni tuberkulozi se zdravljenja ne sme uvesti, pri neaktivni ('latentni') tuberkulozi pa je treba pred uvedbo zdravljenja in v skladu z nacionalnimi priporočili začeti zdravljenje latentne tuberkuloze s tuberkulostatiki. Vsem bolnikom je treba naročiti, naj poiščejo zdravniško pomoč, če se med zdravljenjem ali po njem pojavijo znaki/simptomi tuberkuloze. Reaktivacija hepatitisa B: Pri bolnikih, ki so kdaj že bili okuženi s HBV in so se zdravili z antagonisti TNF, vključno z Enbrelom, so poročali o reaktivaciji hepatitisa B. Pred uvedbo zdravljenja je treba bolnike preiskati na okužbo s HBV. Če je bolnik pozitiven na HBV, je pred uvedbo zdravljenja priporočljivo posvetovanje s specialistom za zdravljenje hepatitisa B. Pri dajanju Enbrela bolnikom, ki so že bili okuženi s HBV, je potrebna previdnost. Take bolnike je treba ves čas zdravljenja in še več tednov po prekinitvi spremljati glede znakov in simptomov aktivne okužbe s HBV. Če se razvije okužba s HBV, je treba zdravljenje prekiniti in uvesti učinkovito protivirusno ter ustrezno podporno zdravljenje. Hepatitis C: Poročali so o poslabšanju hepatitisa C, potrebna je previdnost. Alergijske reakcije: poročali so o alergijskih reakcijah, vključno z angioedemom in urtikarijo, opisani pa so tudi primeri resnih reakcij. Če se pojavi kakršnakoli resna alergijska ali anafilaktična reakcija, je treba zdravljenje prekiniti in uvesti ustrezno zdravljenje. (2,4) Pokrovček igle vsebuje lateks, ki lahko povzroči preobčutljivostne reakcije, če z Enbrelom ravna oseba z znano ali možno preobčutljivostjo na lateks ali če ga damo taki osebi. Imunosupresija: Za antagoniste TNF, vključno z Enbrelom, velja, da lahko vplivajo na naravno odpornost bolnika proti okužbam in maligním obolenjem. Bolniki, zelo izpostavljeni virusu noric, naj začasno prekinejo zdravljenje. Maligne in limfoproliferativne bolezni: Tveganja za razvoj limfomov, levkemije ali drugih hematopoetičnih ali čvrstih rakavih obolenj ni mogoče izključiti. Previdnost je potrebna pri razmisleku o uporabi antagonistov TNF pri bolnikih z anamnezo malignosti ali pri razmisleku o nadaljevanju zdravljenja pri bolnikih, pri katerih se pojavi malignost. *Kožni rak:* Pri bolnikih, zdravljenih z antagonisti TNF, vključno z Enbrelom, so poročali o melanomu in nemelanomskem kožnem raku. Priporočamo občasen pregled kože. Cepljenja: Med zdravljenjem bolnik ne sme prejeti živih cepiv. Tvorba avtoprotiteles: Zdravljenje lahko sproži nastajanje avtoimunskih protiteles. Hematološke reakcije: Poročali so o redkih primerih pancitopenije in zelo redkih primerih aplastične anemije, tudi s smrtnim izidom. Previdnost je potrebna pri bolnikih, ki imajo krvno diskrazijo v anamnezi. Vse bolnike in starše/skrbnike je treba opozoriti, da morajo v primeru pojava znakov ali simptomov, ki kažejo na krvno diskrazijo ali okužbo, med zdravljenjem takoj poiskati zdravniško pomoč. V primeru krvne diskrazije je treba zdravljenje prekiniti. Nevrološke bolezni: Pri bolnikih z demielinizirajočimi obolenji, ali pri tistih, ki imajo povečano tveganje zanje, je treba pred zdravljenjem skrbno pretehtati tveganja in koristi, vključno z nevrološko oceno. Kongestivno srčno popuščanje (KSP): Pri predpisovanju bolnikom s KSP je potrebna previdnost. O nastanku KSP so redko poročali tudi pri bolnikih brez predhodne srčno-žilne bolezni. Izsledki sicer še niso dokončni, vendar podatki kažejo na morebitno tendenco k poslabšanju popuščanja pri bolnikih, zdravljenih z Enbrelom. Alkoholni hepatitis: Ne sme se uporabljati za zdravljenje alkoholnega hepatitisa. Previdnost je potrebna pri uporabi pri bolnikih, ki imajo tudi zmeren do hud alkoholni hepatitis. Wegenerjeva granulomatoza: Enbrela ni priporočljivo uporabljati za zdravljenje te bolezni. Hipoglikemija pri bolnikih, ki se zdravijo zaradi sladkorne bolezni: Po uvedbi zdravljenja so poročali o hipoglikemiji, zato bo morda treba zmanjšati odmerek zdravila za zdravljenje sladkorne bolezni. Starostniki: Potrebna je previdnost, posebno pozornost je treba posvetiti pojavljanju okužb. Pediatrična populacija: Priporočamo, da pred začetkom zdravljenja, če je le mogoče, opravite vsa cepljenja v skladu z veljavnimi smernicami. Pri bolnikih z JIA, ki so se zdravili z Enbrelom, so poročali o kroničnì vnetni črevesni bolezni in uveitisu. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Sočasno zdravljenje z anakinro ali z abataceptom: klinična korist teh dveh kombinacij ni dokazana, zato nista priporočljivi. Sočasno zdravljenje s sulfasalazinom: potrebna je previdnost. **Plodnost, nosečnost in dojenje:** Ženske v rodni dobi morajo med zdravljenjem in še tri tedne po prenehanju le-tega uporabljati ustrezno metodo kontracepcije. Uporaba med nosečnostjo ni priporočljiva. Etanercept prehaja placento. Uporaba živih cepiv v prvih 16 tednih po tem, ko so matere dojenčkov prejele zadnji odmerek Enbrela, pri dojenčkih običajno ni priporočljiva. Bolnica mora med zdravljenjem prenehati dojiti ali pa prekiniti zdravljenje, pri čemer je treba upoštevati tako korist dojenja za otroka kot korist zdravljenja za mater. **Neželeni učinki:** Odrasli: *Zelo pogosti (≥ 1/10):* Okužbe (vključno z okužbami zgornjih dihal, bronhitisom, cistitisom in kožnimi okužbami), reakcije na mestu injiciranja (vključno s krvavitvijo, podplutbami, eritemom, srbenjem, bolečino in oteklino). *Pogosti (≥ 1/100 do < 1/10):* alergijske reakcije, nastanek avtoprotiteles, pruritus, zvišana telesna temperatura. Pediatrična populacija: Na splošno so bili neželeni učinki po vrsti in pogostnosti podobni tistim pri odraslih. Vrste okužb, opaženih v kliničnih preskušanjih pri bolnikih z JIA, starih 2-18 let, so bile na splošno blage do zmerne in skladne s tistimi, ki jih pogosto vidimo pri skupinah ambulantnih pediatričnih bolnikov. Hudi neželeni učinki so bili: norice z znaki in simptomi aseptičnega meningitisa, ki je izzvenel brez posledic, vnetje slepiča, gastroenteritis, depresija/osebnostne motnje, kožne razjede, ezofagitis/gastritis, streptokokni septični šok (streptokoki skupine A), sladkorna bolezen tipa I in okužbe mehkih tkiv ter postoperativnih ran. V kliničnih preskušanjih pri bolnikih z JIA so poročali o 4 primerih sindroma aktivacije makrofagov. Viri iz obdobju trženja so pri bolnikih z JIA poročali o kroničnì vnetni črevesni bolezni in uveitisu. **Način in režim izdaje:** Rp/Spec. **Imetnik dovoljenja za promet:** Pfizer Limited, Ramsgate Road, Sandwich, Kent CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 01.04.2016
Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

Pfizer Luxembourg SARL, GRAND DUCHY OF LUXEMBOURG, 51, Avenue J.F. Kennedy, L – 1855,
Pfizer, podružnica Ljubljana, Letališka cesta 3c, 1000 Ljubljana

"Samo za stokovno javnost !"

ENB-04-16

Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2016;25:73-76
doi: 10.15570/actaapa.2016.21

# Microbiological characteristics of perianal streptococcal dermatitis: a retrospective study of 105 patients in a 10-year period

Anja Šterbenc[1], Katja Seme[1], Liza Lea Lah[2], Olga Točkova[3], Tina Kamhi Trop[4], Nataša Švent-Kučina[1], Mateja Pirš[1] ✉

**Abstract**

Beta-hemolytic streptococci (BHS) are the most common causative agents of perianal streptococcal dermatitis (PSD). This study evaluates the distribution of BHS isolates in perianal bacterial cultures. We retrospectively reviewed microbiological results for perianal BHS that were isolated in our laboratory between 2006 and 2015. We identified a total of 105 BHS isolates from rectal swabs and swabs of clinically intact perianal skin. The majority of BHS were of group A (GABHS) (73/105; 69.5%), followed by group B BHS (GBBHS) (27/105; 25.7%), and non-group A or B BHS (5/105; 4.8%). The distribution of GABHS was age-specific, with the majority of GABHS obtained from young children. All BHS isolates were susceptible to penicillin. GABHS were universally susceptible to clindamycin, whereas 1.4% were resistant to erythromycin. GBBHS were resistant to erythromycin and clindamycin in 14.8% and 7.4% of cases. In addition, we wanted to emphasize the importance of correct diagnosis of PSD. Hence, we provide a review of protocols that can decrease the time to diagnosis and treatment of PSD, reduce patients' discomfort, and prevent unnecessary diagnostic procedures.

**Keywords:** beta-hemolytic streptococci, perianal swab samples, culture, streptococcal dermatitis

**Received:** 23 November 2016 | **Returned for modification:** 26 November 2016 | **Accepted:** 30 November 2016

## Introduction

Beta-hemolytic streptococci (BHS) are well-known causative agents of cutaneous, oropharyngeal, and invasive infections (1). Perianal streptococcal dermatitis (PSD) typically affects children 6 months to 10 years old and is usually caused by group A BHS (GABHS) with the species name *Streptococcus pyogenes* (2, 3). In contrast, PSD has rarely been reported in adults (4, 5), where group B BHS (GBBHS) with the species name *Streptococcus agalactiae* are regarded as the most common causative agents (6). In rare cases, BHS of groups C (GCBHS) and G (GGBHS) as well as *Staphylococcus aureus* can also cause perianal disease (6, 7). PSD can be diagnosed relatively easily if physicians are familiar with the classical presentation of the disease, which includes perianal erythema, edema, and itching together with rectal pain and blood-streaked stools (2, 6, 8). Infants typically also present with episodes of intermittent irritability (9). Diagnosis can be confirmed using swabs of the perianal area for bacterial culture; another possibility in some cases is the use of rapid antigen detection tests (RADTs) (10, 11). Initiation of an appropriate antibiotic treatment rapidly and drastically improves patients' symptoms. However, treatment is often delayed because differential diagnosis of PSD includes a variety of clinical conditions (e.g., irritant diaper dermatitis, candidiasis, infection with *Enterobius vermicularis*, inverse psoriasis, seborrheic dermatitis, chronic inflammatory bowel disease, histiocytosis, zinc deficiency, and, rarely, sexual abuse) (2, 8, 12). In addition to oral penicillin and amoxicillin, PSD can also be treated with clindamycin, erythromycin, clarithromycin, or cefuroxime (1, 6, 13, 14).

This study evaluates the distribution of BHS isolates from rectal and perianal skin swab samples and provides a review of protocols that could potentially decrease the time to diagnosis and treatment of PSD, reduce patients' discomfort, and prevent unnecessary diagnostic procedures.

## Materials and methods

We retrospectively reviewed microbiology laboratory records and searched for BHS isolates in the perianal area. BHS isolates from rectal or perianal skin swabs that were submitted to the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, between January 2006 and December 2015 were included in the study. Only the first BHS isolate from the perianal region of each patient was included in the study. Sampling sites included the rectum, perianal skin, and perineum. For each patient with a positive BHS culture, data were collected on patient age and sex, streptococcal species, and antimicrobial susceptibility. Identification was confirmed to the species level by colony morphology, catalase test, and a commercial latex agglutination test (PathoDxtra Strep Grouping Kit, Thermo Fisher Scientific, Waltham, MA, USA) or matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility was determined using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines until April 2014 and afterwards the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (15, 16).

## Results

In the 10-year study period, we identified a total of 105 BHS isolates isolated from the rectum or perianal skin region of the same number of patients. GABHS, GBBHS, and non-group A or B BHS were cultured in a total of 73/105 (69.5%), 27/105 (25.7%), and 5/105 (4.8%) cases, respectively (Fig. 1). The distribution of GABHS, GBBHS, and non-group A or B BHS according to age groups

[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. [2]Department of Infectious Diseases, Ljubljana University Medical Center, Ljubljana, Slovenia. [3]Department of Dermatology, Ljubljana University Medical Center, Ljubljana, Slovenia. [4]Department of Gastroenterology, Hepatology, and Nutrition, Children's Hospital, Ljubljana University Medical Center, Ljubljana, Slovenia. ✉ **Corresponding author: mateja.pirs@mf.uni-lj.si**

is presented in Figure 2. The majority of streptococcal isolates were obtained from children younger than 15 (89/105; 84.7%). The median age of patients was 5 (average 11.8 years, age range 1 to 84 years) and 73 out of 105 (69.5%) were male (Fig. 3).

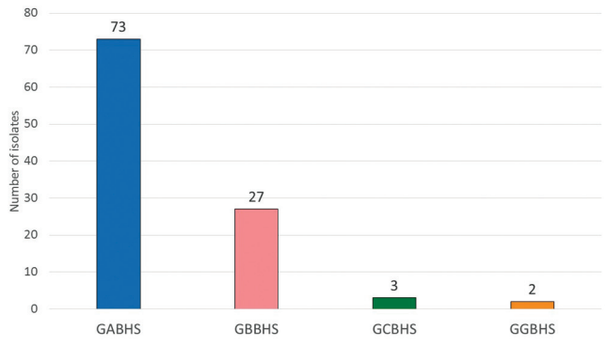



Figure 1 | Proportion of BHS in perianal isolates. GABHS = group A beta-hemolytic streptococci. GBBHS = group B beta-hemolytic streptococci. GCBHS = group C beta-hemolytic streptococci. GGBHS = group G beta-hemolytic streptococci.

