Table of Contents

Original articles

Epidemiology and resistance trends of Staphylococcus aureus isolated from vaginal samples: a 10-year retrospective study in Hungary
Márió Gajdács, Edit Urbán

XRCC1 variants do not represent a risk for dermatomyositis and systemic lupus erythematosus in Bulgarian patients
Zornitsa Kamenarska, Maria Hristova, Rumyana Dodova, Anton Vinkov, Radka Kaneva, Joana Pozharashka, Lyubomir Dourmishev

A descriptive, historical, and thematic analysis of Acta Dermatovenerologica Alpina, Pannonica et Adriatica
Jovan Miljković, Mario Poljak, Anja Šterbenc, Peter Kokol

Review article

An update on prophylactic human papillomavirus (HPV) vaccines: a review of key literature published between September 2018 and September 2019
Anja Šterbenc, Tina Triglav, Mario Poljak

Short report

Non-ablative radio frequency for the treatment of androgenetic alopecia
Yimei Tan, Liu Wei, Yiyi Zhang, Andy Goren, John McCoy, Andrija Stanimirovic, Torello Lotti, Maja Kovacevic

Case reports

Pemphigus vulgaris possibly associated with application of a tissue expander in a patient with Crohn’s disease and primary sclerosing cholangitis
George Badavanis, Efstathia Pasmatzi, Nikiforos Kapranos, Alexandra Monastirli, Pavlos Constantinou, George Psaras, Panayioti Protopapa, Dionysios Tsambaos

Primary cutaneous plasmacytosis: a case report
Adelina Costin, João Alves, Diogo Cerejeira

Papular eruption on UV-exposed skin in a 7-year-old boy caused by Enterobius vermicularis infection
Mateja Starbek Zorko, Anja Trajber Horvat

Asymptomatic reactivation of hepatitis B virus after prolonged treatment with etanercept
Nuno Gomes, Beatriz Leão, Cândida Abreu, Filomena Azevedo, Sofia Magina

Targetoid erythema surrounding multiple seborrheic keratoses induced by chemotherapy with gemcitabine
Chiara Retrosi, Roberta Vezzoni, Claudio Conforti, Paola Corneli, Giovanni Magaton-Rizzi, Iris Zalaudek, Nicola Di Meo
Correspondences

Is there a relationship between environmental factors and pityriasis rosea? Reply to Singh et al.
*Francesco Drago, Giulia Ciccarese, Aurora Parodi*

Reply to: comment on "Is there a relationship between environmental factors and pityriasis rosea?"
*Mehak Singh, Manoj Pawar, Antonio Chuh, Vijay Zawar*
Epidemiology and resistance trends of Staphylococcus aureus isolated from vaginal samples: a 10-year retrospective study in Hungary

Márió Gajdács1,2, Edit Urbán3

Abstract

Introduction: The vaginal flora is a complex microbial environment. The disruption of this niche usually leads to a pathological state and symptoms in patients. Aerobic vaginitis is a distinct form of vaginal inflammation, mainly caused by aerobic/facultative anaerobic bacteria (Staphylococcus aureus, Streptococcus agalactiae, and members of the Enterobacteriaceae). This study describes the prevalence and antibiotic susceptibility patterns of S. aureus isolated from vaginal samples from females at a tertiary-care teaching hospital in Hungary.

Methods: This retrospective study was carried out using data collected at the Albert Szent-Györgyi Clinical Center (University of Szeged) corresponding to a 10-year period (2008–2017). Antimicrobial susceptibility testing was performed using the disk diffusion method and gradient diffusion, using EUCAST interpretative standards. Methicillin-resistant S. aureus (MRSA) was detected on mannitol salt agar using cefoxitin disks, the PBP2’ Latex Agglutination Test Kit, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Results: The median age of affected patients was 31 years. Most (93.95%) of the samples received were vaginal swabs. A total of 3,356 individual isolates were recorded (315.6 ± 89.10/year, range: 213–480 isolates). In 91.4% of samples, S. aureus was the only pathogen isolated. The highest levels of resistance were detected against erythromycin (11.11 ± 3.65%, range: 6.76–17.17%) and clindamycin (10.85 ± 3.36%, range: 6.49–15.54%), whereas resistance rates against doxycycline, ciprofloxacin, chloramphenicol, sulfamethoxazole-trimethoprim, and gentamicin were much lower (0–4.48%). Susceptibility to cefoxitin was observed in 97.79% of the isolates; 74 strains were MRSA. All MRSA strains were susceptible to the antibiotics used for therapy for multidrug-resistant Gram-positive infections.

Conclusions: A slow and steady increase in the prevalence of resistance could be observed (mainly corresponding to MRSA isolates). Although the present resistance trends are still advantageous (compared to European resistance levels) and do not hinder adequate therapy, continuous surveillance of resistance levels is recommended. Macrolides and clindamycin should be used with caution, and, if available, only when susceptibility to these drugs has been verified.

Keywords: Staphylococcus aureus, epidemiology, antimicrobial resistance, vaginal samples

Received: 3 June 2019 | Returned for modification: 6 August 2019 | Accepted: 9 September 2019

Introduction

The vaginal flora is a complex microbial environment, consisting of a multitude of microbial species in variable quantities and proportions (1, 2). This collection of microorganisms is very distinct from other anatomical regions of the human body. In premenarcheal girls, Lactobacillus species are infrequent or minor constituents of the vaginal microbiota, with other facultative and strict anaerobes being in abundance. This corresponds to a neutral or slightly alkaline vaginal pH during early childhood. In healthy adult women, the microbial diversity is greatly reduced, with an abundance of Lactobacillus species, responsible for the acidification (due to lactic acid and H2O2 production) of this environment (3, 4). The colonization of the vaginal flora by lactobacilli is influenced by their adhesion levels to vaginal epithelial cells; in addition, some of the nutrients are derived from these dead epithelial cells of the female hosts (and others can be found in some secretions of the glands in the lower reproductive tract) (5, 6). Following puberty, the thicker stratified epithelium and higher levels of glycogen in the reproductive tract of females also favor Lactobacillus colonization (2–4). In addition to lactobacilli, other microorganisms commonly identified as commensals in the female genital tract include Staphylococcus aureus, Streptococcus agalactiae (group B Streptococcus), Enterococcus faecalis, Escherichia coli, and Candida albicans (7–9). After menopause, estrogen levels drop in the female body, dramatically affecting the vaginal microbiota; the pH rises to 6.0 to 8.0, corresponding to the reduction or elimination of lactobacilli and the increased colonization of the vaginal tract by members of the Enterobacteriaceae family, Gram-positive cocci, and Bacteroides and Prevotella species (fecal flora) (2–6). However, if the normal vaginal flora is disrupted, these bacteria become pathogenic, causing vaginal discharge (often with a putrid odor) and other symptoms such as itching, which is detrimental to patients’ quality of life (QoL) (10, 11). If there is a multitude of bacterial species present in the vaginal flora, that usually corresponds to a disrupted, pathological state of the vaginal microbiome.

Aerobic vaginitis (AV) was first characterized in 2002 as a distinct form of vaginal inflammation from bacterial vaginosis (BV) (12–14). Although both conditions are characterized by the disruption of the female genital Lactobacillus flora, BV is a non-inflammatory condition, distinguished by the high quantity of anaerobic bacteria, whereas in AV there is significant inflammation and it is mainly caused by aerobic/facultative anaerobic bacteria (13–14).
The main causes of AV include overpopulation of the vaginal flora with *S. aureus*, *S. agalactiae*, or *E. coli*, typically concurring with an increased inflammatory response or epithelial atrophy (or both) (18). The diagnosis of AV should be performed on the basis of wet mount microscopy, and it should be differentiated from BV on the basis of Nugent’s method or Gram-staining (12–13, 16). Antimicrobial susceptibility testing results aid the choice of the appropriate antibiotic; however, therapy on the basis of culture results only is not recommended. The prevalence of AV is around 8 to 11% in pregnant women, and 5 to 24% in women reporting complaints regarding the reproductive system (12–17).