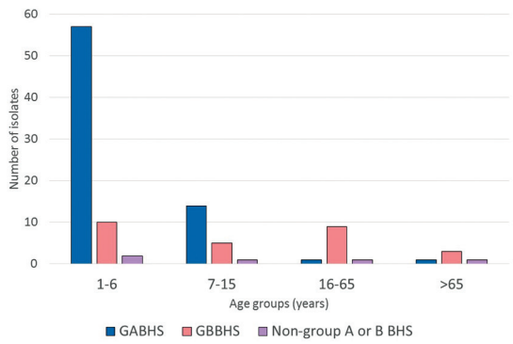



Figure 2 | Age-related distribution of a total of 105 streptococcal perianal isolates. GABHS = group A beta-hemolytic streptococci. GBBHS = group B beta-hemolytic streptococci. Non-group A or B BHS = non-group A or B beta-hemolytic streptococci (groups C and G).

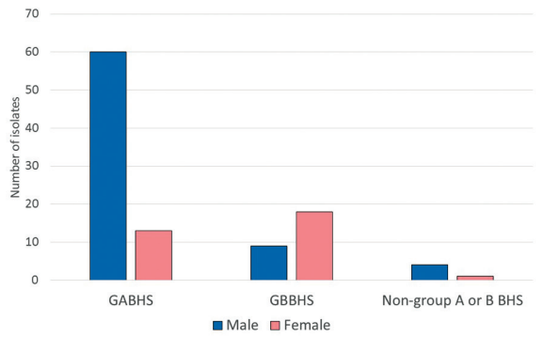



Figure 3 | Distribution of a total of 105 streptococcal perianal isolates according to the patient's sex. GABHS = group A beta-hemolytic streptococci. GBBHS = group B beta-hemolytic streptococci. Non-group A or B BHS = non-group A or B beta-hemolytic streptococci (groups C and G).

The seasonal distribution of all GABHS, GBBHS, and non-group A or B BHS perianal cultures is presented in Figure 4. Almost half (46.6%) of the GABHS perianal cultures were obtained during the spring (between March and June), and the second peak was observed in December. Similar to GABHS, the number of GBBHS perianal cultures peaked in the spring and winter months. Only a small number of non-group A or B BHS belonging to groups C and G were isolated. All GABHS isolates were susceptible to penicillin and clindamycin, whereas the rate of erythromycin resistance was 1.4%. All GBBHS isolates were susceptible to penicillin, whereas 14.8% and 7.4% were resistant to erythromycin and clindamycin, respectively. The number of non-group A or B BHS isolates was too low to reliably assess antimicrobial susceptibility; nonetheless, all of the isolates were susceptible to penicillin.

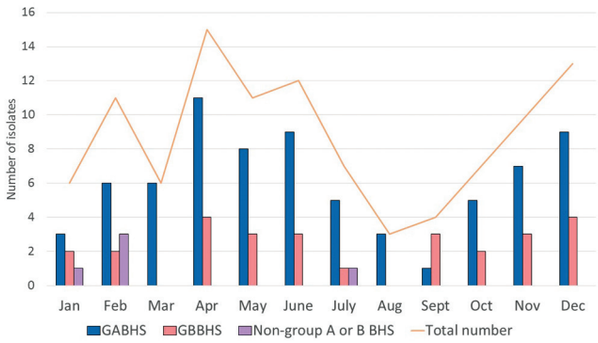



Figure 4 | Seasonal distribution of a total of 105 streptococcal perianal isolates. GABHS = group A beta-hemolytic streptococci. GBBHS = group B beta-hemolytic streptococci. Non-group A or B BHS = non-group A or B beta-hemolytic streptococci (groups C and G).

## Discussion

Although perianal dermatitis caused by infection with BHS is a well-described clinical entity in children, its incidence is most likely significantly underestimated in both children and adults due to frequent misdiagnosis (6). This study emphasizes the importance of correct and rapid diagnosis of PSD.

PSD classically presents as a well-demarcated perianal erythema with or without exudate, which may centrifugally spread to the penis or vulva and is present in more than 90% of patients with PSD (1, 2, 17–19). It is often accompanied by edema, infiltration, and tenderness (3). According to the literature (2, 3, 17–20), patients' signs and symptoms include perianal itching (78–100%), pain on defecation (52%), constipation (47%), blood-streaked stools (20–35%), and anal fissures (26%). PSD mostly occurs in children 6 months to 10 years old and is more common in boys (2, 3, 20). Differential diagnosis of PSD is vast and, unfortunately, patients are commonly overlooked (2, 8, 12). Patients can present with symptoms that have lasted for several years and may have even undergone several unnecessary diagnostic procedures, such as colonoscopy or rectoscopy (3, 8, 12). Inappropriate treatments with topical antifungal agents and steroids or oral preparations for pinworms obscure the typical clinical presentation of PSD and worsen its symptoms (8). Due to prolonged and inappropriately treated or untreated PSD, patients may develop anal fissures, which can result in painful defecation, leading to constipation and toilet avoidance (11, 19).

Our study evaluated a 10-year distribution of BHS in swabs from the rectum and perianal skin that were submitted to our laboratory for bacterial culture. As shown in Figure 1, the majority of isolates were GABHS, followed by GBBHS and non-group A or B BHS (groups C and G). BHS were isolated from perianal cultures obtained from patients of all age groups except for children younger than 1; however, we observed significant differences in species distribution (Fig. 2). In preschool and primary school age groups, the majority of perianal streptococcal cultures grew GABHS, whereas GBBHS were the most commonly isolated BHS in adults. Non-group A or B BHS isolates obtained from swabs of clinically intact perianal skin were rare. Based on our results, we can assume that GABHS represents the most probable cause of PSD in children, whereas GBBHS is the presumed causative agent of PSD in adults. As shown in Figure 2, more than 84% of perianal

streptococcal cultures were obtained from children under 15. Our results are in accordance with previous studies, suggesting that, although PSD predominantly occurs in young children, it should not be considered an exclusively pediatric disease (1, 3, 6). More than 69% of BHS perianal isolates obtained in our study were from male patients, which is in agreement with previous observations suggesting male predilection of PSD (Fig. 3) (2, 3, 20).

Seasonal distribution of BHS isolates observed in our study showed remarkable consistency with previous reports of PSD in children (3, 17). Interestingly, seasonal distribution of PSD cases exhibits a characteristic pattern of pharyngeal GABHS infections in temperate climates and supports the idea of autoinoculation through digital contamination or ingestion of GABHS (3, 17). In our study, we were not able to assess the proportion of concurrent pharyngeal GABHS carriage, but it has previously been shown that up to 92% of individuals with PSD test positive for pharyngeal GABHS (17).

Although appropriate sampling is crucial for laboratory confirmation of etiology in PSD, there are currently no clear recommendations regarding the adequacy of different clinical samples used in diagnosing PSD. The affected area(s) should be cleaned with saline and thoroughly swabbed. Anal, perianal, and perineal swabs represent preferred clinical samples, whereas stool samples are not recommended. Needle aspiration of a leading edge of the inflamed area can also be used; however, the low sensitivity for detecting causative agents and its relative invasiveness limit its role in routine practice (21, 22). Processing of swab samples obtained for RADT must be performed in accordance with the manufacturer's instructions. Swab samples obtained for bacterial culture should be placed in a transport medium (e.g., Stuart's or Amies) and sent at room temperature to the microbiology laboratory as soon as possible. Standard laboratory procedure is cultivation of BHS on blood agar (21). As emphasized by some authors, high clinical suspicion of PSD should encourage physicians to specifically ask for BHS culture because stool culture might fail to detect BHS (3, 12, 20). In addition, cultivation allows isolation and identification of all BHS as well as *S. aureus*, which is also a possible etiologic agent.

Alternatively, GABHS- and GBBHS-RADTs are sometimes used as a point-of-care test; however, GBBHS-RADTs should be avoided due to their low sensitivity and specificity (23). Unfortunately, only a few studies have evaluated the clinical sensitivity and specificity of RADTs for detecting extrapharyngeal GABHS infection (2, 10, 11). Depending on the RADT used, the sensitivity for extrapharyngeal GABHS ranged from 77.9% to 98.0%, suggesting that these tests may represent a rapid, practical, and accurate alternative diagnostic tool for point-of-care differentiation of GABHS-associated PSD from other conditions with similar presentations (e.g., irritant dermatitis, candidiasis, and pinworm infestation) (10). Nevertheless, physicians should be aware of the age-specific distribution of GABHS and GBBHS infection and must account for these differences when deciding to use GABHS-RADT for screening PSD. One of the major caveats of using GABHS-RADT in diagnosing GABHS-associated PSD is the lack of formal approval for extrapharyngeal testing (11, 12). In addition, a negative GABHS-RADT result warrants additional testing by conventional bacterial culture especially in adults, where PSD is most commonly caused by GBBHS (1, 6, 10). A subset of perianal dermatitis cases can also be caused by non-group A or B BHS (groups C and G), as well as *S. aureus* (6, 7). Thus, in children, GABHS-RADT can be used as a point-of-care test, whereas RADTs are not recommended in adults. Cultivation of BHS is the preferred microbiological method for diagnosing PSD in adults and in children with perianal dermatitis and a negative GABHS-RADT result.

Early initiation of antibiotic treatment provides rapid improvement of symptoms (8). Our study has shown that susceptibility of BHS to penicillin remains excellent. GABHS isolates are rarely resistant to erythromycin or clindamycin, whereas higher resistant rates for both antibiotics were observed in GBBHS isolates. A 7- to 10-day course of oral penicillin V (50,000 to 100,000 IU/kg) is considered to be the initial treatment of choice for pediatric GABHS-associated PSD (2, 3, 13, 14, 24, 25). However, recurrence of the disease may occur in up to 39% of children treated and a repeated course of antibiotics is necessary, whereas some advocate prolonged treatment (e.g., 14–21 days) (26–30). Unfortunately, studies comparing the optimal duration of antibiotic therapy are lacking. Alternatively, children can be treated with oral amoxicillin (50 mg/kg/day) and, if compliance is an issue, one dose of penicillin G 1.2 M IU im can be used in children weighing > 27 kg, and one dose of penicillin G 600,000 IU im in children weighing < 27 kg (24, 25). In children with penicillin allergy, midecamycin (40 mg/kg/day), clarithromycin (15 mg/kg/day), or clindamycin (30 mg/kg/day) can be used (24, 25), although data regarding their efficacy rely solely on a subset of treated children (2, 4, 14, 20, 31). To date, cefuroxime is the only alternative antibiotic in treatment of PSD that has been assessed in a randomized controlled trial (13). In comparison to penicillin, an increased efficacy of a 7-day course of cefuroxime (20 mg/kg/day) was observed, with shorter duration of symptoms and faster bacterial eradication (13, 14). However, the study was not blinded and, because no follow-up was performed after the end of the treatment, optimal duration of antibiotic therapy with cefuroxime could not be evaluated (13). Furthermore, usage of cephalosporins is not recommended for treatment of BHS due to their broad spectrum of activity, which can lead to the development of antibiotic resistance in other bacteria (24). In addition to oral therapy, patients can also receive topical treatment with antiseptics (e.g., chlorhexidine) or antibiotics (e.g., bacitracin, mupirocin, fusidic acid, erythromycin, and gentamicin), although their usefulness remains uncertain (2, 3, 14, 19, 30). Unfortunately, no controlled trials were conducted to evaluate the efficacy of antimicrobial therapy for non-GABHS-associated PSD. In adults with predominantly GBBHS-induced PSD, a 7- to 10-day treatment with oral penicillin V (1–1.5 M IU/day) is considered standard therapy (1, 6, 24, 25). Alternatively, patients can receive one dose of penicillin G 1.2 M IU/day im or, when penicillin allergy is suspected, oral midecamycin (400 mg tid), clarithromycin (250–500 mg bid), and azithromycin (500 mg 1st day, 250 mg 2nd–5th day) (25). However, physicians should be aware of important differences between pediatric and adult cases of PSD. Only 42% of adult patients with PSD are successfully treated with the first course of oral antibiotics, possibly due to the higher minimal inhibitory concentration for penicillin in GBBHS compared to GABHS, hence a higher dosage of the same antibiotic might be necessary, whereas some advocate prolonged treatment (6, 32). Kahlke et al. (6) have clearly shown that the presence of concomitant dermatological and/or anorectal conditions that have not yet developed in children (e.g., hemorrhoids, skin tags, anogenital warts, and anal cancer) contribute to reduced rates of successfully treated infections in adults (1, 6). In addition, these conditions can present with symptoms that are otherwise observed in PSD (e.g., perianal erythema in patients with hemorrhoids) and may be the reason for frequent misdiagnosis in adults

(6). Although complications of PSD are rare, a urine analysis may be performed to screen for possible post-streptococcal glomerulonephritis (19, 30).

In both children and adults, follow-up is crucial due to frequent relapses of the disease (8). Repeated antibiotic treatment of these cases is usually successful (3, 8). Short-term recurrence of PSD might be caused by poor compliance with the antibiotic therapy, inappropriate dosage, and intra-familial or close contact transmissions, especially in children (2). Thus, screening and eventual treatment of symptomatic family members of patients with recurrent PSD may be warranted (12). A change of personal hygiene tools (e.g., toothbrush and towels) should be recommended after completion of antibiotic treatment. Patients should be advised not to share personal hygiene items with family members that could be BHS carriers. Simple measures such as thorough handwashing can be effective in preventing further infections (24).

Our study is based solely upon a retrospective review of laboratory records with BHS isolates from rectal and perianal skin swab samples and presumed diagnosis of perianal streptococcal infections, which is its main limitation. No data on clinical presentation and diagnosis of PSD, antibiotic treatment, and potential relapse(s) or the presence of concomitant diseases were collected. Further studies with clinically and microbiologically confirmed cases of PSD are needed to confirm our observations.

To conclude, we observed seasonal and age-specific distribution of GABHS, GBBHS, and non-group A or B BHS in rectal and perianal skin bacterial cultures. Thus, symptoms that include perianal itching, rectal pain, and blood-streaked stools, as well as bright red, well-demarcated perianal erythema with edema, infiltration, and tenderness on a clinical examination of a preschool child are highly suspicious of PSD caused by GABHS. Based on our data, a subset of PSD cases can also be diagnosed in adulthood, where GBBHS are the most likely causative agents. Anal, perianal, or perineal swabs are preferred clinical samples for microbiological confirmation of diagnosis of PSD, whereas stool samples are not recommended. GABHS-RADTs enable rapid diagnosis especially in children; however, a negative result warrants further testing with cultivation of BHS. In adult patients, cultivation of BHS is always necessary due to the poor performance of GBBHS-RADTs. Swab samples of perianal lesions obtained for conventional bacterial culture are the most reliable diagnostic tool for diagnosing perianal dermatitis because they also enable detection of less common causative agents. As shown in our study, BHS are universally susceptible to penicillin and, because symptoms improve dramatically with appropriate antibiotic therapy, treatment with oral penicillin should not be delayed.

## References


1. Zhang C, Haber RM. The ABCs of Perineal Streptococcal Dermatitis: Case Series and Review of the Literature. J Cutan Med Surg. 2016; [Epub ahead of print].
2. Kokx NP, Comstock JA, Facklam RR. Streptococcal perianal disease in children. Pediatrics. 1987;80:659-63.
3. Jongen J, Eberstein A, Peleikis HG, Kahlke V, Herbst RA. Perianal streptococcal dermatitis: an important differential diagnosis in pediatric patients. Dis Colon Rectum. 2008;51:584-7.
4. Neri I, Bardazzi F, Marzaduri S, Patrizi A. Perianal streptococcal dermatitis in adults. Br J Dermatol. 1996;135:796-8.
5. Bafounta ML, Bloch P, Kernbaum S, Saiag P. Group A beta-hemolytic Streptococcus: an unusual etiology of perianal dermatitis in an adult? Ann Dermatol Venerol. 1998;125:902-4.
6. Kahlke V, Jongen J, Peleikis HG, Herbst RA. Perianal streptococcal dermatitis in adults: its association with pruritic anorectal diseases is mainly caused by group B Streptococci. Colorectal Dis. 2013;15:602-7.
7. Heath C, Desai N, Silverberg NB. Recent microbiological shifts in perianal bacterial dermatitis: Staphylococcus aureus predominance. Pediatr Dermatol. 2009;26:696-700.
8. Brilliant LC. Perianal streptococcal dermatitis. Am Fam Physician. 2000;61:391-7.
9. Shouval DS, Schurr D, Nussinovitch M. Presentation of perianal group A streptococcal infection as irritability among children. Pediatr Dermatol. 2008;25:568-70.
10. Clegg HW, Dallas SD, Roddey OF, Martin ES, Swetenburg RL, Koonce EW, et al. Extrapharyngeal group A Streptococcus infection: diagnostic accuracy and utility of rapid antigen testing. Pediatr Infect Dis J. 2003;22:726-31.
11. Cohen R, Levy C, Bonacorsi S, Wollner A, Koskas M, Jung C, et al. Diagnostic accuracy of clinical symptoms and rapid diagnostic test in group A streptococcal perianal infections in children. Clin Infect Dis. 2015;60:267-70.
12. Block SL. Perianal dermatitis: much more than just a diaper rash. Pediatr Ann. 2013;42:12-4.
13. Meury SN, Erb T, Schaad UB, Heininger U. Randomized, comparative efficacy trial of oral penicillin versus cefuroxime for perianal streptococcal dermatitis in children. J Pediatr. 2008;153:799-802.
14. Olson D, Edmonson MB. Outcomes in children treated for perineal group A beta-hemolytic streptococcal dermatitis. Pediatr Infect Dis J. 2011;30:933-6.
15. Clinical and Laboratory Standards Institute. (M100–S24). Performance Standards for Antimicrobial Susceptibility Testing – Twenty- Fourth Informational Supplement, vol. 34. Wayne, PA: Natl Comm Clin Lab Stand; 2014.
16. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. [cited 2016 Nov 06]. Available from: http://www.eucast.org.
17. Mogielnicki NP, Schwartzman JD, Elliott JA. Perineal group A streptococcal disease in a pediatric practice. Pediatrics. 2000;106:276-81.
18. Echeverría Fernández M, López-Menchero Oliva JC, Marañón Pardillo R, Míguez Navarro C, Sánchez Sánchez C, Vázquez López P. [Isolation of group A beta-hemolytic Streptococcus in children with perianal dermatitis]. An Pediatr (Barc). 2006;64:153-7.
19. Lehman R, Pinder S. Streptococcal perianal infection in children. BMJ. 2009;338: b1517.
20. Wright JE, Butt HL. Perianal infection with beta haemolytic streptococcus. Arch Dis Child. 1994;70:145-6.
21. Garcia LS, Isenberg HD. Clinical Microbiology Procedures Handbook. 3rd ed. Washington, DC: ASM Press; 2010.
22. Piso RJ, Pop R, Wieland M, Griesshammer I, Urfer M, Schibli U, et al. Low sensitivity of needle aspiration cultures in patients with cellulitis/erysipelas. Springerplus. 2016;5:1578.
23. Donders GG, Vereecken A, Salembier G, Spitz B. Accuracy of rapid antigen detection test for group B streptococci in the indigenous vaginal bacterial flora. Arch Gynecol Obstet. 1999;263:34-6.
24. Tomažič J, Strle F, s sod. Infekcijske bolezni. Ljubljana: Združenje za infektologijo, Slovensko zdravniško društvo; 2014/2015. Poglavje 6, Okužbe kože in mehkih tkiv; p. 152-62.
25. Gilbert DN, Moellering RC Jr, Eliopoulos GM, Chambers HF, Saag MS. The Sanford Guide to Antimicrobial Therapy 2010. 40th ed. Sperryville, VA: Antimicrobial Therapy, Inc.; 2010.
26. Marks VJ, Maksimak M. Perianal streptococcal cellulitis. J Am Acad Dermatol 1988;18:587-826.
27. Duhra P, Ilchyshyn A. Perianal streptococcal cellulitis with penile involvement. Br J Dermatol. 1990;123:793-6.
28. Teillac-Hamel D, de Prost Y. Perianal streptococcal dermatitis in children. Eur J Dermatol 1992;2:71-4.
29. Morgenroth HHA. Perianale Streptokokkeninfektionen, eine mögliche Ursache für den Pruritus ani, perianale Dermatitis, Defäkationsschmerzen, Stuhl-verhalt und blutige Stuhlauflagerungen. Monatsschr Kinderheilkd; 1995. 144, p. 55-58.
30. Herbst R. Perineal streptococcal dermatitis/disease: recognition and management. Am J Clin Dermatol. 2003;4:555-60.
31. Rehder PA, Eliezer ET, Lane AT. Perianal Cellulitis. Cutaneous Group A Streptococcal Disease. Arch Dermatol. 1988; 124:702–4.
32. Farley MM. Group B streptococcal disease in nonpregnant adults. Clin Infect Dis. 2001;33:556-61.

Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2016;25:77-78
doi: 10.15570/actaapa.2016.22

# Coexistence of erythema dyschromicum perstans and vitiligo: a case report and review of the literature

Funda Tamer[1] ✉

**Abstract**

Erythema dyschromicum perstans is a rare, chronic, pigmentary disorder with unknown etiology. It clinically presents with oval to round, gray, blue, or brown macules of various sizes. The etiology remains unknown; however, cobalt allergy, radio contrast media, intestinal parasites, human immunodeficiency virus, and hypothyroidism have been proposed as causative factors. In addition, vitiligo is characterized by depigmented macules and patches that are widely and symmetrically distributed. It has been suggested that autoimmune mechanisms play an important role in the etiopathogenesis of vitiligo. Physical and emotional stress can trigger vitiligo in genetically predisposed patients. However, coexistence of erythema dyschromicum perstans and vitiligo is extremely rare, and similar immune mechanisms have been implicated in the pathogenesis of these cutaneous pigmentary disorders.

**Keywords:** erythema dyschromicum perstans, vitiligo

**Received:** 26 August 2016 | **Returned for modification:** 4 November 2016 | **Accepted:** 17 November 2016

## Introduction

Erythema dyschromicum perstans (EDP) is a rare, chronic, pigmentary disorder with unknown etiology (1). It clinically presents with oval to round, gray, blue, or brown macules of various sizes. Symmetrically distributed lesions usually appear on the face, neck, trunk, and extremities (1, 2). The etiology remains unknown; however, cobalt allergy, radio contrast media, intestinal parasites, human immunodeficiency virus, and hypothyroidism have been proposed as causative factors (2, 3). Moreover, it has been suggested that EDP is associated with lichen planus. Patients that have lichen planus and EDP together have been reported previously (1, 3). There is controversy whether EDP is a subtype of lichen planus or a distinct entity. Histopathological findings usually show perivascular lymphocytic infiltration, melanophages, vacuolization of the basal layer, and necrotic keratinocytes (4). The disease should be differentiated from lichen planus pigmentosus, postinflammatory hyperpigmentation, fixed drug eruption, and Addison's disease. Antibiotics, corticosteroids, antihistamines, dapsone, chloroquine, clofazimine, and isotretinoin are the treatment of choice. However, none of them provide an effective treatment (5).

Vitiligo is characterized by depigmented macules and patches that are widely and symmetrically distributed. Autoimmune mechanisms play an important role in the etiology. Physical and emotional stress can trigger vitiligo in genetically predisposed patients. Furthermore, oxidative stress can increase melanocyte destruction. Corticosteroids, calcineurin inhibitors, vitamin D analogues, oral vitamins, phototherapy, and laser therapy are the treatment options (6).

Coexistence of erythema dyschromicum perstans and vitiligo is extremely rare. However, similar immune mechanisms have been implicated in the pathogenesis of these cutaneous pigmentary disorders.

## Coexistence of erythema dyschromicum perstans and vitiligo

A 23-year-old Caucasian male patient complaining of changes in skin color was admitted for further clinical evaluation. Dermatological examination revealed grayish, hyperpigmented, excoriated macules and plaques on the back and occipital region, and mild hyperpigmentation on the upper chest. In addition, there was a well-demarcated, oval, depigmented patch 10 cm in diameter on his left shoulder (Figs. 1a–b).

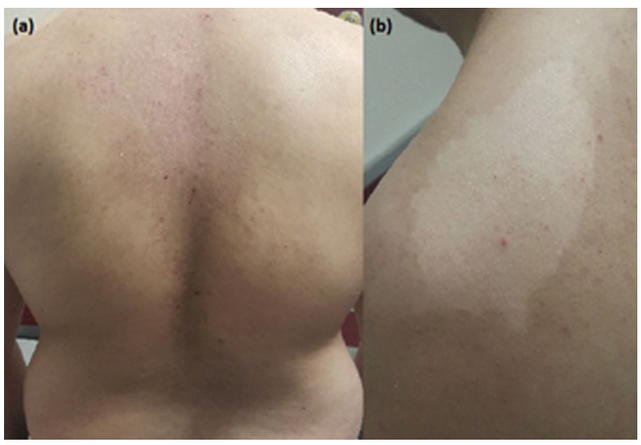



Figure 1 | a) Hyperpigmented, grayish, excoriated macules and plaques on the back; b) Oval depigmented patch on the left shoulder.

Wood's lamp examination showed discrete depigmentation with sharp borders. The patient admitted that the depigmented lesion had been present for the last 6 months. Moreover, the lesion appeared as a small macule and it gradually increased in size. It was asymptomatic and there were no other depigmented macules elsewhere. Thus, the diagnosis of vitiligo was made based on the clinical features and Wood's lamp examination. Furthermore, the patient stated that the hyperpigmented lesions first appeared on the back and had extended to the occipital region and chest over the last 5 years. He had used topical corticosteroids previously, but no clinical response had been achieved. The past medical history was unremarkable. He denied taking any medication. The skin biopsy was taken from the hyperpigmented lesions on the middle of the back. Histopathological examination revealed vacuolar degeneration on the basal layer, melanophages, and

[1]Medical Park Hastanesi, Yenimahalle, Ankara, Turkey. ✉ Corresponding author: fundatmr@yahoo.com

lymphocytic infiltration in the upper dermis. Periodic acid–Schiff, crystal violet, and Congo red stains were performed and the specimen did not show any amyloid deposits or metachromasia (Fig. 2). Therefore we confirmed the diagnosis of erythema dyschromicum perstans. The patient was put on topical steroid and oral antihistaminic treatment.

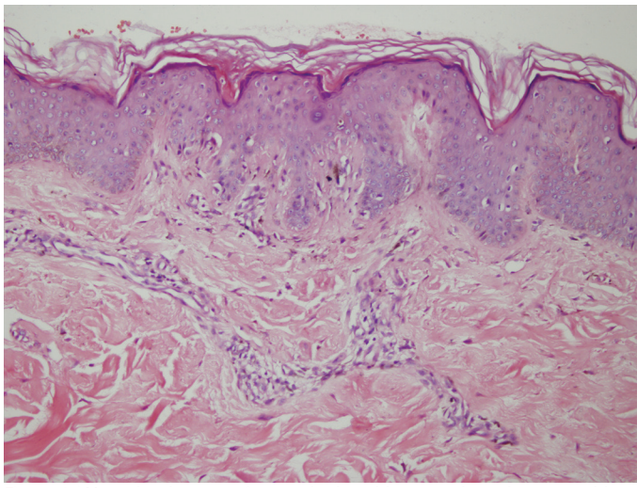


Figure 2 | Vacuolar degeneration on the basal layer, melanophages, and lymphocytic infiltration in the upper dermis (H&E ×200).

The patient we presented above had both EDP and vitiligo at the same time. Only two similar cases have been reported previously. Henderson et al. presented a 31-year-old man that had EDP and developed depigmented patches on his elbows and knees in the previous year. The patient stated that the slowly progressive, hyperpigmented lesions had been present for a long time. The patient had cosmetic concerns and he did not have any other illnesses. Dermatological examination revealed gray macules with erythematous borders on the chest and proximal aspect of the arms and legs. In addition, there were depigmented macules with sharp borders on the knees, elbows, and distal site of the legs. The skin biopsies were performed from a hyperpigmented and a depigmented lesion. Histopathological examination showed an absence of melanin in the depigmented area. However, there was melanin in the upper dermis and basal area, vacuolization of the basal layer, and pigment incontinence in the hyperpigmented lesion. Therefore the diagnosis of EDP and vitiligo were both confirmed histopathologically (7).

Naik reported a 33-year-old man with a 6-month history of EDP and a 20-year history of depigmented patches on the trunk and extremities. The patient had been treated with light exposure 5 years previously. The patient revealed that he did not have any preceding lesions or hypoesthesia in the affected areas. The past medical history and family history were both unremarkable. Dermatological examination revealed scaly, gray-blue patches on the trunk and arms. Furthermore, there were depigmented patches on the dorsal site of the legs, hands, upper chest, back, and lips. Wood's lamp examination confirmed depigmentation, but hyperpigmented patches did not fluoresce under Wood's lamp. A skin biopsy was performed from the active border of a gray-blue patch. Histopathological examination revealed superficial, perivascular infiltration of lymphocytes and melanophages. However, the number of melanocytes in the basement membrane was normal. Therefore, the patient was diagnosed with EDP and vitiligo (1).