Infections of the vagina have been associated with a significantly increased risk of preterm labor and low birth weight; in addition, other sequelae may include pelvic inflammatory disease (PID), which can lead to ectopic pregnancies, tubal infertility, and other dysfunctions of the female reproductive system (18, 19). The role of bacterial infections in the vagina has also been described in the progression of cervical dysplasia and the transmission/acquisition of various viral infections (HIV and herpes simplex virus 2) (20).

The epidemiology of genitourinary pathogens varies significantly with respect to the healthcare institution in question and the geographical localization (21). The emerging threat of methicillin-resistant *S. aureus* (MRSA) infections is a grave concern for clinicians (22). This study describes the prevalence and antibiotic susceptibility patterns of *S. aureus*, a significant pathogen in AV, isolated from vaginal samples at a tertiary-care teaching hospital in Hungary during a 10-year study period (2008–2017).

**Methods**

**Study design, data collection**

This retrospective study was carried out using data collected between January 1st, 2008 and December 31st, 2017 at the microbiology laboratory of the Albert Szent-Györgyi Clinical Center. This teaching hospital annually serves more than 400,000 patients in the Southern Great Plain of Hungary, according to the national insurance data (23). An electronic search in the records of the MedBakter laboratory information system (LIS) for samples processed at our laboratory that were positive for *S. aureus* was conducted by the authors.

For the purposes of data analyses, the 10-year study period was divided into two 5-year periods (2008–2012 and 2013–2017, respectively). In the data analysis, the authors included samples with 105 or more colony-forming units (CFU) for *S. aureus* (except in cases where international guidelines recommend otherwise). Only the first isolate per patient was included in the study. In addition, *S. aureus* isolates with divergent susceptibility patterns were considered different isolates. In addition, patient data were collected regarding demographic characteristics (age and reason for sample submission as indicated on the request forms for microbiological analysis). The study was deemed exempt from an ethics review by the Institutional Review Board and informed consent was not required because data anonymity was maintained.

**Identification of relevant isolates**

Sample processing was carried out according to guidelines in routine clinical bacteriology. All culture media (5% sheep blood agar, chocolate agar, and eosin methylene blue agar) were incubated at 37 °C for 24 to 48 hours in a 5% CO₂ atmosphere. If *S. aureus* was detected from the relevant samples, the plates were passed on for further processing. Between 2008 and 2012, presumptive phenotypic (biochemical reaction-based) methods and VITEK 2 ID (bioMérieux, Marcy-l’Étoile, France) were used for bacterial identification, whereas after 2013 this was complemented by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Germany) (24). The methodology of sample preparation for MALDI-TOF MS measurements was described elsewhere (25).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method and, when appropriate, E-test (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar (MHA) plates. In addition, for the verification of discrepant results, VITEK 2 AST (bioMérieux, Marcy-l’Étoile, France) was also utilized. The interpretation of the results was based on EUCAST breakpoints (http://www.eucast.org). *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 were used as quality control strains.

To evaluate the resistance trends of isolated strains, erythromycin (ERI), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), doxycycline (DOX), gentamicin (GEN), and sulfamethoxazole/trimethoprim (SXT) were chosen as indicator antibiotics, based on the local antibiotic utilization data method. MRSA was detected using mannitol salt agar (MSA) using cefoxitin (FOX) disks (< 22 mm zone diameter) and PBPl2 Latex Agglutination Test Kit (Thermo Fisher Scientific Hungary GmbH, Budapest, Hungary). After 2013, a combined MALDI-TOF MS and PBPl2 latex agglutination protocol was introduced in our laboratory (26). MRSA-positive isolates were considered to be resistant to all β-lactam antibiotics. In the case of MRSA-positivity, susceptibility testing for additional antibiotics (vancomycin, VAN; linezolid, LZO; daptomycin, DAP; mupirocin, MUP; and fusidic acid, FZA) was performed (22). During data analysis, intermediate results were grouped with and reported as resistant. Inducible CLI resistance was detected using the D test, and these strains were also reported as resistant.

**Statistical analysis**

Descriptive statistical analysis (including means or medians with ranges and percentages to characterize data) was performed using Microsoft Excel 2013 (Microsoft Corp., Redmond, WA). Statistical analyses were performed with SPSS software version 24 (IBM SPSS Statistics for Windows 24.0, IBM Corp., Armonk, NY), using the χ² test, Student’s *t*-test, and Mann–Whitney U test. The normality of variables was tested using Shapiro–Wilks tests. *P* values < 0.05 were considered statistically significant.

**Results**

**Demographic characteristics, sample types**

The median age of affected patients was 31 years in both the first (2008–2012) and second (2013–2017) half of the study period (range 2008–2012: 2–91 years, range 2013–2017: 9–83 years; *p* > 0.05), and the detailed age distribution of patients is presented in Figure 1. Most (93.95%) of the samples received were vaginal swabs, and 5.62% were high cervical swabs, 0.29% were explanted intrauterine devices (IUDs), and 0.14% were urethral swabs.

The main indications for the sample submission associated
with samples positive for *S. aureus* included vaginitis/vulvitis (49.79%) and high-risk pregnancy (31.02%); less common indications included nonspecific abdominal pain (1.73%), symptoms of menopause (1.31%), suspicion of a urinary tract infection (0.66%), unspecified pathology of the cervix (0.39%), amenorrhea/dysmenorrhea (0.33%), polycystic ovary syndrome (PCOS) or other cysts of the female genitourinary tract (0.30%), endometriosis (0.30%), or other nonspecific reasons (14.84%).

**Distribution of *S. aureus* isolates among vaginal samples from women**

During the 10-year surveillance period (January 1st, 2008 – December 31st, 2017), the Institute of Clinical Microbiology received 4,012 samples from outpatient clinics and inpatient departments that turned out to be positive for *S. aureus*; after data consolidation, 3,395 individual isolates were recorded (373.6 ± 89.10/year, range: 213–480 isolates; highest in 2016, lowest in 2008) during the study period (the detailed isolation frequency is presented in Fig. 2). A considerable but not significant increase (p = 0.182) was observed in the isolation frequency in the second part of the study period (the detailed isolation frequency is presented in Fig. 2). A considerable but not significant increase (p = 0.182) was observed in the isolation frequency in the second part of the study period (range 0–5.28%, highest in 2017). In addition to MRSA-positive isolates (range 0–1.61%), and GEN (0.20 ± 0.14%, range: 0–0.59%) were observed in 97.79% of the isolates over the 10-year study period.

Overall, 74 strains were detected that were MRSA-positive (FOX resistant and positive for PBP2’ latex agglutination; the first isolate was recovered in 2012), representing 2.21 ± 1.91% of the isolates (range 0–5.28%, highest in 2017). In addition to MRSA-positivity, 56 (75.7%) strains were also resistant to ERI, 38 (51.4%) to CLI, 31 (41.9%) to DOX, 11 (14.9%) to CIP, and 6 (8.1%) to GEN. No MRSA-positive strains were resistant to CHL and SXT; in addition, all MRSA strains were susceptible to the antibiotics specifically used for the therapy for MDR Gram-positive infections or MRSA-decolonization (namely VAN, LZD, DAP, MUP, and FZA).

### Antibiotic susceptibility trends among *S. aureus* strains in the study period

The resistance levels of the individual *S. aureus* isolates are summarized in Table 1. The highest levels of resistance were detected against ERI (11.11 ± 3.65%, range: 6.76–17.17%) and CLI (10.85 ± 3.36%, range: 6.49–15.54%), whereas resistance rates against DOX (2.19 ± 2.10%, range: 0–4.48%), CIP (1.50 ± 1.04%, range: 0–3.41%), CHL (0.67 ± 0.40%, range: 0–1.90%), SXT (0.31 ± 0.81%, range: 0–1.61%), and GEN (0.20 ± 0.14%, range: 0–0.59%) were much lower in comparison. Statistically significant differences during the two halves of the study period could only be observed in the case of CLI (p < 0.001), and no such difference was noted for other antibiotics (p = 0.138, p = 0.565, p = 0.345, p = 0.147, p = 0.693, and p = 0.292 for ERI, CHL, CIP, DOX, GEN, and SXT, respectively). Susceptibility to FOX was observed in 97.79% of the isolates over the 10-year study period.