Gross et al. performed immunocytochemical analysis of leukocyte infiltrates in the affected skin of EDP and vitiligo patients. They showed similar subpopulations including CD3+, CD8+, T-suppressor, macrophages, and T-cytotoxic cells in the epidermis and Ia antigen positivity of the dendritic cells and lymphoid cells in the infiltrates of both diseases. Therefore, they suggest similar immune mechanisms in these cutaneous pigmentary disorders (8).

## Conclusion

This article has reported an extremely rare case of EDP coexisting with vitiligo. To the best of our knowledge, no new cases have been reported since 2003. It has been considered that EDP may be a form of lichen planus because of the similar immunopathological features. However, these cases suggest that common immunological mechanisms may also be responsible for the coexistence of EDP and vitiligo. It should be considered that patients with EDP may develop other dermatological disorders, including lichen planus and vitiligo.

## References


1. Naik NS. Erythema dyschromicum perstans and vitiligo. Dermatol Online J. 2003;9:25.
2. Tlougan BE, Gonzalez ME, Mandal RV, Kundu RV, Skopicki D. Erythema dyschromicum perstans. Dermatol Online J. 2010;16:17.
3. Schwartz RA. Erythema dyschromicum perstans: the continuing enigma of Cinderella or ashy dermatosis. Int J Dermatol. 2004;43:230-2.
4. Vásquez-Ochoa LA, Isaza-Guzmán DM, Orozco-Mora B, Restrepo-Molina R, Trujillo-Perez J, Tapia FJ. Immunopathologic study of erythema dyschromicum perstans (ashy dermatosis). Int J Dermatol. 2006;45:937-41.
5. Wang F, Zhao YK, Wang Z, Liu JH, Luo DQ. Erythema dyschromicum perstans response to isotretinoin. JAMA Dermatol. 2016;152:841-2.
6. Manga P, Elbuluk N, Orlow SJ. Recent advances in understanding vitiligo. F1000Res. 2016:6;5.
7. Henderson CD, Tschen JA, Schaefer DG. Simultaneously active lesions of vitiligo and erythema dyschromicum perstans. Arch Dermatol. 1988;124:1258-60.
8. Gross A, Tapia FJ, Mosca W, Perez RM, Briceno L, Henriquez JJ, et al. Mononuclear cell subpopulations and infiltrating lymphocytes in erythema dyschromicum perstans and vitiligo. Histol Histopathol. 1987;2:277-83.

Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2016;25:79-81
doi: 10.15570/actaapa.2016.23

# Methotrexate-induced panniculitis in a patient with rheumatoid arthritis

Raghda Al Maashari[1] ✉, Mowafak M. Hamodat[2]

**Abstract**

Methotrexate-induced accelerated nodulosis (MIAN) is not an uncommon adverse effect associated with the use of the methotrexate in rheumatoid arthritis. Limited case reports describe panniculitis as a pathological finding in this setting. A 31-year-old female with seropositive rheumatoid arthritis on methotrexate therapy presented with a 2-week history of sudden onset of painful infiltrated subcutaneous nodules on both forearms. Based on clinical and histological findings, a diagnosis of methotrexate-induced panniculitis was made. The majority of MIAN case reports that we reviewed showed characteristic pathological findings of classic rheumatoid nodules; few reported panniculitis as a finding. This case illustrates the importance of recognizing this phenomenon as methotrexate-induced panniculitis should be considered in the differential diagnosis of any patient receiving methotrexate presenting with a recent history of accelerated nodulosis. Discontinuation of methotrexate remains controversial.

**Keywords:** panniculitis, methotrexate, rheumatoid arthritis, nodulosis

**Received:** 19 July 2016 | **Returned for modification:** 18 August 2016 | **Accepted:** 29 August 2016

## Introduction

Methotrexate is one of the most widely used anti-rheumatic drugs in the management of rheumatoid arthritis. Methotrexate-induced accelerated nodulosis (MIAN) is not an uncommon adverse effect associated with the use of methotrexate in rheumatoid arthritis. There are limited case reports that describe panniculitis as a pathological finding in this setting. We report a case of panniculitis in a patient with rheumatoid arthritis on methotrexate therapy.

## Case history

A 31-year-old female developed symmetric arthritis in her hands, ankles, knees, and lower back and was initially diagnosed as a case of seropositive rheumatoid arthritis in 2009. Her arthritis was poorly responsive to treatment with several agents including azathioprine and sulfasalazine. In October 2011, she was started on methotrexate at a dosage of 20 mg per week. During the course of the treatment, other agents used in combination with methotrexate included adalimumab and tocilizumab, which were both discontinued at the patient's personal preference. Since October 2013, methotrexate has been used as monotherapy and it achieved partial control of her symptoms.

In January 2014, the patient presented to the clinic with a 2-week history of sudden onset of painful infiltrated subcutaneous nodules that developed on both forearms. Physical examination revealed well-circumscribed, indurated, tender, subcutaneous nodules localized over the lateral proximal aspect of the forearms bilaterally.

At that time, the laboratory data were as follows: white blood cell count 8.4 × $10^9$/l (normal range 4.5–11 × $10^9$/l) , hemoglobin 127 g/l (117–155 g/l) , platelet count 406 × $10^9$ (normal range 140–450 × $10^9$/l), erythrocyte sedimentation rate 10 mm/hr (normal range 0–20 mm/hr), and a positive antinuclear antibody titer 1:80 speckled pattern. Rheumatoid factor, cyclic citrullinated peptide, and extractable nuclear antigen were all negative.

A 5 mm punch biopsy taken from the left forearm showed septal panniculitis and fibrosis (Fig. 1). The septa were infiltrated by lymphocytes and histiocytes, with areas of hemorrhage (Fig. 2), microcyst formation, membranous fat necrosis, and lipophages (Fig. 3). A few eosinophils were seen (Fig. 4). High-power magnification showed lipophages and membranous fat necrosis. Focally, a small vein was noted cuffed by lymphocytes (Fig. 5). No definite granuloma was identified, nor leukocytoclastic vasculitis (Fig. 6). High-power magnification showed microcyst formation. Based on the clinical and histological findings, methotrexate was stopped with no additional drugs started.

Within one month of methotrexate cessation, the lesion completely resolved, although clinically her peripheral arthritis worsened. Tofacitinib was later introduced and helped in controlling the patient's arthritis.

## Discussion

Rheumatoid arthritis is a chronic inflammatory disease affecting about one percent of the general population (1). Methotrexate is an anti-metabolite that inhibits dihydrofolate reductase and is considered one of the most frequently used drugs for rheumatoid arthritis and many other immune diseases due to its beneficial anti-inflammatory and immunosuppressive effects (2).

One to 10 percent of patients on methotrexate may develop cutaneous lesions, which may include cutaneous ulcerations, photosensitivity, alopecia, macular punctate rash, hypersensitivity vasculitis, and lower leg ulcers. Adverse effects associated with the use of methotrexate also include the development of accelerated nodulosis, also known as methotrexate-induced accelerated nodulosis (MIAN).

The first report that documented the occurrence of MIAN was published in 1986 (3) and since then a number of case reports and systematic studies have reported this phenomenon, which describes the development or acceleration of nodulosis in patients receiving methotrexate therapy for autoimmune conditions. This phenomenon is thought to occur in eight to 10 percent of rheumatoid arthritis patients (1). The time period between the beginning

[1]Dermatology Department, Sheikh Khalifa Medical City (SKMC), Abu Dhabi, UAE. [2]Pathology and Laboratory Medicine Department Sheikh Khalifa Medical City (SKMC), Abu Dhabi, UAE. ✉Corresponding author: raalmaashari@seha.ae

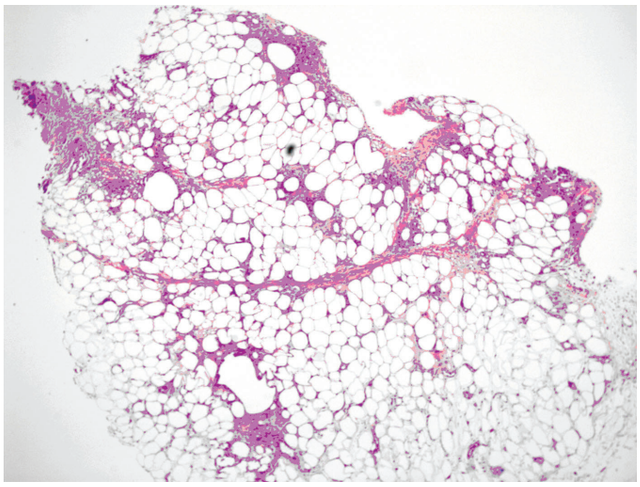


Figure 1 | Septal panniculitis and fibrosis.

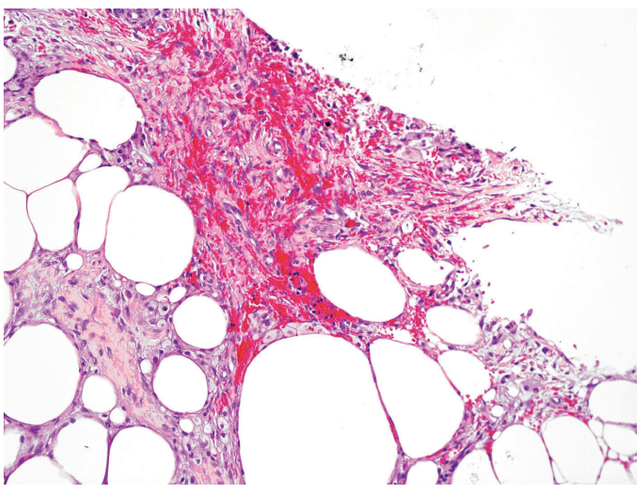


Figure 2 | Infiltration of the septa by lymphocytes and histiocytes, with areas of hemorrhage.

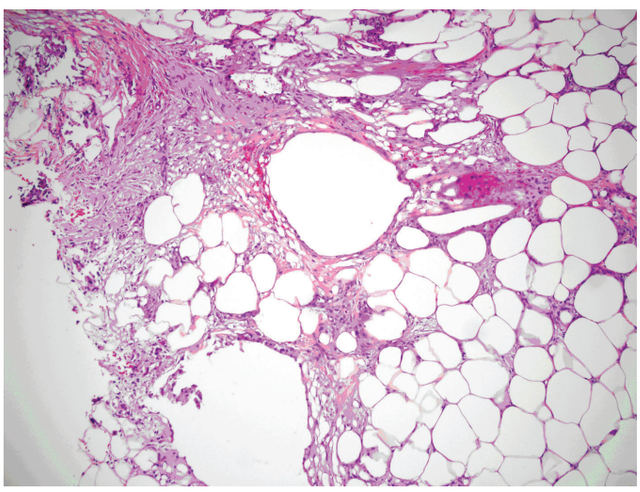


Figure 3 | Lipophages, microcyst formation, and membranous fat necrosis.

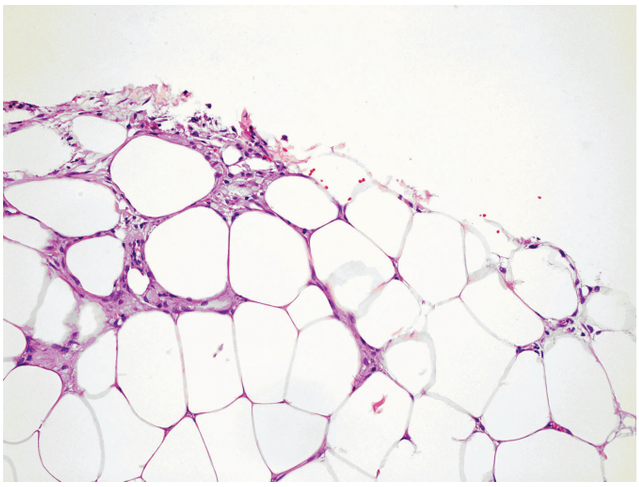


Figure 4 | A few eosinophils identified in this image.

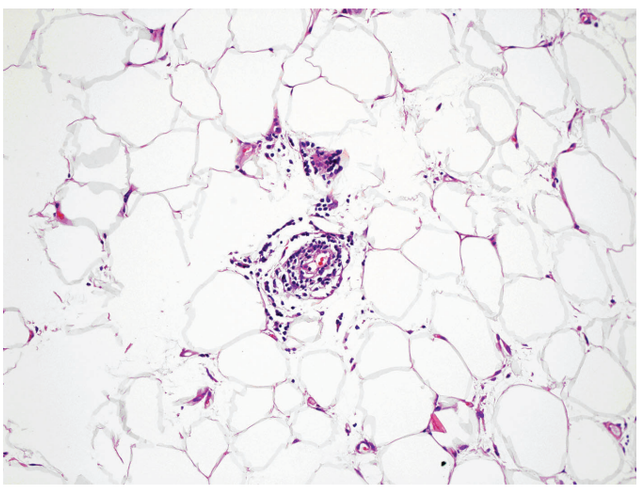


Figure 5 | High-power image of lipophages and membranous fat necrosis. Focally, a small vein was noted cuffed by lymphocytes.

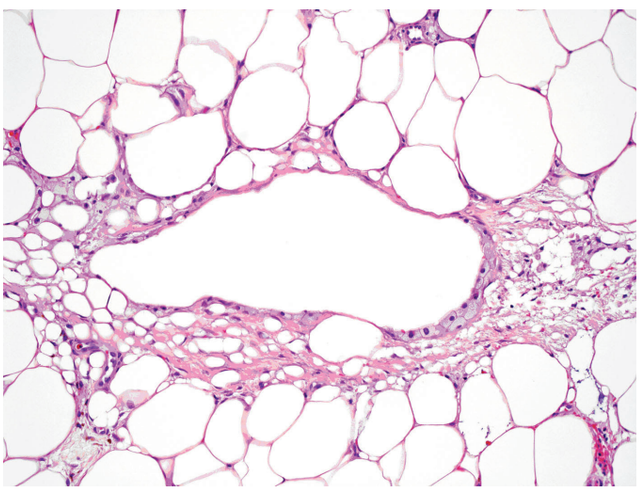


Figure 6 | Leukocytoclastic vasculitis and a definite granuloma are absent.

of methotrexate administration and the development of the nodules is variable (weeks to years) (4).

Panniculitis has several causes, including various infections, malignancies, and connective tissue disease, and drugs such as steroids, sulfonamides, and oral contraceptives, as well as MINE chemotherapy in rare cases (5). Methotrexate was listed by Brissaud in 2000 among one of the possible causes (6). The pathogenesis of methotrexate-induced panniculitis remains obscure.

The pathological findings in the majority of the MIAN case reports that we reviewed showed the classic findings of the classic rheumatoid nodule. However, a few reported panniculitis as a finding, as in the case of our patient.

To date, autoimmune conditions that have been reported to be associated with methotrexate-induced panniculitis include rheumatoid arthritis, dermatomyositis (2), and MCTD (7). Interestingly, there has been a report of MAIN in a patient with psoriatic arthri-

tis in which the histopathological findings were consistent with septal panniculitis (8).

It is unclear what factors determine predisposition of a certain category of rheumatoid arthritis patient to develop accelerated nodulosis because many reports of its occurrence are limited to case reports or are inconclusive due to a small sample size (3). Both the HLA-DRB1*0401 allele (3) and MTR 2756GG genotype (9) have been proposed to be associated with MIAN. Moreover, cumulative methotrexate dosage (3) and treatment efficacy (6) do not appear to affect the occurrence of methotrexate-induced nodulosis.
Methotrexate-induced nodules can present as an isolated finding or associated with systemic symptoms. They are commonly seen in the fingers and are usually smaller in size (< 5 mm in diameter) than rheumatoid nodules, though they may be clinically indistinguishable. Histologically, some methotrexate-induced nodules are characterized by septal panniculitis (7).

Clues that favor the diagnosis of MIAN involve the occurrence of skin lesions simultaneously with methotrexate use and its disappearance upon drug withdrawal. In some cases, methotrexate rechallenge can be performed to confirm the diagnosis. However, the absence of nodule recurrence with methotrexate re-challenge cannot rule out the role of methotrexate as an inciting agent (6). In the case of our patient, it was not performed.

Histological findings associated with drug-induced panniculitis can range from septal panniculitis with a lympho-histiocytic infiltrate to lobular panniculitis with a mixed or mostly neutrophilic infiltrate particularly with tyrosine kinase inhibitors (10).

The management of panniculitis depends on the cause. In methotrexate-induced panniculitis, controversy remains regarding whether methotrexate should be discontinued or not. In the case of our patient, the nodules resolved within 1 month of methotrexate cessation without the use of any additional drugs. Clearance of nodules remains variable, although it has been reported that nodules may clear after 6 months (3) of methotrexate discontinuation but sometimes recur when the drug is restarted. In cases in which methotrexate needs to be continued, additional drugs such as (11) hydroxychloroquine, colchicine, sulfasalazine, azathioprine, or D-penicillamine should be started because nodulosis can persist for 3.5 years if methotrexate is continued (3).

## References


1. Pugner KM, Scott DI, Holmes JW, Hieke K. The costs of rheumatoid arthritis: an international long-term view. Semin Arthritis Rheum. 2000;29:305-20.
2. Jang KA, Choi JH, Moon KC, Yoo B, Sung KJ, Koh JK. Methotrexate nodulosis. J Dermatol. 1999;26:460-4.
3. Ahmed SS, Arnett FC, Smith CA, Ahn C, Reveille JD. The HLA-DRB1*0401 allele and the development of methotrexate-induced accelerated rheumatoid nodulosis: a follow-up study of 79 Caucasian patients with rheumatoid arthritis. Medicine. 2001 Jul;80:271-8.
4. Patatanian E, Thompson DF. A review of methotrexate-induced accelerated nodulosis. Pharmacotherapy. 2002;22:1157-62.
5. Saint-Cyr I, Vezon G, Boisseau-Garsaud AM, Calès-Quist D, Panelatti G, Ossondo M. Panniculitis induced by MINE chemotherapy. Ann Dermatol Venereol. 2001;128:756-8.
6. de Sèze S, Ryckewaert A, Kahn MF, Kuntz D, Meyer O, Bardin T, Orcel P, editors. Actualité Rhumatologique. Paris (France): Expansion Scientifique Française; c2000. Brissaud P, Grossin M. Panniculites systémiques; p. 80-106. French.
7. Nezondet-Chetaille AL, Brondino-Riquier R, Villani P, Bouvenot G. Panniculitis in a patient on methotrexate for mixed connective tissue disease. Joint Bone Spine. 2002;69:324-6.
8. Berris B, Houpt JB, Tenenbaum J. Accelerated nodulosis in a patient with psoriasis and arthritis during treatment with methotrexate. J Rheumatol. 1995;22:2359-60.
9. Berkun Y, Atta IA, Rubinow A, Orbach H, Levartovsky D, Suhail A, et al. 2756GG genotype of methionine synthase reductase gene is more prevalent in rheumatoid arthritis patients treated with methotrexate and is associated with methotrexate-induced nodulosis. J Rheumatol. 2007;34:1664-9.
10. Kerl K. Histopathological patterns indicative of distinct adverse drug reactions. Chem Immunol Allergy. 2012;97:61-78
11. Patatanian E, Thompson DF. A review of methotrexate-induced accelerated nodulosis. Pharmacotherapy. 2002;22:1157-62.

# Canes-Nail™

NOVO

## Do zdravih nohtov v dveh korakih in le 6-tih tednih

**1. korak** **Odstranjevanje okuženega nohta**

2-3 tedne


**2. korak** **Nadaljevanje zdravljenja okuženega dela kože s protiglivično kremo**

4 tedni


## Zdravljenje v dveh korakih omogoča:

- Hitro in temeljito odstranjevanje okuženega dela nohta
- Dnevno viden napredek[1]
- Enostavno zdravljenje brez bolečin[1]
- Globinsko odstranjevanje glivic[2]

Podrobni prikaz zdravljenja okuženega dela nohta si lahko ogledate na www.canesnail.si

**Skrajšan povzetek glavnih značilnosti zdravila**
**Ime zdravila:** Canespor 10 mg/g krema. **Sestava:** 1 g kreme vsebuje 10 mg bifonazola. **Terapevtske indikacije:** za zdravljenje kožnih mikoz, ki jih povzročajo dermatofiti, kvasovke, plesni in druge glivice (npr. Malassezia furfur) ter okužbe s Corynebacteriumom minutissimum: tinea pedum, tinea manuum, tinea corporis, tinea inguinalis, pityriasis versicolor, površinske kandidoze in eritrazma. **Odmerjanje in način uporabe:** Kremo Canespor uporabljamo enkrat na dan, najbolje zvečer pred spanjem. Na prizadeto kožo nanesemo tanko plast zdravila in ga vtremo. Učinek je trajnejši, če kremo Canespor uporabljamo pravilno in dovolj dolgo. Običajno traja zdravljenje: mikoz na stopalu in med prsti (tinea pedum, tinea pedum interdigitalis) - 3 tedne; mikoz po telesu, rokah in v kožnih gubah (tinea corporis, tinea manuum, tinea inguinalis) – 2 do 3 tedne; okužb rožene plasti kože, blagih, kroničnih, površinskih okužb (pityriasis versicolor, eritrazma) – 2 tedna; površinskih kandidoz kože – 2 do 4 tedne. Za površino v velikosti dlani zadostuje večinoma že majhna količina kreme. Otroci: Pregled kliničnih podatkov kaže, da uporaba bifonazola pri otrocih ne povzroča škodljivih učinkov. Kljub temu naj se bifonazol pri dojenčkih uporablja le pod zdravniškim nadzorom. **Kontraindikacije:** Preobčutljivost za bifonazol, cetil in stearilalkohol ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Bolniki z anamnezo preobčutljivostnih reakcij na druge imidazolske antimikotike (npr. ekonazol, klotrimazol, mikonazol) morajo previdno uporabljati zdravila, ki vsebujejo bifonazol. Paziti je treba, da zdravilo ne pride v stik z očmi. Krema Canespor vsebuje cetil in stearilalkohol, ki lahko povzroči lokalne kožne reakcije (npr. kontaktni dermatitis). Pri bolnikih, ki so preobčutljivi za cetil in stearilalkohol, je priporočljivo, da namesto kreme Canespor uporabljajo raztopino Mycospor. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Ni podatkov o medsebojnem delovanju z drugimi zdravili. **Nosečnost in dojenje:** Prve 3 mesece nosečnosti smejo ženske bifonazol uporabiti šele potem, ko zdravnik oceni razmerje koristi in tveganja. Dojenje: Ni znano, ali se bifonazol pri človeku izloča v materinem mleku. Doječe matere smejo bifonazol uporabiti šele potem, ko zdravnik oceni razmerje koristi in tveganja. Med obdobjem dojenja ženska bifonazola ne sme uporabljati v predelu prsi. Plodnost: Predklinične študije niso pokazale, da bi bifonazol vplival na plodnost samcev ali samic. **Neželeni učinki:** Splošne težave in spremembe na mestu aplikacije: bolečine na mestu uporabe, periferni edemi (na mestu uporabe); bolezni kože in podkožja: kontaktni dermatitis, alergijski dermatitis, eritem, srbenje, izpuščaj, urtikarija, mehur, eksfoliacija kože, ekcem, suha koža, draženje kože, maceracija kože, pekoč občutek na koži. Ti neželeni učinki po prekinitvi zdravljenja izginejo. **Način in režim izdaje:** Izdaja zdravila je brez recepta v lekarnah. **Imetnik dovoljenja za promet:** Bayer d. o. o., Bravničarjeva 13, 1000 Ljubljana. **Datum zadnje revizije:** 20.10.2011. **Datum priprave informacije**: april 2012. **Vse informacije o zdravilu dobite pri Bayer d.o.o.**


Literatura:
1. Canes-Nail; Navodila za uporabo.
2. Canespor krema; Povzetek glavnih značilnosti zdravila.


Samo za strokovno javnost.

SI.BCC.11.2012.0042

Bayer HealthCare

Acta Dermatovenerol APA

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica

2016;25:83-84
**doi: 10.15570/actaapa.2016.24**

# Erythema exsudativum multiforme induced by a taurine-containing energy drink

Antigona Mithat Begolli Gerqari[1], Mybera Ferizi[2], Sadije Halimi[2], Aferdita Daka[2], Syzana Hapciu[2], Ilir Mithat Begolli[1]✉, Mirije Begolli[3], Idriz Hysen Gerqari[4]

**Abstract**

Erythema exsudativum multiforme is an immunologically mediated skin condition caused by viruses, bacteria, food, and drugs. There are different forms, and depending on the severity of the disease there is a major and minor form. Whereas the minor form passes without consequences, the major form and Stevens–Johnson syndrome affect the mucosa and may result in death. The disease affects all age groups but is more often observed in young individuals. Typical signs of the disease are skin lesions termed herpes iris. Taurine is an organic compound used in energy drinks and food that can cause many forms of hypersensitivity reactions, and one of these is erythema exsudativum multiforme. As consumption of energy drinks containing taurine increases, the problem of an increase in cases presenting with various forms of hypersensitivity reactions should be considered. Here we present the case of a 19-year-old man with erythema exsudativum multiforme caused by a drink containing taurine. We excluded all other factors that may have caused erythema multiforme and the patient was hospitalized, having been referred to us for the second time presenting with the same problem caused twice by the same drink.

**Keywords:** Erythema exsudativum multiforme, taurine, vasculitis

**Received:** 24 August 2016 | **Returned for modification:** 22 September 2016 | **Accepted:** 10 October 2016

## Introduction

Erythema multiforme (EM) is an acute (based on hypersensitivity) immune-mediated disease sometimes presenting as a recurrent skin condition (1–10). It is classified as a type IV hypersensitivity immunological reaction. In most cases, erythema exsudativum multiforme is triggered by infections with bacteria, primarily streptococcus, and viruses, especially herpes simplex virus, cytomegalovirus, hepatitis virus, HIV, parapoxviruses, and adenoviruses (1–10).

Other factors causing erythema multiforme are drugs and food. The disease affects all age groups; however, it is more often observed in young people. Typical signs of the disease are skin lesions termed herpes iris. The severity of the disease varies and in some cases may also involve the mucosa, in which case one should consider the major form of the disease or Stevens–Johnson syndrome (2, 3, 10). By consensus definition in 1993, Stevens–Johnson syndrome was classified separately from the erythema multiforme spectrum and listed under toxic epidermal necrolysis (3).

Taurine is an organic compound, albeit not a free amino acid in the usual biochemical meaning of the term (4). It is naturally found and widely distributed in mammalian tissues (5). In the food industry there are some energy drinks that contain taurine, and there have been reports of hypersensitivity reaction with synthetic taurine (11). Furthermore, taurine has been associated with numerous side effects, and data suggest that taurine can cause reactions such as urticaria, anaphylaxis, and rarely erythema exsudativum multiforme.

## Case report

Here we present the case of 19-year-old student referred to our dermatology clinic for the appearance of sharply demarcated round red macules several centimeters in size (Fig. 1). In some areas of the skin, the macules formed confluent plaques (Fig. 2) localized on both buttocks. In the crural and femoral parts of the skin, target lesions were observed, presenting with a sharp margin round and oval in shape, some of which had a red central blister. Similar lesions, but fewer in number and isolated, were localized on the skin of the abdomen and back. There was no involvement of the mucosa. The patient complained of itching, a local temperature, and discomfort. Corticosteroids and antibiotics were immediately administrated, and topical corticosteroids were applied. Further routine analyses were carried out: sedimentation, hemogram and peripheral blood smear, urea, creatinine, transaminases and bilirubin, glycaemia, and CRP. The values for the parameters measured were within normal range. The TORCH helicobacter pylori and rheumatic factors were also negative. During the medical history, the patient confirmed that this was the second time that he had experienced the same skin changes following consumption of the same taurine-containing energy drink. We carried out a 6-month follow-up of the patient, and there were no recurrences of the disease.