### Discussion

This study reports on the epidemiological trends and resistance levels of *S. aureus* in gynecological samples in the Southern Great Plain of Hungary over a long surveillance period (10 years). To date, this is the first and longest such study in Hungary. There are

**Table 1** Table 1. Percentage of *S. aureus* resistant isolates to the tested antibiotics.

<table>
<thead>
<tr>
<th>Study year</th>
<th>ERI</th>
<th>CHL</th>
<th>CIP</th>
<th>CLI</th>
<th>DOX</th>
<th>GEN</th>
<th>SXT</th>
<th>MRSA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>8.45%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>7.98%</td>
<td>6.10%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2009</td>
<td>11.40%</td>
<td>0.65%</td>
<td>1.63%</td>
<td>9.45%</td>
<td>0.65%</td>
<td>0.33%</td>
<td>2.61%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2010</td>
<td>6.76%</td>
<td>0.54%</td>
<td>0.27%</td>
<td>6.49%</td>
<td>2.63%</td>
<td>0.54%</td>
<td>0.27%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2011</td>
<td>8.86%</td>
<td>0.74%</td>
<td>0.74%</td>
<td>8.12%</td>
<td>2.95%</td>
<td>0.00%</td>
<td>0.37%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2012</td>
<td>9.29%</td>
<td>1.24%</td>
<td>3.41%</td>
<td>8.36%</td>
<td>3.72%</td>
<td>0.31%</td>
<td>0.00%</td>
<td>3.41%</td>
</tr>
<tr>
<td>2013</td>
<td>13.00%</td>
<td>0.45%</td>
<td>0.90%</td>
<td>11.21%</td>
<td>4.48%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>1.79%</td>
</tr>
<tr>
<td>2014</td>
<td>12.50%</td>
<td>0.82%</td>
<td>2.45%</td>
<td>11.14%</td>
<td>0.27%</td>
<td>0.27%</td>
<td>0.00%</td>
<td>3.53%</td>
</tr>
<tr>
<td>2015</td>
<td>17.17%</td>
<td>1.30%</td>
<td>1.96%</td>
<td>15.43%</td>
<td>1.09%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>2.83%</td>
</tr>
<tr>
<td>2016</td>
<td>7.29%</td>
<td>0.63%</td>
<td>1.88%</td>
<td>14.79%</td>
<td>0.21%</td>
<td>0.00%</td>
<td>0.42%</td>
<td>3.13%</td>
</tr>
<tr>
<td>2017</td>
<td>16.42%</td>
<td>0.29%</td>
<td>1.76%</td>
<td>15.54%</td>
<td>0.00%</td>
<td>0.59%</td>
<td>0.00%</td>
<td>5.28%</td>
</tr>
<tr>
<td>10-year average</td>
<td>11.11%</td>
<td>0.67%</td>
<td>1.50%</td>
<td>10.85%</td>
<td>2.19%</td>
<td>0.20%</td>
<td>0.37%</td>
<td>2.21%</td>
</tr>
<tr>
<td>SD ±</td>
<td>3.65%</td>
<td>0.40%</td>
<td>1.04%</td>
<td>3.36%</td>
<td>2.10%</td>
<td>0.24%</td>
<td>0.81%</td>
<td>1.91%</td>
</tr>
</tbody>
</table>

*Represents FOX resistant and PBP2’ latex agglutination-positive isolates. ERI = erythromycin, CHL = chloramphenicol, CIP = ciprofloxacin, CLI = clindamycin, DOX = doxycycline, GEN = gentamicin, SXT = sulfamethoxazole/trimethoprim, MRSA* = methicillin-resistant *S. aureus*, SD = standard deviation.

Values in boldface represent peak resistance levels.
scopic data available in the literature regarding the susceptibility patterns of Staphylococcus aureus isolates from gynecological samples, and therefore additional data are necessary to provide a comprehensive picture of resistance trends (15, 22, 27–28). As a general rule, the susceptibility patterns are advantageous, and the resistance rates were below 20% in the case of every antibiotic tested. However, a slow and steady increase in resistance levels could be observed (corresponding to MRSA isolates, which are resistant to all β-lactam antibiotics, apart from fifth-generation cephalosporins), especially since 2012, when the first MRSA isolate was reported (22, 29–30). In a similar study in the same geographical region and time period (2008–2017), the resistance trends of Staphylococcus aureus were described from male STI samples: more than 98% of isolates were methicillin-susceptible, and the highest levels of resistance were detected against macrolides (15–28%) and clindamycin (13–30%) (31). Staphylococcus aureus as a urinary pathogen was also characterized in the southern part of Hungary: in these studies, 9.8 to 11.6% of isolates were MRSA between 2013 and 2017, and a numerically increasing tendency was observed (unpublished results). Based on the results of the European Antimicrobial Resistance Surveillance Network (EARS-Net), the European average for the percentage of MRSA was 16.9%, showing a decreasing tendency with large inter-country variation (1.0–44.0%) (32). Regarding Hungary, MRSA levels have been over 20% since the 2010s, and were fluctuating between 21 and 27% in the last 3 years of surveillance (2014–2017) (32, 33). Of course, the resistance situation is not as dramatic in the Staphylococcus aureus isolates from vaginal samples (with the highest levels of MRSA detected around 5%) compared to invasive isolates; nevertheless, the obvious increase in resistance should be noted.

There is no generally accepted clinical strategy for therapy for AV; however, several proposals have been published. The distinction between AV and BV is crucial for the choice of appropriate therapy (3–13). Metronidazole has no effect on AV, unlike in BV and Trichomonas vaginalis infections, for which this drug is routinely used (6–9). Clindamycin may be considered as a valid therapeutic option (especially in pregnancy), whereas fluoroquinolones are recommended in non-pregnant females because their effect on the vaginal microbiota (Lactobacillus) seems to be minimal (3–13). However, antibiotic therapy should be complemented if the inflammation (topical steroids) or atrophy (estrogen) is pronounced. In addition, the use of vaginal probiotics should also be encouraged. Regarding the use of estrogen, in some patient populations (breast cancer patients and postmenopausal women) its use is contraindicated; in these cases, a very low dose of local estriol should be used in combination with probiotics (3–13). Based on our results, the therapy for these genitourinary infections will not be hindered by antibiotic resistance for now; however, close surveillance should be performed for Staphylococcus aureus isolates from all anatomical sites to monitor the changes in resistance trends (22). On the other hand, macrolides and CLI should be used with caution, and, if available, only when susceptibility to these drugs has been verified.

Limitations of this study must be acknowledged. First, the design of the study is retrospective and we could not access the medical records of the individual patients affected by these infections. For this reason, the correlation between the existence of relevant risk factors and underlying illnesses (apart from age) and the isolation of Staphylococcus aureus could not be assessed. There is a risk of selection bias because studies describing the prevalence of various infectious diseases and resistance trends are mainly from tertiary-care centers, which generally correspond to patients with more severe conditions or underlying illnesses. Finally, a molecular characterization of the resistance determinants in the individual isolates was not performed (i.e., mecA or mecc genes), only to the extent of FOX resistance and latex agglutination tests.

**Conclusions**

AV is a distinct form of vaginal inflammation from BV, predominantly caused by Staphylococcus aureus, *Agalactiae*, and members of the Enterobacteriaceae family, which are abundantly present in case of vaginal dysbiosis. Because Staphylococcus aureus is a significant pathogen in AV, the aim of this study was to evaluate its relevance and frequency in vaginal samples at our tertiary-care teaching hospital during a 10-year study period (2008–2017). The highest levels of resistance were associated with CLI and macrolides (around 10%), whereas MRSA-levels were below 5% up until 2017. Continuous monitoring of resistance trends and the introduction of antimicrobial stewardship is recommended in the therapy for AV.

**Acknowledgements**

The authors would like to thank Tünde Déák and Erika Karasz for their excellent laboratory assistance during the routine diagnostic work. Márió Gajdács was supported by the National Youth Excellence Scholarship (Grant Number NTP-NTFÖ-18-C-0225) and the ESCMID Mentorship and Observership Programme.

**References**


Možne uporabne kontraindikacije in previdnostni ukrepi:

- Če se pojavi preobčutljivostna reakcija ali hudo draženje, je treba uporabo zdravila prekiniti.
- Zdravilo Belakne ne sme priti v stik z očmi, usti, robovi nosu ali mukoznimi membranami. Če zdravilo po nesreči pride v stik z očmi, usti, robovi nosu ali mukoznimi membranami, je treba zdravilo Belakne najprej prekiniti. Kliknite v oči in uste, s nakupljenimi oči v vodo. Če so oči naraščene ali menjačne, je treba in naščrteno usta v vodo. Ne sme se zdravilo Belakne uporabiti ali najedom, če je koža v drugih delih tega preden uporabite zdravilo.
- Zdravilo Belakne ne sme uporabiti na narastni osebi, ki je v sladkovodnem ali boljšem sladkovodnem vodnem okolju, saj je celotno zdravilo Belakne znan kot priročna in rokodobna voda.

Zdravilo Belakne ne sme uporabiti na bolnikih s hudimi akenami ali aknami na večjih površinah telisa ali mučimočnem dermatizmu, saj je celotno znan kot priročna in rokodobna voda.

Zdravilo Belakne je kontraindicirano med nosečnostjo in pri ženskah, ki načrtujejo nosečnost. Zdravilo Belakne lahko uporabljate med dojenjem, vendar se zdravila ne sme nanašati na predel prsniho koša, da se ne pride v stik s dojenčkom. Zdravilo Belakne je kontraindicirano pri ženskah, ki pričajata za dojenje ali so v dojenju, saj je celotno znan kot priročna in rokodobna voda.
XRCC1 variants do not represent a risk for dermatomyositis and systemic lupus erythematosus in Bulgarian patients

Zornitsa Kamenarska1, Maria Hristova2, Rumyana Dodova3, Anton Vinkov4, Radka Kaneva5, Joana Pozharashka1, Lyubomir Dourmishev1

Abstract

Introduction: Systemic lupus erythematosus (SLE) and dermatomyositis (DM) share a similar pathogenesis, and genetic, hormonal, and environmental factors are known to trigger the autoimmune process. The X-ray repair cross-complementing genes (XRCC1 and XRCC3) are known to play a central role in mammalian DNA repair processes. Evidence suggests that impaired DNA repair efficiency is implicated in the development of autoimmune diseases. This case-control study investigates the association between the XRCC1 Arg194Trp (C>T) and Arg399Gln (G>A) polymorphisms and the susceptibility to DM and SLE in Bulgarian patients.

Methods: Altogether 88 patients, 55 with SLE and 33 with DM, and 94 unrelated healthy controls were included in this study.

Results: None of the polymorphisms showed an association with SLE, DM, or their clinical parameters. The allele and genotype frequency of the two single nucleotide polymorphisms was similar to those found in other healthy Caucasian populations.

Conclusions: Our results indicate that the XRCC1 rs1799782 Arg194Trp and rs25487 Arg399Gln polymorphisms do not play a role in the susceptibility to SLE and DM.

Keywords: XRCC1, polymorphisms, risk, dermatomyositis, systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that belongs to the group of autoimmune connective tissue diseases and affects multiple organs and systems (1), whereas dermatomyositis (DM) is a rare idiopathic inflammatory myopathy with a specific skin syndrome (2). Both SLE and DM are triggered by similar genetic, hormonal, and environmental factors.

An impaired DNA repair process is considered to represent a major risk factor for autoimmune disease susceptibility. There are two main mechanisms involved in single-stranded DNA damage repair: base excision repair (BER) and nucleotide excision repair (3). The X-ray repair cross-complementing gene 1 (XRCC1) belongs to the BER pathway (4). Some XRCC proteins were found at the DNA damage sites, which confirms their role as factors in the incidence of various autoimmune disorders (5–7).

There are two common polymorphisms in the XRCC1 gene (Arg194Trp exon 6, C>T, rs1799782 and Arg399Gln exon 10, G>A, rs25487) that lead to amino acid replacement. Although the precise effects of these polymorphisms remain unknown, it is suggested that amino acid changes at the preserved regions may alter their function (8). Changes in protein biochemistry lead to the assumption that the variant alleles may reduce the kinetics of the repair process, thus contributing to disease susceptibility (9).

This study investigates the role of XRCC1 polymorphisms as risk factors for the development of DM and SLE in Bulgarian patients and defines their contribution to increased risk.

Materials and methods

Patients and controls

The patient group consisted of 33 patients with DM who fulfilled the modified classification criteria of Targoff et al. (10) and 55

Table 1 | Demographic and clinical data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DM</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female/male</td>
<td>20/13</td>
<td>45/10</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>52 ± 14.7 years</td>
<td>40 ± 12.4 years</td>
</tr>
<tr>
<td>Clinical, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous disease</td>
<td>25 (75.8)</td>
<td>33 (60.0)</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>27 (81.8)</td>
<td>11 (20.0)</td>
</tr>
<tr>
<td>Elevated muscle enzymes</td>
<td>19 (57.6)</td>
<td>38 (69.1)</td>
</tr>
<tr>
<td>EMG findings</td>
<td>19 (57.6)</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>19 (57.6)</td>
<td>31 (56.4)</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>9 (27.3)</td>
<td>14 (25.5)</td>
</tr>
<tr>
<td>Malar rash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discoid rash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photosensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serositis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunological disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation, EMG = electromyography, ANA = antinuclear antibodies, DM = dermatomyositis, SLE = systemic lupus erythematosus.
patients with SLE who fulfilled the revised American College of Rheumatology (ACR) criteria (11).

Among the DM patients, 20 were female and 13 male. Patients’ age varied from 18 to 82 years, with a mean age of 52. SLE patients were predominantly female (45), and only 10 male patients were included. The mean age of the SLE group was 40 with a range between 15 and 78 years. The follow-up was conducted at the Department of Dermatology and Department of Nephrology, Medical University of Sofia and at the Department of Nephrology, Ministry of Interior Hospital in Sofia for a mean period of 10 years. Ninety-four healthy sex-, age-, and ethnicity-matched controls were selected from the Biobank of the Molecular Medicine Center and National Genetic Laboratory. Table 1 presents their demographic and clinical data.

The investigation presented in this article was performed in line with the Declaration of Helsinki for research involving humans and was approved by the local ethics committee. Venous blood samples were drawn for DNA isolation after written informed consent signed by all participants.

Genetic analysis

DNA was extracted using the Chemagen DNA purification kit (Chemagen AG, San Francisco, USA).

The analysis of the polymorphisms was performed using the TaqMan genotyping assay, and the allele calling was carried out with Applied Biosystems 7500 software version 2.0.5.

Catalogue primers ordered by Thermo Fisher Scientific were used for the analysis. The rs1799782 context sequence (VIC/FAM) is TACCTGGGAGATCTGTTGATCC[J/G]GCTGAAAGAGAGCCCGCCGGCTC (https://www.thermofisher.com/order/genome-database/details/genotyping/C_11463404_10?CID=&ICID=&subtitle=pe=) and the rs25487 context sequence (VIC/FAM) is GGTTGGCCGTCCTACCTCC[T/C]GGGAGGGGCAAGGCCGACGCA-TGGG (https://www.thermofisher.com/order/genome-database/details/genotyping/C_622564_10?CID=&ICID=&subtitle=pe=). The TaqMan reaction was performed according to the instructions provided by Thermo Fisher Scientific in a volume of 5 µl containing 2.5 µl 2X TaqMan® Master Mix, 0.12 µl 40X Assay Working Stock, 0.88 µl dH2O, and 1.5 µl DNA template (10 ng/µl). The amplification conditions were initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s.

Working Stock, 0.88 µl dH2O, and 1.5 µl DNA template (10 ng/μl). The amplification conditions were initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s.

Internal positive controls were used to confirm the results.

Statistical analysis

Statistics were calculated using SPSS 22.0 software (SPSS Inc, Chicago, USA). The test for Hardy–Weinberg equilibrium was performed using chi-square (χ²) statistics. Changes in allele frequencies in DM, SLE cases, and controls were compared using Fisher’s exact test, and their significance was expressed as p-value and odds ratios (OR) with exact 95% confidence intervals (CI).