## Discussion

Erythema exsudativum multiforme is an immunologically mediated skin reaction or a reaction to viruses or bacteria (10), classified in the group of type-IV delayed cell-mediated hypersensitivity. The minor form is localized on the skin and the mucosa are not involved (1, 9). The typical skin sign is herpes iris, or target lesions with a red to reddish-blue color. The localization of the changes occur in photo-exposed areas. The main causes are various drugs, food containing some additives, bacteria, especially streptococcus, and some viruses, such

[1]University of Prishtina, Faculty of Medicine, Prishtina, Kosovo. [2]Department of Dermatology, University Clinical Center of Kosovo Prishtina, Kosovo. [3]Department of Pediatrics, University Clinical Center of Kosovo, Prishtina, Kosovo. [4]Department of Nuclear Medicine, University Clinical Center of Kosovo, Prishtina, Kosovo. ✉**Corresponding author: ilirbegolli@gmail.com**

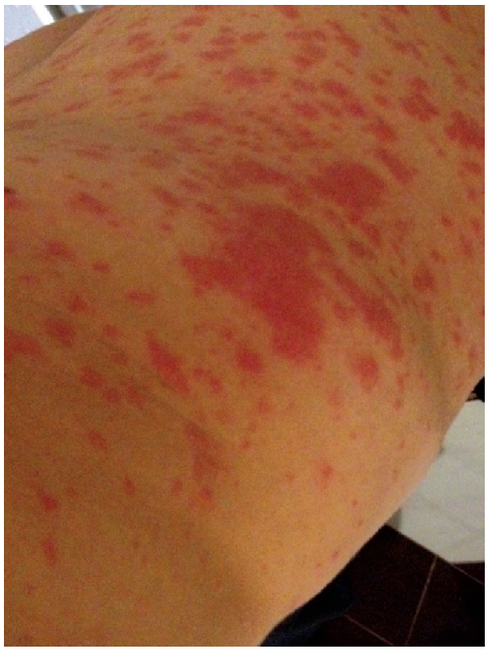


Figure 1 | Erythema exsudativum multiforme.

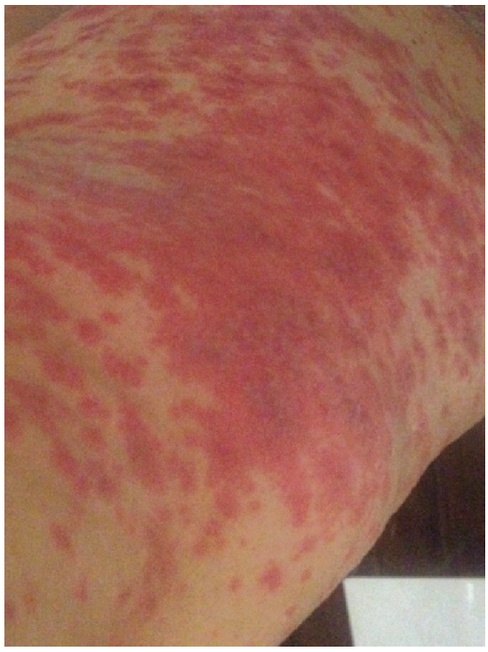


Figure 2 | Erythema exsudativum multiforme: confluent plaques.

as herpes simplex virus and Epstein Barr virus in particular. Patients may experience several recurrences per year. Furthermore, the major form of erythema exsudativum multiforme is a rare and life-threatening disease that presents with both skin and mucosal involvement (2, 3, 10). In such cases, the lesions begin on the face and develop on the trunk with blisters on macular skin lesions (2, 3). Hence, the same disease appears at different stages and with different severity. Although Stevens–Johnson syndrome mostly appears as a reaction to some medicines, it has been separated from the erythema multiform spectrum and added to toxic epidermal necrolysis (3). Mucosal involvement in a situation in which erythema multiforme is caused by herpes simplex must be taken into consideration when differentiating between erythema multiforme and Stevens–Johnson syndrome (2, 3, 10). The prognosis of the disease varies and depends on the cause and the state of the patient's immune system. Energy drinks containing taurine have recently been blamed for causing hypersensitivity reactions such as urticaria, in some cases anaphylaxis, and in rare cases even erythema exsudativum multiforme (8). Taurine, an organic compound found in animal tissues (5), has been studied in the medical and pharmaceutical industry as a food and drink supplement that lowers the risk of cardiovascular disease, mostly via a mechanism that prevents hypertension and decreases blood cholesterol (6). Nevertheless, it is important to emphasize the potential hypersensitivity reaction to synthetic taurine (11).

## References


1. Sokumbi O, Wetter DA. Clinical features, diagnosis, and treatment of erythema multiforme: a review for the practicing dermatologist. Int J Dermatol. 2012;51: 889-902.
2. Assier H, Bastuji-Garin S, Revuz J, Roujeau JC. Erythema multiforme with mucous membrane involvement and Stevens–Johnson syndrome are clinically different disorders with distinct causes. Arch Dermatol. 1995;131:539-43.
3. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. Arch Dermatol. 1993;129:92-6.
4. Lombardini JB, Schaffer SW. Special issue: Taurine: discovered 185 years ago and still intrigues the scientific community. Amino Acids. 2002;23:343.
5. Lambert IH. Regulation of the cellular content of the organic osmolyte taurine in mammalian cells. Neurochem Res. 2004;29:27-63.
6. Choi MJ, Kim JH, Chang KJ. The effect of dietary taurine supplementation on plasma and liver lipid concentrations and free amino acid concentrations in rats fed a high-cholesterol diet. Adv Exp Med Biol. 2006;583:235-42.
7. Huston RK, Baxter LM, Larrabee PB. Neonatal parenteral nutrition hypersensitivity: a case report implicating bisulfite sensitivity in a newborn infant. JPEN J Parenter Enteral Nutr. 2009;33:691-3.
8. Meng WJ, Li Y, Zhou ZG. Anaphylactic shock and lethal anaphylaxis caused by compound amino acid solution, a nutritional treatment widely used in China. Amino Acids. 2012;42:2501-5.
9. Huff JC. Erythema multiforme and latent herpes simplex infection. Semin Dermatol. 1992;11:207-10.
10. Fitzpatrick TB et al., editors. Fitzpatrick's dermatology in general medicine. 4th ed. New York (USA): McGraw-Hill; c1993. Fritsh PO, Elias PM. Erythema multiforme and toxic epidermal necrolysis; p. 585-600.
11. Lee SE, Lee SY, Jo EJ, Kim MY, Yang MS, Chang YS, et al. A case of taurine-containing drink induced anaphylaxis. Asia Pac Allergy. 2013;3:70-3.

Acta Dermatovenerol APA

**Acta Dermatovenerologica**
Alpina, Pannonica et Adriatica

2016;25:85-86
**doi: 10.15570/actaapa.2016.25**

# Neutrophilic dermatosis of the dorsal hands: a restrictive designation for an acral entity

Miguel Costa-Silva[1]✉, Ana Pedrosa[1,2], Filomena Azevedo[1], Alberto Mota[1,2]

**Abstract**

In 2000, Galaria et al. proposed the designation *neutrophilic dermatosis of the dorsal hands* (NDDH). The authors describe a case of NDDH with predominant involvement of the palmar aspect of the hands in a patient suffering from lung cancer, a possible paraneoplastic manifestation. Therefore, the term *NDDH* is not accurate because palmar manifestations of this dermatosis are also possible.

**Keywords:** neutrophilic dermatosis of the dorsal hands, Sweet's syndrome

**Received:** 11 October 2016 | **Returned for modification:** 4 November 2016 | **Accepted:** 10 November 2016

## Introduction

In 1995, Strutton et al. (1) reported six patients with a dermatosis limited almost entirely to the dorsal aspects of the hands resembling Sweet's syndrome (SS) but differing histologically by the presence of leukocytoclastic vasculitis. In 2000, Galaria et al. (2) further described similar cases but lacking the vasculitis component; he proposed the designation *neutrophilic dermatosis of the dorsal hands* (NDDH). Currently, NDDH is viewed as a subset of neutrophilic dermatosis and is recognized as a localized variant of SS due to the similarities between these two entities (3).

## Case description

A 63-year-old male patient was referred to our dermatology clinic due to painful violaceous bullous plaques located on the palms and dorsal aspect of the fingers of both hands with 2 weeks' evolution (Figure 1). The patient had suffered from an advanced epidermoid carcinoma of the lung for 2 years. The treatment included a cycle of gemcitabine 1 month before the clinical presentation of the lesions. At time of our observation, the patient was admitted due to an episode of hemoptysis complicated by a respiratory infection. Investigations revealed a total leukocyte count of 11.31 × 109/l with 86.9% neutrophils and elevated C-reactive protein (176.8 mg/l). Blood cultures and pus culture from the cutaneous lesions showed no growth. Cutaneous biopsy demonstrated a dense neutrophilic infiltrate in the dermis with evidence of neutrophilic vasculitis, consistent with the diagnosis of NDDH (Figure 2). A treatment with a course of prednisolone, 20 mg daily, and topical betamethasone valerate resulted in a significant improvement at 1 month follow-up. The patient died 5 months after presentation due to epidermoid carcinoma of the lung with liver and brain metastases.

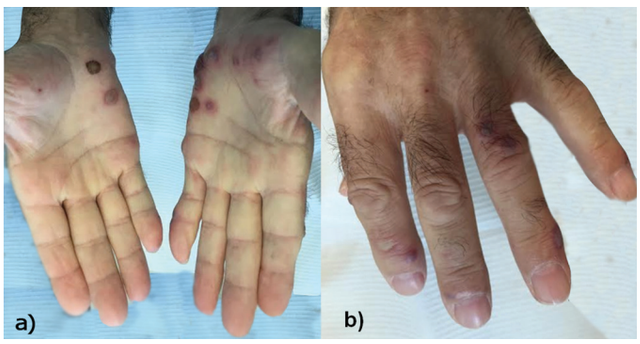



Figure 1 | Bullous plaques located on the palms (A) and dorsal aspect of the fingers (B) of both hands.

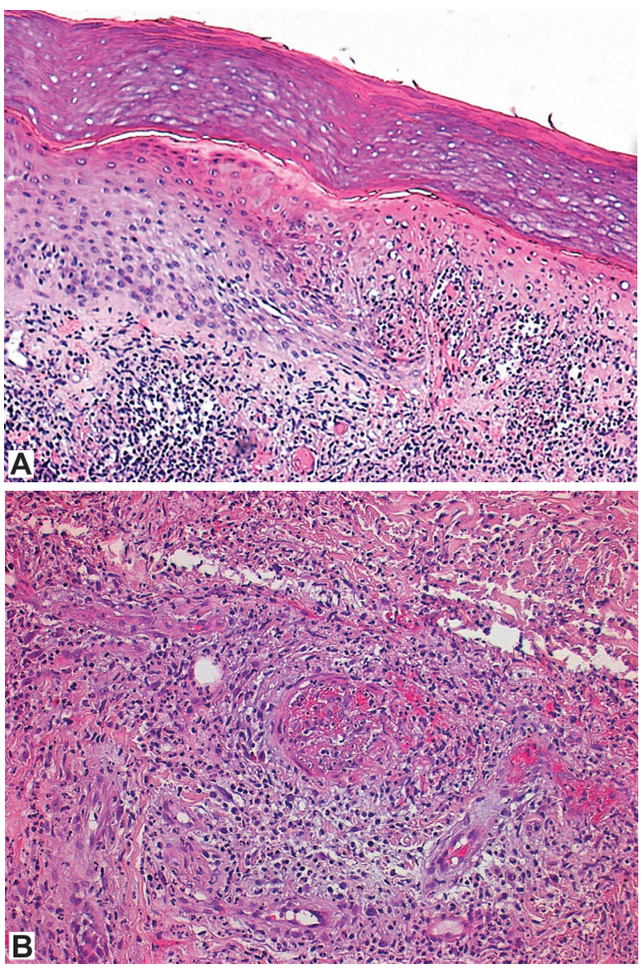



Figure 2 | A dense neutrophilic infiltrate in the dermis (A) with evidence of neutrophilic vasculitis (B)

[1]Department of Dermatology and Venereology, Centro Hospitalar São João, EPE Porto, Portugal. [2]Faculty of Medicine, University of Porto, Porto, Portugal.
✉ **Corresponding author: miguelcostaesilva.dermato@gmail.com**

## Discussion

NDDH and SS share similar clinical, laboratory, and histological findings (3). The most commonly associated disorders are hematological (myelodysplasia, IgA gammopathy, and B-cell lymphoma) and inflammatory (ulcerative colitis, Crohn's disease, seropositive arthritis, and sarcoidosis) (3, 4). There are also reports of NDDH associated to a lesser extent with infectious agents, trauma, and drugs (3). However, many cases of NDDH do not fulfill all of the SS criteria. In fact, the occurrence of constitutional signs and symptoms and the elevation of serum inflammatory markers are inconsistent in NDDH (3, 4). Despite the fact that our patient had elevated serum inflammatory markers, he also suffered from a respiratory infection, and so it is difficult to ascribe the relative contribution to this elevation, the infection, the NDDH, or both. As with SS, most patients are treated with systemic corticosteroids (5).

Vasculitis is present in about 30% of NDDH cases, in contrast to the lack of this feature in the cases originally described as SS (5). Some authors have argued that the vascular damage in these cases is probably a secondary event related to the intensity of the neutrophilic infiltrate and do not represent true vasculitis (3–5).

Our patient predominantly had involvement of the palmar aspect of the hands in contrast to the majority of the NDDH cases reported. Nonetheless, a small number of cases involving the lateral or palmar aspect of the hands have been found in some reports (3, 4, 6–8). This has led some authors to propose dropping dorsal from the designation of NDDH and changing it to *neutrophilic dermatosis of the hands* or even *acral neutrophilic dermatosis* (4, 6). In this respect, it is possible that NDDH as a possible paraneoplastic manifestation may in fact have a more atypical presentation, as in the case of our patient. However, an etiologic role of gemcitabine cannot be entirely ruled out in this particular case because NDDH has been associated with chemotherapy drugs (3).

## Conclusion

NDDH is associated with potentially serious systemic conditions. This warrants awareness of NDDH in the dermatological community in order to facilitate clinical recognition and a prompt workup. The term *NDDH* gives the false impression that this disease is strictly located on the dorsal aspect of the hands, which is not always the case, as demonstrated in this report. This may result in a more underdiagnosed disorder, and it is possible that a name change will contribute to identifying more cases.

## Acknowledgment

We thank Jennifer Costa and Aurea Canellhas for their contribution to this work; in particular, the pathology investigation and photos.