Results

Altogether 88 patients were analyzed: 33 with DM and 55 with SLE. Women prevailed in both groups, which is typical for autoimmune diseases. The mean age was lower among patients with SLE than among patients with DM (40 vs. 52, Table 1). Arg399Gln did not show any association with the development of the diseases, although the major genotype Arg/Arg is more frequent among patients with DM compared to controls (48.5% vs. 38.4%, Table 2).

Arg194Trp did not show any association with the development of DM and SLE. The major genotype Arg/Arg has a frequency of about 80% in all groups (Table 2). The Arg399Gln polymorphisms did not show any association with the ACR criteria for SLE or the DM criteria (Tables 3, 4). The Arg194Trp polymorphisms did not show any association with the ACR criteria (Table 5). Due to its low frequency, the association with the DM clinical parameters was not studied.

Discussion

XRCC1 is a DNA repair protein that makes complexes with various polymerases and ligases to repair DNA single-strand breaks (12–14). Thus, it seems logical to hypothesize that individuals with impaired XRCC1 function would show an elevated risk of developing diseases and their clinical manifestations.

| Genotype and allele frequencies of the XRCC1 rs1799782 C/T (Arg194Trp) and rs25487 G/A (Arg399Gln) polymorphisms among patients with dermatomyositis (DM), systemic lupus erythematosus (SLE), and controls.

Table 2 | Genotype and allele frequencies of the XRCC1 rs1799782 C/T (Arg194Trp) and rs25487 G/A (Arg399Gln) polymorphisms among patients with dermatomyositis (DM), systemic lupus erythematosus (SLE), and controls.

Table 3 | Comparison between rs25487 G/A (Arg399Gln) alleles and genotypes and the American College of Rheumatology criteria for systemic lupus erythematosus.

Table 4 | Comparison between rs25487 G/A (Arg399Gln) alleles and genotypes and the American College of Rheumatology criteria for systemic lupus erythematosus.

Table 5 | Comparison between rs25487 G/A (Arg399Gln) alleles and genotypes and the American College of Rheumatology criteria for systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GG (n = 19)</th>
<th>GA (n = 24)</th>
<th>AA (n = 11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>12 (63.2%)</td>
<td>13 (54.2%)</td>
<td>70 (73.6%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>2 (10.5%)</td>
<td>5 (20.8%)</td>
<td>2 (18.2%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>10 (52.6%)</td>
<td>12 (50.0%)</td>
<td>7 (63.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>0 (0.0%)</td>
<td>2 (8.3%)</td>
<td>1 (9.1%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Arthritis</td>
<td>12 (63.2%)</td>
<td>18 (75.0%)</td>
<td>7 (63.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Serositis</td>
<td>4 (21.1%)</td>
<td>6 (25.0%)</td>
<td>3 (27.3%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Renal disease</td>
<td>19 (100.0%)</td>
<td>24 (100.0%)</td>
<td>11 (100.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Neurological disease</td>
<td>6 (31.6%)</td>
<td>4 (16.7%)</td>
<td>1 (9.1%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Hematological disease</td>
<td>9 (47.4%)</td>
<td>8 (33.3%)</td>
<td>5 (45.5%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Immunological disease (Anti-dsDNA, Anti-Sm, Anti-phospholipid)</td>
<td>11 (57.9%)</td>
<td>16 (66.7%)</td>
<td>8 (72.7%)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

ANA = antinuclear antibodies.
The frequency of the Arg399Gln genotype in the Bulgarian population is close to that found in the Polish population (15). The frequency of the minor allele (38.5%) is in the range found for Caucasian populations (15, 16). A recent meta-analysis showed that Arg399Gln might play a role in susceptibility to SLE in the Asian population, but its role in the Caucasian population is still disputable (17). To date there have been two studies concerning the Caucasian population, which showed inconsistent results (15, 16). The Gln/Gln + Arg/Gln genotypes and the 399Gln allele were found associated with SLE in Polish patients, and the Gln/Gln + Arg/Gln genotypes were also found associated with malar rash and photosensitivity (15). Similar to our results, no association was found with susceptibility to SLE in Brazilian patients, but the XRCCIArg399Gln was found associated with a higher prevalence of anti-dsDNA antibodies in sera. The concomitant presence of XRCCIArg399Gln with other DNA repair polymorphic sites was associated with increased prevalence of nervous system pathology and antiphospholipid syndrome (16). Our results and the results obtained by other authors suggest that XRCC1Arg399Gln is not an independent factor in susceptibility to SLE in the Caucasian population, but it may confer a risk in combination with other DNA repair polymorphic sites.

The frequency of 399 Gln/Gln + Gln/Arg was higher among Taiwanese Han Chinese SLE patients, but the differences were not statistically significant. This functional single nucleotide polymorphism (SNP) was associated with malar rash, discoid rash, photosensitivity, antinuclear antibody, hematologic manifestations and joint involvement (18).

Interestingly, it was the 399Arg/Arg genotype and the Arg allele that were related to SLE and malar rash in Iranian patients (19). Such discrepancies are explained by the fact that autoimmune diseases are complex and multifactorial and are triggered by the interplay of both genetic and environmental causes. Furthermore, a different association was reported between XRCC1 gene polymorphisms and cancer in various ethnic groups, which suggests that the interactions between various environmental factors and genes may differ (20). Diversity in the clinical picture and some demographic factors can also lead to inconsistent results.

The Arg194Trp allele and genotype frequency was also similar in all groups. The frequency of the minor allele (11.2%) is comparable with previous data for Caucasian populations (21). Our results support the findings that there is no significant association between this polymorphism and the autoimmune diseases in different genetic models (17, 22). Despite this, the 194Trp/Trp genotype and the Trp allele have been related to discoid rash, anti-DN, and anti-Scl70 in Brazilian patients (21). The allele frequencies of Arg194Trp have been correlated with detectable autoantibodies and damage to the hematological system in Chinese Han SLE patients (23). The results suggest that this polymorphism may have a disease-modifying role in some populations.

Conclusions

Our results could not confirm the independent role of the XRCC1 SNPs to the susceptibility and clinical course of DM and SLE in Bulgarians. The major drawback of our study is the limited number of patients and controls, which affects the statistical significance of the results. Further studies in a larger cohort are needed to elucidate the role of these two polymorphisms for susceptibility and clinical manifestations of SLE and DM.

Acknowledgments

This work was funded by Grant - 2017 of Medical University-Sofia (contract 115, project 8519/2016).

Table 4 | Comparison between the rs25487 G/A (Arg194Gln) alleles and genotypes and dermatomyositis clinical parameters.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GG (n = 16)</th>
<th>GA (n = 10)</th>
<th>AA (n = 7)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weakness</td>
<td>15 (93.8%)</td>
<td>5 (50.0%)</td>
<td>7 (100.0%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>9 (56.3%)</td>
<td>7 (70.0%)</td>
<td>3 (42.9%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Elevated muscle enzymes</td>
<td>10 (62.5%)</td>
<td>4 (40.0%)</td>
<td>5 (71.4%)</td>
<td>0.7</td>
</tr>
<tr>
<td>EMG findings</td>
<td>8 (50.0%)</td>
<td>4 (40.0%)</td>
<td>7 (100.0%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cutaneous disease</td>
<td>14 (87.5%)</td>
<td>5 (50.0%)</td>
<td>6 (85.7%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Antibodies</td>
<td>5 (31.3%)</td>
<td>1 (10.0%)</td>
<td>3 (42.9%)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

EMG = electromyography.