## References

1. Strutton G, Weedon D, Robertson I. Pustular vasculitis of the hands. J Am Acad Dermatol. 1995;32:192-8.
2. Galaria NA, Junkins-Hopkins JM, Kligman D, James WD. Neutrophilic dermatosis of the dorsal hands: pustular vasculitis revisited. J Am Acad Dermatol. 2000; 43:870-4.
3. Cravo M, Cardoso JC, Tellechea O, Cordeiro MR, Reis JP, Figueiredo A. Neutrophilic dermatosis of the dorsal hands associated with hypopharyngeal carcinoma. Dermatol Online J. 2008;14:5.
4. Weenig RH, Bruce AJ, McEvoy MT, Gibson LE, Davis MD. Neutrophilic dermatosis of the hands: four new cases and review of the literature. Int J Dermatol. 2004;43:95-102.
5. Walling HW, Snipes CJ, Gerami P, Piette WW. The relationship between neutrophilic dermatosis of the dorsal hands and Sweet syndrome: report of 9 cases and comparison to atypical pyoderma gangrenosum. Arch Dermatol. 2006; 142:57-63.
6. Nofal A, Assaf M, Elakad R, Fawzy M, Nofal E. Neutrophilic dermatosis of the dorsal hands: a localized variant of Sweet's syndrome or a distinct entity? Int J Dermatol. 2015;54:e66-7.
7. DiCaudo DJ, Connolly SM. Neutrophilic dermatosis (pustular vasculitis) of the dorsal hands: a report of 7 cases and review of the literature. Arch Dermatol. 2002;138:361-5.
8. Del Pozo J, Sacristán F, Martínez W, Paradela S, Fernández-Jorge B, Fonseca E. Neutrophilic dermatosis of the hands: presentation of eight cases and review of the literature. J Dermatol. 2007;34:243-7.


# Hitrost v zdravljenju aktinične keratoze

**Rp** **V***

* le za zdravljenje kože pri nehiperkeratotični, nehipertrofični aktinični keratozi pri odraslih bolnikih: le po priporočilu dermatologa ali onkologa

## Enkrat dnevno, 3 zaporedne dni

*Aktinična keratoza na obrazu in lasišču pri odraslih bolnikih*
Eno tubo zdravila Picato® 150 µg/g gel (ki vsebuje 70 µg ingenol mebutata) je treba enkrat dnevno nanesti na prizadeti predel in postopek ponavljati 3 zaporedne dni.

**Terapevtske indikacije:** Zdravilo Picato® je indicirano za zdravljenje kože pri nehiperkeratotični, nehipertrofični aktinični keratozi pri odraslih bolnikih.

Natančno preberite skrajšan povzetek lastnosti o zdravilu!

LEO®

LEO

**Ime zdravila** Picato 150 mikrogramov/gram gel

**Kakovostna in količinska sestava** 1 g gela vsebuje 150 mg ingenol mebutata. Vsaka tuba vsebuje 70 µg ingenol mebutata v 0,47 g gela.

**Terapevtske indikacije** Zdravilo Picato je indicirano za zdravljenje kože pri nehiperkeratotični, nehipertrofični aktinični keratozi pri odraslih bolnikih.

**Odmerjanje in način uporabe**

Odmerjanje: *Aktinična keratoza na obrazu in lasišču pri odraslih bolnikih* Eno tubo zdravila Picato 150 µg/g gel (ki vsebuje 70 µg ingenol mebutata) je treba enkrat dnevno nanesti na prizadeti predel in postopek ponavljati 3 zaporedne dni. *Pediatrična populacija* Zdravilo Picato ni primerno za uporabo pri pediatrični populaciji. *Starejši bolniki* Prilagoditev odmerka ni potrebna

Način uporabe: Vsebina tube zadošča za zdravljenje površine 25 cm² (npr. 5 cm x 5 cm). Vsebino tube je treba nanesti na eno zdravljeno površino velikosti 25 cm². Tuba je namenjena samo enkratni uporabi, zato jo po uporabi zavrzite. Gel iz tube iztisnite na konico prsta, ga enakomerno porazdelite po celotni površini prizadetega mesta in počakajte 15 minut, da se posuši. Vsebino ene tube lahko uporabite za zdravljenje enega mesta v velikosti 25 cm². Samo za enkratno uporabo.

Za zdravljenje vratu: če je več kot polovica zdravljenega mesta na zgornjem delu vratu, je treba uporabiti odmerjanje za obraz in lasišče. Če je več kot polovica zdravljenega mesta na spodnjem delu vratu, je treba uporabiti odmerjanje za trup in okončine. Bolnikom naročite, naj si po nanosu zdravila Picato nemudoma umijejo roke z milom in vodo. Če se zdravi roke, je treba umiti samo prst, s katerim se je nanesel gel. 6 ur po nanosu zdravila Picato ne umivajte mesta zdravljenja in se ga ne dotikajte. Po preteku tega časa lahko zdravljeno mesto umijete z blagim milom in vodo.

Zdravila Picato ne nanašajte takoj po prhanju ali manj kot 2 uri pred spanjem.

Po nanosu zdravila Picato zdravljenega mesta ne pokrivajte z neprepustnimi povoji. Optimalne učinke zdravljenja je mogoče oceniti približno 8 tednov po zdravljenju. Če se pri kontrolnem pregledu ugotovi nepopoln učinek, je treba znova skrbno oceniti zdravljenje in razmisliti o ponovni obravnavi. Klinični podatki o zdravljenju za več kot en cikel zdravljenja, ki traja 2 ali 3 zaporedne dni, niso na voljo. Klinični podatki o zdravljenju več kot enega mesta niso na voljo. Klinični podatki o zdravljenju pri imunokomprimiranih bolnikih niso na voljo, vendar ni pričakovati sistemskih tveganj, saj se ingenol mebutat ne absorbira sistemsko.

**Kontraindikacije** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov.

**Posebna opozorila in previdnostni ukrepi**

Izpostavljenost oči Stik z očmi je treba preprečiti. Če pride do nenamerne izpostavitve, je treba oči nemudoma izprati z velikimi količinami vode in bolnik naj čim prej poišče zdravniško pomoč. Pričakovati je da se bodo v primeru nenamerne izpostavitve oči zdravilu Picato pojavile težave z očmi, kot so bolečina očesa, edem vek in periorbitalni edem.

Zaužitje Zdravila Picato se ne sme zaužiti. Če pride do nenamernega zaužitja, naj bolnik spije veliko vode in poišče zdravniško pomoč.

Splošno Nanašanje gela Picato se ne priporoča, dokler koža, zdravljena s predhodnimi zdravili ali kirurško, ni zaceljena. Zdravila se ne sme nanašati na odprte rane ali dele kože s poškodovano kožno pregrado. Zdravilo Picato se ne sme uporabljati v bližini oči, na notranjem predelu nosnic, na notranjem predelu ušes ali na ustnicah.

Lokalni odzivi kože Pričakuje se, da se bodo po nanosu zdravila Picato na koži pojavili lokalni odzivi, kot so eritem, prhljaj/luščenje in nastajanje krast . Lokalizirani odzivi kože so prehodni in se običajno pojavijo v 1 dnevu od začetka zdravljenja, največjo intenzivnost pa dosežejo en teden po zaključku zdravljenja. Pri zdravljenju obraza in lasišča lokalizirani kožni odzivi običajno izzvenijo v 2 tednih od začetka zdravljenja, pri zdravljenju predelov na trupu in okončinah pa v 4 tednih. Učinka zdravljenja morda ne bo mogoče ustrezno oceniti, dokler se ne pozdravijo lokalni odzivi kože.

Izpostavljenost soncu Izvedene so bile študije, ki so ocenile vpliv UV-sevanja na kožo po enkratni ali večkratni uporabi gela z ingenol mebutatom, 100 µg/g. Gel z ingenol mebutatom ni pokazal nobenega potenciala za draženje zaradi svetlobe ali za fotoalergijske učinke. Vendar pa se je treba zaradi narave bolezni izogibati čezmerni izpostavitvi sončni svetlobi (tudi porjavitvenim svetilkam in solarijem) ali izpostavitev čim bolj zmanjšati.

Obravnava aktinične keratoze Pri lezijah, ki so klinično atipične za aktinično keratozo ali so sumljive za malignost, je treba opraviti biopsijo, za določitev primernega zdravljenja.

**Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** Študij medsebojnega delovanja niso izvedli. Menijo, da interakcije s sistemsko absorbiranimi zdravili niso verjetne, saj se zdravilo Picato ne absorbira sistemsko.

**Plodnost, nosečnost in dojenje**

Nosečnost Podatkov o uporabi ingenol mebutata pri nosečnicah ni. Študije na živalih so pokazale blago toksičnost za zarodek/plod (glejte poglavje 5.3). Tveganja za ljudi, ki prejemajo kožno zdravljenje z ingenol mebutatom, so malo verjetna, saj se zdravilo Picato ne absorbira sistemsko. Iz previdnostnih razlogov se je uporabi zdravila Picato med nosečnostjo bolje izogibati.

Dojenje Učinkov na dojene novorojence/otroke se ne pričakuje, ker se zdravilo Picato ne absorbira sistemsko. Doječim materam je treba dati navodilo, da novorojenček/dojenček še 6 ur po nanosu zdravila Picato ne sme priti v telesni stik z zdravljenim mestom.

Plodnost Študij plodnosti z ingenol mebutatom niso izvedli.

**Neželeni učinki**

Povzetek varnostnega profila Neželeni učinki, o katerih so najpogosteje poročali, so lokalni kožni odzivi, vključno z eritemom, prhljajem/luščenjem, krastami, otekanjem, vezikulacijo/pustulacijo in erozijo/ulceracijo na mestu uporabe gela z ingenol mebutatom: glejte preglednico 1 za izraze po MedDRA. Po nanosu gela z ingenol mebutatom se je pri večini bolnikov (> 95 %) pojavil en ali več lokalnih kožnih odzivov. Pri zdravljenju obraza in lasišča so poročali o okužbi na mestu nanosa.

Seznam neželenih učinkov v obliki preglednice V preglednici 1 je prikazana izpostavitev 499 bolnikov z aktinično keratozo zdravilu Picato 150 µg/g ali 500 µg/g v štirih z vehiklom nadzorovanih študijah 3. faze, v katere sta bila skupaj vključena 1002 bolnika. Bolniki so enkrat dnevno prejemali lokalno zdravljenje (površine 25 cm²) z zdravilom Picato v koncentraciji 150 µg/g 3 zaporedne dni ali 500 µg/g 2 zaporedna dneva ali lokalno zdravljenje z vehiklom. V preglednici so predstavljeni neželeni učinki v skladu z MedDRA, razvrščeni po organskih sistemih in anatomski umestitvi.

Pogostnost neželenih učinkov je opredeljena kot:

zelo pogosti (≥ 1/10); pogosti (≥ 1/100 do < 1/10); občasni (≥ 1/1.000 do < 1/100); redki (≥ 1/10.000 do < 1/1.000); zelo redki (< 1/10.000) in neznana (ni mogoče oceniti iz razpoložljivih podatkov).

V razvrstitvah pogostnosti so neželeni učinki navedeni po padajoči resnosti.

Opis izbranih neželenih učinkov Lokalni kožni odzivi pri zdravljenju »obraza/lasišča« oziroma »trupa/okončin«, pri katerih je bila incidenca > 1-odstotna, so: eritem na mestu uporabe (94 % oz. 92 %), luščenje kože na mestu uporabe (85 % oz. 90 %), krasta na mestu uporabe (80 % oz. 74 %), oteklina na mestu uporabe (79 % oz. 64 %), vezikule na mestu uporabe (13 % oz. 20 %), pustule na mestu uporabe (43 % oz. 23 %) in erozija mesta uporabe (31 % oz. 25 %).

Incidenca hudih lokalnih odzivov na koži obraza in lasišča je bila 29-odstotna, na koži trupa in okončin pa 17-odstotna. Hudi lokalni odzivi na koži pri zdravljenju »obraza/lasišča« oziroma »trupa/okončin«, pri katerih je bila incidenca > 1-odstotna, so: eritem na mestu uporabe (24 % oz. 15 %), luščenje kože na mestu uporabe (9 % oz. 8 %), krasta na mestu uporabe (6 % oz. 4 %), oteklina mesta uporabe (5 % oz. 3 %) in pustule na mestu uporabe (5 % oz. 1 %).

Dolgotrajno sledenje Spremljali so celokupno 198 bolnikov s popolno ozdravitvijo lezij na 57. dan (184 se jih je zdravilo z zdravilom Picato in 14 z vehiklom) še 12 mesecev. Rezultati niso spremenili varnostnega profila zdravila Picato

**Preveliko odmerjanje** Preveliko odmerjanje zdravila Picato lahko povzroči povečano incidenco lokalnih odzivov kože. Obravnava prevelikega odmerjanja naj obsega zdravljenje kliničnih simptomov.

**Posebna navodila za shranjevanje** Shranjujte v hladilniku (2 °C - 8 °C). Odprte tube po prvem odprtju zavrzite.

**Vrsta ovojnine in vsebina** Večplastne eno odmerne tube z notranjo plastjo iz polietilena velike gostote (HDPE) in aluminijasto pregradno membrano. Pokrovčki iz HDPE.

Zdravilo Picato 150 µg/g gel je pakirano v škatli s 3 tubami, od katerih vsaka vsebuje 0,47 g gela.

**Imetnik dovoljenja za promet** LEO Pharma A/S, Industriparken 55, 2750 Ballerup, Danska

**Datum zadnje revizije** 15. 11. 2012

**Zastopnik v Sloveniji** Pharmagan, d.o.o., Vodopivčeva 9, 4000 Kranj

**Preglednica 1 Neželeni učinki po organskih sistemih v skladu z MedDRA**

| Pogostnost | | |
|---|---|---|
| **Organski sistem** | **Obraz in lasišče** | **Trup in okončine** |
| **Infekcijske in parazitske bolezni** | | |
| pustule na mestu nanosa | zelo pogosti | zelo pogosti |
| okužba na mestu nanosa | pogosti | |
| **Bolezni živčevja** | | |
| glavobol | pogosti | |
| **Očesne bolezni*** | | |
| edem veke | pogosti | |
| bolečina v očesu | občasni | |
| periorbitalni edem | pogosti | |
| **Splošne težave in spremembe na mestu aplikacije** | | |
| erozija na mestu nanosa | zelo pogosti | zelo pogosti |
| vezikule na mestu nanosa | zelo pogosti | zelo pogosti |
| oteklina na mestu nanosa | zelo pogosti | zelo pogosti |
| luščenje kože na mestu nanosa | zelo pogosti | zelo pogosti |
| krasta na mestu nanosa | zelo pogosti | zelo pogosti |
| eritem na mestu nanosa | zelo pogosti | zelo pogosti |
| bolečina na mestu nanosa** | zelo pogosti | pogosti |
| pruritus na mestu nanosa | pogosti | pogosti |
| draženje na mestu nanosa | pogosti | pogosti |
| izcedek na mestu nanosa | občasni | |
| parestezija na mestu nanosa | občasni | občasni |
| razjeda na mestu nanosa | občasni | občasni |
| občutek toplote na mestu nanosa | | občasni |

*: Oteklina na mestu nanosa na obrazu ali lasišču se lahko razširi na predel oči.
**: Vključno s pekočim občutkom na mestu nanosa.