Table 5 | Comparison between the rs1799782 C/T (Arg194Trp) alleles and genotypes and the American College of Rheumatology criteria for systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CC (n = 44)</th>
<th>CT (n = 1)</th>
<th>TT (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>26 (51.9%)</td>
<td>0 (0.0%)</td>
<td>70 (70.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>10 (22.7%)</td>
<td>1 (10.0%)</td>
<td>1 (10.0%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>27 (61.4%)</td>
<td>0 (0.0%)</td>
<td>4 (40.0%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>3 (6.8%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Arthritis</td>
<td>31 (70.5%)</td>
<td>1 (25.0%)</td>
<td>1 (10.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Serositis</td>
<td>12 (27.3%)</td>
<td>1 (100.0%)</td>
<td>10 (100.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Renal disease</td>
<td>44 (100.0%)</td>
<td>1 (100.0%)</td>
<td>1 (100.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Neurological disease</td>
<td>9 (20.5%)</td>
<td>0 (0.0%)</td>
<td>2 (20.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Hematological disease</td>
<td>18 (40.9%)</td>
<td>1 (100.0%)</td>
<td>5 (50.0%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Immunological disease (Anti-dsDNA, Anti-Sm, Anti-phospholipid)</td>
<td>27 (61.4%)</td>
<td>0 (0.0%)</td>
<td>7 (70.0%)</td>
<td>1</td>
</tr>
</tbody>
</table>

ANA = antinuclear antibodies.
References


A descriptive, historical, and thematic analysis of *Acta Dermatovenerologica Alpina, Pannonica et Adriatica*

Jovan Miljković1,2, Mario Poljak3,4, Anja Šterbenc3,5, Peter Kokol6✉

Introduction

Single journal studies have a long history in bibliometric research. In general, they deal with bibliometric, citation, and content analyses, as well as with other bibliometric aspects of a single journal in order to present the journal’s characteristics and inform potential authors. Single journal studies have mostly been devoted to sciences and technology, and medical sciences, followed by library and information science, arts, humanities, and social sciences (1). The journals analyzed have been indexed by major databases, such as Web of Science, Scopus, or PubMed.

Studies on single journals present various types of bibliometric measures, including the productivity of authors and their affiliations; authors’ sex, profession, and rank; degree of collaboration among authors; affiliations and countries; keyword analysis; keyword co-occurrences; impact factor (IF) (2); thematic analysis (3); “sleeping beauties” (4); and characteristics of editorial boards (5). Some recent examples of single journal studies were performed for *Computers in Human Behavior* (6), *Journal of Advanced Nursing* (3), *Forests* (7), *Remote Sensing* (8), and *Tissue Engineering* (9).

Interestedly, recent single journal studies have extended to include multiple journal studies; namely, nursing journals (10), medical informatics journals (11), and sports science journals (12). The publication of the first issue of *Acta Dermatovenerologica Alpina, Pannonica et Adriatica* (ADAPA) in 1992 was the visionary outcome of one of the leading researchers in the history of dermatology in central Europe, Aleksej Kansky. His vision was to establish a journal that would serve as a forum for research and discussion, sharing the ideas and experience of professionals in the region and beyond. He was also the first editor-in-chief until 2009, when he stepped down and became editor emeritus. He was followed by Jovan Miljković, who is currently serving as acting editor-in-chief, assisted by Mario Poljak, Aleksandar Godić, and Boštjan Luzar as editors. Since 2005, the journal has been indexed in Index Medicus/MEDLINE, EMBASE/Excerpta Medica, and Biomedicina Slovenica, and it started being covered by Emerging Sources Citation Index (ESCI), the Thomson Reuters Web of Science Core Collection index, in 2016 (13).

Despite the fact that ADAPA is a “small journal from a small country,” it is believed that it has significantly improved its quality and international profile since 1992 and fulfilled and possibly exceeded the vision and expectations of its founding father, Aleksej Kansky. ADAPA history and its achievements have been presented and briefly analyzed previously (13–15); however, no full single journal analysis has been performed to date. Hence, in this study we performed an extensive bibliometric analysis of ADAPA publishing history, covered by SCOPUS (Elsevier, Netherlands), focusing on descriptive bibliometric measures, evolution of topics, co-authorship cooperation, and identification of hot topics.
Methods

The search was conducted on July 21st, 2019 in the bibliographical database Scopus, using the search string Acta Dermato-venerologica Alpina, Pannonica et Adriatica in the Source title field, for the period from January 1st, 1994 to July 19th, 2019. To perform the descriptive bibliometric analysis (including distribution of types of documents) and identify the most prolific organizations and countries publishing in ADAPA, we used the Scopus built-in analysis services. For the trends analyses of ADAPA publication characteristics (number of publications, average number of pages, references, authors, organizations, and citations per publication), we exported the publication metadata to Excel (Microsoft, USA) and calculated the averages with the Excel built-in text and statistical functions.

To analyze the historical and thematic context of ADAPA literature production, we induced various scientific landscapes, as well as coauthors and keyword co-occurrence networks, using VOSviewer software V1.6.11 (Leiden University, Netherlands). We analyzed publication titles and abstracts; however, we omitted general/common terms, such as study, significance, method, baseline, and time stamps. To analyze the evolution of terms, we used the author-keyword timeline landscape. We assessed country cooperation by using the country co-authorship network. To identify hot topics, we used the approach recently proposed by Kokol et al. (16).

Results and discussion

General information

From 1994 to 2019, a total of 759 publications were published in ADAPA. Among them there were 557 (73.4%) research articles, 100 (13.2%) review articles, 54 (7.1%) conference papers, 19 (2.5%) letters to the editor, 14 (1.8%) editorials, eight (1.1%) retractions and errata, and seven (1.0%) short reports.

Despite the fact that ADAPA started being published in 1992, Scopus only covers it from 1994 (Volume 3) onward. As shown in Figure 1, the trend in the number of publications has not been steady; it somewhat increased until 2009, and then decreased until 2015, when it reached the lowest annual number of published articles. Following intense efforts to increase the visibility of ADAPA and announcement of free-of-charge publication for all article types in 2017 in order to promote original clinical and scientific research that would not be submitted otherwise because of authors’ financial restrictions, the number of published articles began to rise again, reaching a peak in 2018, with 46 articles per year. However, because of a large increase in the number of case reports and letters to the editor submitted to ADAPA in 2018, the editors decided to reinstate article processing charges for case reports and letters to the editor in January 2018 in order to ensure timely publication of the most influential research. Interestingly, it seems that this measure did not deter authors from submitting to ADAPA because 26 contributions were published in the first half of 2019, indicating that the positive trend will most probably continue.

The dynamics of source normalized impact per paper trend

Scopus started to calculate ADAPA’s source normalized impact per paper (SNIP) in 2002. The SNIP is calculated as the ratio of the journal’s average citation count per publication to the average number of references in reference lists of citing articles, and it may be a more appropriate tool for assessing a journal’s influence than an official IF. This is due to two reasons: i) the SNIP evaluates contextual citation impact, accounting for the citation potential of the respective field, and ii) on some occasions, SNIP values can predate IF or are the only metric to assess journals that have not yet been granted an official IF (as in the case of ADAPA) (17). For ADAPA, the SNIP rapidly increased from less than 0.2 to almost 1.2 in 2010, and then started to decrease to 0.46 in 2018 (Fig. 2). The previous ADAPA citation analyses performed in 2012 and 2015 (18, 19) showed positive dynamics of the predicted IF with the highest values (> 1.0) achieved in 2013. However, SNIP values have been relatively stable since then, suggesting that the future of ADAPA is encouraging. Whereas important modifications in editorial policy were applied only recently, it might still be too early to notice the full impact of these interventions (e.g., discontinuation of publishing fees for original articles and reviews). The editors of ADAPA sincerely hope that Thomson Scientific will soon recognize our efforts and award ADAPA an official IF (15, 19).

Geographical distribution of ADAPA publications

The publications originated from 55 different countries, mostly from Slovenia (n = 210), Italy (n = 88), Turkey (n = 48), Austria (n = 46), and Germany (n = 34). Out of 177 institutions, the Ljubljana University Medical Center, Slovenia (n = 106) was the most productive, followed by the University of Ljubljana, Slovenia (n = 74), University of Trieste, Italy (n = 29), Rutgers New Jersey Medical School, United States (n = 27), Karl Franzens University, Graz, Austria (n = 21), and University of Maribor, Slovenia (n = 16) and Maribor University Medical Center, Slovenia (n = 16). Surprisingly, only six publications had officially declared funding sources. The most prolific among the four funding agencies was the Slovenian Research Agency, with three funded publications.

Our results further confirm the regional importance of ADAPA (15) and show that the journal is an attractive platform for promot-
ing science among Slovenian researchers and clinicians. The visibility of ADAPA is increasing, and authors from various countries are submitting their work to ADAPA. Since 2017, we have noticed an increase in publications originating from the Middle East and India (data not shown), which is probably a direct result of dropping article processing charges because publishing in standard open-access journals (with relatively high open-access fees) may not be financially feasible for researchers with limited funds.

**Bibliometric characteristics of CSN publications**

Figure 3 shows trends in bibliometric characteristics for ADAPA. The average number of authors and institutions per published article is increasing, contrary to the average number of pages per article, which is slightly decreasing. It appears that in recent years collaboration between authors and institutions is becoming increasingly popular and is most likely a result of the “globalization” of science and the surge in available centers providing observerships to researchers from abroad. The average number of citations per article curve is typically bell shaped, with the peak values in the period between 2005 and 2008, reaching around 15 citations per publication.

The number of references varied largely between years, with no clear trend (Fig. 3). Review articles (dotted line in Fig. 3; their number is shown on the secondary axis on the right) tend to include more references than other types of articles and, because ADAPA does not have a fixed number of review articles published per issue, this may be reflected in the observed curve with no clear trends.

**Topic evolution**

The analysis of chronological evolution of topics was based on the timeline landscape of author keywords occurring at least five times (Fig. 4). Using this approach, a total of 44 author keywords emerged on the landscape. As shown in Figure 4, the topic evolution proceeded through four periods. In the first period, from 1994 to 2000 (violet), research focused on AIDS, *Chlamydia trachomatis*, and *Lyme borreliosis* and *erythema migrans* and their diagnosis. Anti-mycotic therapy (including *itraconazole* and *terbinafine*) in children also appeared to be popular topics during this period. The research in the second period, from 2001 to 2005 (blue), focused on skin, syphilis, sexually transmitted diseases, *PCR*, treatment, genetics, and epidemiology. The third period, from 2006 to 2009 (green), focused on HIV, psoriasis, mycosis fungoides, vitiligo alopecia, and *lichen planus*. After 2009, the research focus shifted to HPV, *dermoscopy*, *squamous cell carcinoma*, and *hidradenitis suppurativa*.

Interestingly, with chronological and technological advancement, the repertoire of highlighted topics appears to decrease. This could be due to use of more popular and/or broad-spectrum keywords or a shift in the research community’s attention with respect to dermatological research.

The analysis may be somewhat hindered by the use of interchangeable terms, creating two separate results for the same term. For example, on some occasions the authors list *human papillomavirus* as a keyword whereas others use HPV. Moreover,
keywords with a comparatively low frequency that did not reach the threshold for analysis presentation might still be of clinical or scientific importance.

**Hot topics**

The hot topic analysis revealed five hot topics, including Koebner phenomenon, psychodermatology, psoriasis, adalimumab, and hidradenitis suppurativa. A hot topic is defined as a theme or a term that commonly appears over a period of time. Two variables influence the “hotness” of a topic; namely, how often the term is used in the literature and how many articles contain the relevant term (20). However, one must be aware that hot topics are not permanent: they evolve and are almost inevitably replaced by other, more up-to-date themes. With soaring amounts of available journals and published research articles, it might prove to be impractical and too time-consuming as well as strenuous to recover pertinent data on what topic is currently considered “hot.” Nevertheless, based on our results, it appears that Koebner phenomenon, psychodermatology, psoriasis, adalimumab, and hidradenitis suppurativa are currently considered “hot” and might also be interesting topics for future research.

**Cooperation based on co-authorship**

Cooperation based on co-authorship is shown in Figure 5. The size of the circle represents the number of documents per country, with larger circles representing higher numbers of documents per country, and the color corresponds to the average number of citations per article, with yellow denoting the most-cited articles. Based on 759 publications analyzed, authors from 44 out of 55 countries cooperated through co-authorships, suggesting that international cooperation is common. International cooperation was most frequently observed for authors from Slovenia, who collaborated with authors from 30 other countries, followed by authors from Serbia and Poland (26 countries), the United States (21 countries), and Italy (18 countries). The strongest cooperation emerged between Slovenia and the United States, and between Slovenia and Serbia. Collaboration with other researchers can be a strategic solution for improving research performance because journal publications as well as article citations may constitute key criteria for obtaining research grants, teaching positions, and promotions (21).

The most cited publications were published by authors from the United Kingdom, Bosnia and Herzegovina, and Egypt. Interestingly, it seems that cooperation is not automatically correlated with a greater research impact because some of the articles with the highest citation figures (Fig. 5) did not result from international cooperation, as shown previously (21), although our findings may not be applicable to other medical fields and/or journals.

The most productive country was found to be Slovenia, with publications from Slovenian authors being cited on average six times. Our study showed that, although Slovenia is a relatively small European country, relevant and internationally recognized high-quality articles are produced by Slovenian researchers that regularly publish in ADAPA.
Conclusions

Our study showed that the visionary aim of ADAPA’s founders has not only been achieved, but admirably exceeded. Whereas initially considered to be of mostly regional importance, ADAPA’s influence has gradually broadened and the journal has become truly international. Specifically, the authors that published in ADAPA are affiliated with 55 countries from all inhabited continents, with the exception of Australasia. ADAPA is indexed by all major indexing services, and the number of publications has steadily increased during recent years. Last but not least, in addition to being a forum for presenting influential research, ADAPA also supports international collaboration, resulting in a high proportion of international co-authorships.

References

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa**

**SKRISAN Povzetek Glavnih Značilnosti Zdravlja**

**Najpomembnejši želji iz NEMŠKEGA REGISTRA PSOBEST®**

- **Popolnoma očistiti kožo**
- **Hitro izboljšanje kožnih lezij**

**Možnost očiščenja od glave do pet z VIDNIMI REZULTATI**

- **v 2 tednih**1
- **v dolgotrajno učinkovitostjo v obdobju 4 LET2**

**TALTZ®**

(ikezixumab)

**Hitro1 in TRAJNO2**

**NAJPOMENBNEJŠI ŽELJI ZA ZDRAVLJE**

- **Zdravilo Taltz® je namenjeno za subkutano injiciranje. Mesta injiciranja je mogoča izpustitev**

- **Področja injiciranja je mogoča izpustitev**

- **pod očevim in razmikovanim injiciranjem kod motornega artritispa**

- **zdravilo Taltz® je namenjeno za subkutano injiciranje. Mesta injiciranja je mogoča izpustitev**

- **Področja injiciranja je mogoča izpustitev**

**Kontraindikacije:**

- **Zdravilo Taltz® se ne sme uporabljati skupaj z živimi cepivi.**

**Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:**

- **Terapevtske indikacije:** Zdravilo Taltz® je indikirano za zdravljenje zmerne do hude psoriasis s plaki, ki se primeri za sistemsko zdravljenje. Zdravilo Taltz® je tako pri kombinaciji z metotreksatom indikirano za zdravljenje zmerne psoriasisi artritisa pri obdobju bolnikov, ki so se nedavno odpravili od težave z enim ali večimi acronymičnimi protituberkuloznimi zdravili (DAWAS) ali pa bile težave zdravljenja preobrojene. 

**Osnovna Informatija:** Zdravilo Taltz® je namenjeno za subkutano injiciranje. Mesta injiciranja je mogoča izpustitev pod očevim in razmikovanim injiciranjem kod motornega artritispa, pri obdobju bolnikov, ki so se nedavno odpravili od težave zdravljenja preobrojene. 

**Datum prve odobritve dovoljenja za promet:** 25.4.2016

**Datum zadnje revizije besedila:** 2.12.2019

**Imetnik dovoljenja za promet z zdravilom:** Eli Lilly farmacevtska družba, d.o.o., Dunajska cesta 167, 1000 Ljubljana, telefon 01 / 580 00 10, faks 01 / 569 17 05

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.