Končno.
Prvo zdravilo z odobreno indikacijo za zdravljenje zmerne do hude oblike
Hidradenitis suppurativa
Bližina me osrečuje
Zaradi HS sem se počutila nevredna dotika
Manj abscesov in manj bolečin
Počutim se dobro sama s seboj
Zdravilo Humira je indicirano za zdravljenje aktivne zmerne do hude oblike **hidradenitis suppurativa** (acne inversa) pri odraslih bolnikih, ki se ne odzovejo zadovoljivo na konvencionalno HS zdravljenje.[1]
HUMIRA®
adalimumab
destination you™
abbvie

**SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA Humira 40 mg** raztopina za injiciranje v napolnjeni injekcijski brizgi.**Sestava** Ena 0,8 ml napolnjena injekcijska brizga z enim odmerkom vsebuje 40 mg adalimumaba. Adalimumab je rekombinantno humano monoklonsko protitelo. **Terapevtske indikacije** *Revmatoidni artritis:* v kombinaciji z metotreksatom: zdravljenje zmernega do hudega aktivnega revmatoidnega artritisa pri odraslih bolnikih, kadar odziv na imunomodulirajoča zdravila, vključno z metotreksatom, ni zadosten; zdravljenje hudega, aktivnega in progresivnega revmatoidnega artritisa pri odraslih, ki prej še niso dobivali metotreksata. *Juvenilni idiopatski artritis:* Poliartikularni juvenilni idiopatski artritis (JIA): v kombinaciji z metotreksatom za zdravljenje aktivnega poliartikularnega JIA pri otrocih in mladostnikih od 2.leta starosti, ki se ne odzovejo ustrezno na eno ali več imunomodulirajočih antirevmatičnih zdravil. Artritis, povezan z entezitisom: za zdravljenje aktivnega artritisa, povezanega z entezitisom pri bolnikih, starih 6 let in več, ki so se neustrezno odzvali ali so intolerantni za običajno zdravljenje. *Ankilozirajoči spondilitis:* zdravljenje hudega aktivnega ankilozirajočega spondilitisa pri odraslih, ki se na konvencionalno terapijo ne odzovejo ustrezno. *Aksialni spondiloartritis brez radiografskega dokaza za AS:* zdravljenje odraslih s hudim aksialnim spondiloartritisom brez radiografskega dokaza za AS, toda z objektivnimi znaki vnetja s povišanimi CRP in/ali MRI, ki so nezadostno reagirali na ali ne prenašajo nesteroidnih protivnetnih zdravil. *Psoriatični artritis:* zdravljenje aktivnega in napredujočega psoriatičnega artritisa pri odraslih, če odziv na predhodno zdravljenje z imunomodulirajočimi antirevmatiki ni bil ustrezen. *Psoriaza:* zdravljenje zmerne do hude kronične psoriaze v plakih pri odraslih bolnikih, ki se ne odzovejo na druge sistemske terapije ali imajo kontraindikacije zanje. *Psoriaza v plakih pri pediatričnih bolnikih:* zdravljenje hude psoriaze v plakih pri otrocih in mladostnikih od 4. leta starosti, ki so se neustrezno odzvali na ali niso ustrezni kandidati za topikalno zdravljenje in fototerapije. *Hidradenitis supuprativa:* zdravljenje aktivne zmerne do hude oblike hidradenitis suppurativa (acne inversa) pri odraslih bolnikih, ki se ne odzovejo zadovoljivo na konvencionalno zdravljenje. *Crohnova bolezen:* zdravljenje zmerne do hude, aktivne Crohnove bolezni pri odraslih bolnikih, ki se ne odzovejo na popoln in ustrezen ciklus zdravljenja s kortikosteroidom in/ali imunosupresivom, ali pa takšno zdravljenje ni mogoče. *Crohnova bolezen pri pediatričnih bolnikih:* zdravljenje hude aktivne Crohnove bolezni pri pediatričnih bolnikih (od 6.leta starosti), ki se ne odzovejo zadovoljivo na konvencionalno zdravljenje, vključno s primarno prehransko terapijo, kortikosteroidom in imunomodulatorjem, ali pri tistih, ki imajo intoleranco ali kontraindikacije za tako zdravljenje. *Ulcerozni kolitis:* zdravljenje zmerno do močno aktivnega ulceroznega kolitisa pri odraslih bolnikih, ki se ne odzovejo zadostno na običajno zdravljenje ali le-to ni mogoče. **Odmerjanje in način uporabe** Odmerjanje: Zdravljenje mora uvesti in nadzorovati zdravnik specialist. *Revmatoidni artritis:* odrasli bolnik: 40 mg adalimumaba vsak 2.teden v enkratnem odmerku v subkutani injekciji. *Ankilozirajoči spondilitis, aksialni spondiloartritis brez radiografskega dokaza za AS in psoriatični artritis:* 40 mg adalimumaba v enkratni subkutani injekciji vsak 2.teden. *Psoriaza:* odrasli bolniki: začetni odmerek 80 mg subkutano, ki mu sledi 40 mg subkutano čez en teden in nato 40 mg subkutano vsak 2.teden. Pri bolnikih z nezadostnim odzivom na zdravljenje, se lahko po 16 tednih pokažejo koristi zaradi povečanja pogostnosti odmerjanja na 40 mg vsak teden. *Hidradenitis suppurativa:* 160 mg 1. dan, sledi 80 mg 15. dan in nato 29. dan odmerek 40 mg vsak teden. *Crohnova bolezen:* med indukcijo pri odraslih bolnikih z zmerno do hudo, aktivno Crohnovo boleznijo 80 mg 0. teden in nato 40 mg 2. teden. Po indukcijskem zdravljenju je priporočeni odmerek 40 mg v subkutani injekciji vsak drugi teden. *Ulcerozni kolitis:* med indukcijo pri odraslih bolnikih z zmerno do močno aktivnim ulceroznim kolitisom 160 mg 0. teden in 80 mg 2. teden. Po indukcijskem zdravljenju 40 mg v subkutani injekciji vsak 2.teden. *Pediatrična populacija: Juvenilni idiopatski artritis: Poliartikularni JIA od 2. do 12.leta starosti:* 24 mg/m² telesne površine do največjega enkratnega odmerka 20 mg (za bolnike, stare 2 do < 4 leta) in do največjega enkratnega odmerka 40 mg (za bolnike, stare 4 - 12 let) adalimumaba, vsak 2.teden v subkutani injekciji; *Poliartikularni JIA od 13.leta starosti:* 40 mg adalimumaba vsak 2.teden ne glede na telesno površino. Uporaba zdravila Humira pri bolnikih, starih manj kot 2 leti, za to indikacijo ni primerna. *Pediatrični bolniki s psoriazo ali ulceroznim kolitisom:* Varnost in učinkovitost zdravila Humira pri otrocih, starih 4-17 let, ni bila potrjena. Uporaba pri otrocih, starih manj kot 4 leta, za to indikacijo ni primerna. *Artritis, povezan z entezitisom:* Priporočeni odmerek pri bolnikih, starih 6 let in več, je 24 mg/m² telesne površine do največjega posamičnega odmerka 40 mg adalimumaba vsak drugi teden v subkutani injekciji. *Psoriaza v plakih pri pediatričnih bolnikih:* Priporočeni odmerek je 0,8 mg na kilogram telesne mase (do največ 40 mg na odmerek), ki se ga da subkutano enkrat na teden, v primeru prvih dveh odmerkov, nato pa vsak drugi teden. *Hidradenitis suppurativa pri pediatričnih bolnikih:* Varnost in učinkovitost zdravila. Hidradenitis suppurativa pri pediatričnih bolnikih: Varnost in učinkovitost zdravila Humira pri otrocih, starih 12-17 let, ni bila potrjena. Uporaba pri otrocih, starih manj kot 12 let, za to indikacijo ni primerna. *Pediatrični bolniki s Crohnovo boleznijo:* < 40 kg: 40 mg 0.teden, ki mu sledi 20 mg 2.teden. Po uvodnem zdravljenju je priporočeni odmerek 20 mg vsak drugi teden v obliki subkutane injekcije; ≥ 40 kg: 80 mg 0.teden, ki mu sledi 40 mg 2.teden. Po uvodnem zdravljenju je priporočeni odmerek 40 mg vsak drugi teden v obliki subkutane injekcije. Uporaba pri otrocih, starih manj kot 6 let, za to indikacijo ni primerna. *Pediatrični bolniki s psoriatičnim artritisom in aksialnim spondiloartritisom, vključno z anksiloznim spondilitisom:* Uporaba pri teh bolnikih ni primerna. Način uporabe: uporablja se kot subkutana injekcija. **Kontraindikacije** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Aktivna tuberkuloza ali druge hude okužbe in oportunistične okužbe. Zmerno do hudo srčno popuščanje. **Posebna opozorila in previdnostni ukrepi** *Okužbe:* Bolniki so bolj dovzetni za resne okužbe. Okvarjena pljučna funkcija lahko zveča tveganje za razvoj okužbe. Bolnike je zato treba pred, med in po zdravljenju natančno kontrolirati glede okužb, vključno s tuberkulozo. *Reaktivacija hepatitisa B:* Reaktivacijo hepatitisa B so opažali pri bolnikih, ki so dobivali antagonist TNF in ki so bili kronični nosilci virusa. *Nevrološki zapleti:* Antagonisti TNF so bili v redkih primerih povezani s pojavom ali poslabšanjem kliničnih simptomov in/ali rentgenoloških znakov demielinizirajoče bolezni osrednjega živčnega sistema, vključno z multiplo sklerozo in optičnim nevritisom, in periferne demielinizirajoče bolezni, vključno z Guillain-Barré- jevim sindromom. *Malignomi in limfoproliferativne bolezni:* V kontroliranih delih kliničnih preizkušanj z antagonisti TNF je bilo opaženih več primerov malignomov, vključno z limfomi. *Hematološke reakcije:* Redko opisana pancitopenija, vključno z aplastično anemijo. *Cepljenja:* Uporaba živih cepiv pri dojenčkih, ki so bili izpostavljeni adalimumabu in utero, ni priporočljiva še 5 mesecev po materini zadnji injekciji adalimumaba med nosečnostjo. *Kongestivno srčno popuščanje:* Pri bolnikih z blagim srčnim popuščanjem potrebna previdnost. *Avtoimunska dogajanja:* Zdravljenje lahko povzroči nastanek avtoimunskih protiteles. *Sočasna uporaba bioloških DMARDS ali antagonistov TNF:* Sočasna uporaba z drugimi biološkimi DMARDS (t.j.anakinra in abacept) ali z drugimi antagonisti TNF ni priporočljiva. *Operacije:* Bolnika, ki med zdravljenjem potrebuje operacijo, je treba natančno nadzirati glede okužb. Starejši ljudje: Posebna pozornost glede tveganja okužb. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** V kombinaciji z metotreksatom, je bilo nastajanje protiteles v primerjavi z monoterapijo manjše. Kombinacija zdravila Humira in anakinre ter zdravila Humira in abatacepta ni priporočljiva. **Nosečnost in dojenje** Ženske ne smejo dojiti vsaj pet mesecev po zadnjem zdravljenju z zdravilom Humira. **Neželeni učinki** *Najpogostejši neželeni učinki* so okužbe (kot je nazofaringitis, okužba zgornjih dihal in sinusitis), reakcije na mestu injiciranja (eritem, srbenje, hemoragija, bolečina ali otekanje), glavobol in mišično-skeletne bolečine. Drugi pogostejši neželeni učinki: različne vrste okužb; benigni tumor, karcinom kože; levkopenija, trombocitopenija, levkocitoza; preobčutljivost, alergije; zvišanje lipidov, hipokalemija, hiperurikemija, nenormalni nivo natrija v krvi, hipokalcemija, hiperglikemija, hipofosfotemija, dehidracija; spremembe razpoloženja, anksioznost, nespečnost; glavobol, parestezije, migrena, stisnenje živčnih korenin; motnje vidnega zaznavanja, konjunktivitis, vnetje veke, otekanje oči; vertigo; tahikardija; hipertenzija, zardevanje, hematom; kašelj, astma, dispneja; bolečine v trebuhu, navzeja in bruhanje, gastroinestinalna krvavitev, dispepsija, bolezen gastroezofagealnega refluksa, Sjögrenov sindrom; zvišani jetrni encimi; izpuščaj, poslabšanje ali pojav psoriaze, urtikarija, modrice, dermatitis, oniholiza, čezmerno znojenje, alopecija, srbenje; mišičnoskeletne bolečine, mišični spazmi; hematurija, ledvična okvara; reakcija na mestu injiciranja, bolečina v prsih, edemi, povišana telesna temperatura; koagulacija in motnje krvavenja, prisotnost avtoprotiteles, zvišanje laktat dehidrogenaze v krvi; slabše celjenje. **Način in režim izdajanja** Predpisovanje in izdaja zdravila je le na recept. **Imetnik dovoljenja za promet** AbbVie Ltd, Maidenhead, SL6 4UB Velika Britanija. **Datum revizije besedila:** 19.11.2015.

Vir: 1. Humira Povzetek glavnih značilnosti zdravila

abbvie

HUMIRA®
adalimumab
destination you™

AbbVie Biofarmacevtska družba d.o.o., Dolenjska cesta 242c, Ljubljana, Tel.: 01 320 80 60, Fax.: 01 320 80 61, www.abbvie.si
Samo za strokovno javnost Datum priprave: januar 2016 SIHUD150131

ISD REGIONAL MEETING

# Many faces of Dermatology - Clinical, Surgical & Aesthetical

6TH CONFERENCE OF THE

# EHSF2017

**8–10 FEBRUARY**

COPENHAGEN, DENMARK

# XII INTERNATIONAL CONGRESS OF DERMATOLOGY

**April 18-22, 2017 - Buenos Aires, Argentina**
**Sheraton Hotel & Convention Center**

Organized by

Local Organizer

FUNDERMA
Fundación para la educación, la investigación y el desarrollo de la dermatología

SOCIETAS INTERNATIONALIS DERMATOLOGIAE
AD MCMLX

Host Society

SOCIEDAD ARGENTINA DE DERMATOLOGIA
-SAD-