**PONUDITE MOČNOST ZA POPOLNOMA ČISTO KOŽO.1**

**TALTZ® – IL-17A-inhibitor z visoko afiniteto in visoko specifičnostjo za zdravljenje psorije s plaki in psoriatičnega artritispa1**

---

1. Zdaj hitro izboljšanje kožnih lezij.
2. Hitro izboljšanje kožnih lezij.
An update on prophylactic human papillomavirus (HPV) vaccines: a review of key literature published between September 2018 and September 2019

Anja Šterbenc¹, Tina Triglav², Mario Poljak³✉

Abstract

Prophylactic human papillomavirus (HPV) vaccines have revolutionarily modified our understanding of and efforts in preventing communicable diseases. Undeniably, all three prophylactic HPV vaccines currently available have excellent safety and have substantially contributed to the control of HPV infections and HPV-related diseases during the past decade in the setting where high vaccine coverage has been achieved. This review summarizes and discusses the most influential peer-reviewed literature on HPV vaccines published between September 2018 and September 2019. The review focuses on the current status of HPV vaccination implementation, results from recent clinical trials, updates to recommendations and guidelines, long-term immunogenicity, and evaluations of various dosing schemes and HPV vaccination of alternative populations. In addition, we briefly summarize studies on the real-life effectiveness of prophylactic HPV vaccines from countries with successful HPV vaccination programs, present an update on safety data, and discuss progress and the development of novel prophylactic HPV vaccines, as well as potential future applications and challenges.

Keywords: human papillomavirus, prophylactic HPV vaccines, literature review

Introduction

Currently licensed and available prophylactic human papillomavirus (HPV) vaccines include a bivalent (2vHPV) vaccine against HPV16/18 (Cervarix; GlaxoSmithKline Biologicals, Belgium), a quadrivalent (4vHPV) vaccine against HPV6/11/16/18 (Gardasil; Merck & Co., USA / Silgard; Sanofi Pasteur MSD, France), and a nonavalent (9vHPV) vaccine against HPV6/11/16/18/31/33/45/52/58 (Gardasil; Merck & Co., USA). All three HPV vaccines have been shown to be highly effective and extremely safe, not only in rigorous evaluations within clinical trials but also in real-world settings and post-licensure monitoring (1, 2).

As described in detail previously (1), before the start of the annual national HPV vaccination campaign in Slovenia, the Section for School, University, and Adolescent Health of the Slovenian Medical Society has been holding a yearly professional 1-day “refresher course” dedicated to promotion of and education on HPV vaccination. This year, we celebrated the 10th anniversary since the introduction of free-of-charge HPV vaccination into the Slovenian immunization program, which prompted the coordinators to hold a special meeting entitled “10 years of HPV vaccination” held on September 3rd, 2019 in Ljubljana, Slovenia, with a focus on the successes of prophylactic HPV vaccines. The majority of the publications presented in this literature review were discussed at this meeting. The purpose of the review was to update and improve the knowledge of healthcare workers responsible for HPV vaccination, including school medicine specialists, pediatricians, gynecologists, epidemiologists, and others. This review summarizes the most important peer-reviewed literature published between September 2018 and September 2019, which corresponds to the 2018/2019 school year in Slovenia.

Methods

For the professional meeting and this literature review, we searched PubMed for the terms “human papillomavirus vaccine” and “HPV vaccine” with a custom date range filter of September 1st, 2018 to September 1st, 2019. The main focus of our manual screening of the 1,011 matches retrieved were publications on the HPV vaccine potential and uptake, changes to the existing recommendations and guidelines, efficacy and immunogenicity clinical trials, studies evaluating various dosing schemes and vaccination of alternative populations, studies contributing to the mounting evidence of the real-life effectiveness of prophylactic HPV vaccines even from countries with modest HPV vaccination coverage rates, updates on HPV vaccine safety data, and progress in the development of novel prophylactic HPV vaccines.

HPV vaccination status at the global level

Since 2006, prophylactic HPV vaccines have been increasingly introduced worldwide. As of early 2019, a total of 115 countries or territories have implemented HPV vaccination into their national programs, including 24 countries or territories that provide gender-neutral HPV vaccination. By the end of 2021, 39 countries or territories are additionally projected to have a national HPV vaccination program, provided that the global vaccine supply is sufficient (3).

Recommendations, guidelines, and scientific advice: an update

On October 5th, 2018 the US Food and Drug Administration (FDA)
announced extension of the indications of the 9vHPV vaccine to include men and women age 27 through 45. The approval was largely based on the results of a study of 3,200 women age 27 to 45 in whom the 9vHPV vaccine proved to be 88% effective in preventing persistent HPV infections, genital warts, preneoplastic lesions of the vulva, vagina, and cervix, and cervical cancer (4). After reviewing evidence on HPV vaccination in adults, the US Advisory Committee on Immunization Practices (ACIP) updated the recommendations for catch-up vaccination and vaccination of adults in June 2019. Whereas routine recommendations for adolescents have not changed since the last published recommendations in 2016, ACIP now recommends catch-up HPV vaccination for all individuals through age 26. For adults 27 to 45 years old, catch-up HPV vaccination is not routinely recommended; ACIP recommends shared clinical decision-making regarding HPV vaccination for those that were not adequately vaccinated (5). In their position paper, the European Society of Gynaecological Oncology and the European Federation for Colposcopy also strongly support gender-neutral vaccination programs for children and young adolescents, together with a catch-up program for young adults and, whenever feasible, HPV vaccination on an individual basis (6).

In April 2019, the European Centre for Disease Prevention and Control (ECDC) opened a public consultation on the new guidance for the introduction of HPV vaccines in EU countries, focusing especially on the 9vHPV vaccine, addition of boys to the girls-only HPV vaccination programs, and HPV vaccination of people living with HIV (PLWH). Since all EU/EEA member states successfully implemented HPV vaccination into their national vaccination programs and several countries also expended their recommendations to include boys, questions regarding the effectiveness and especially cost-effectiveness of this approach have been raised. The key conclusions of the draft were: i) the 4vHPV and 9vHPV vaccines are highly efficacious and immunogenic in both women and men, ii) currently there is no direct evidence of clinical efficacy of HPV vaccines in PLWH, although new evidence is emerging from ongoing studies, and iii) the results of cost-effectiveness analysis depend on the endpoint investigated; if the priority is to prevent cervical disease in women, adding boys to current national girls-only HPV immunization programs becomes increasingly more cost-effective if vaccination coverage rates among females are persistently low and if the costs of HPV vaccine are reduced. Nevertheless, increasing vaccination coverage rates among girls might still be more cost-effective; however, if the HPV vaccination program is primarily used to prevent all HPV-related disease, gender-neutral HPV vaccination could be the most cost-effective strategy (7).

Clinical trials

The prevalence of HPV, cytological abnormalities, and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) were assessed using the results of the baseline phase of the BD Onclarity HPV Trial, which included 14,153 US women 21 to 34 years old undergoing routine cervical cancer screening in order to evaluate the impact of largely opportunistic HPV vaccination in the United States. Interestingly, despite lower HPV vaccine coverage rates and vaccination in patients older than the recommended routine age at first dose, there was a statistically significant decrease in the prevalence of overall HPV infections, HPV vaccine targeted types (HPV16 from 31 to 83%, and HPV18 from 73 to 89%), and even HPV types that are not covered by the HPV vaccine (HPV31 and HPV33/58) among vaccinated women regardless of the age group compared to those that did not receive the HPV vaccine. Moreover, compared to unvaccinated women, vaccinated women were less likely to have a cytology result of low-grade squamous intraepithelial lesions (LSIL) or worse and CIN2+, suggesting that “catch-up” vaccination provides benefits for adolescents and young adults (6).

In order to assess the efficacy of the 9vHPV vaccine against cervical disease and cervical surgeries related to the vaccine types, three international, randomized, double-blind trials were conducted using a historic placebo population for comparison. These studies found a 98.2% (95% confidence interval [CI], 93.6–99.7) and 97.8% (95% CI, 93.4–99.4) reduction in the incidence of high-grade cervical disease and cervical surgery, respectively, related to the HPV types included in the 9vHPV vaccine. Although the 9vHPV vaccine did not prevent disease related to the vaccine HPV types that had been detected prior to vaccination, it considerably reduced the incidence of cervical, vaginal, and vulvar disease associated with other vaccine HPV types, suggesting that early vaccination in HPV-naive individuals is most effective, whereas sexually active individuals may still benefit from catch-up vaccination programs (9).

SPERANZA (SPERimentazione Anti HPV Zona Apuana) is a clinical trial designed to assess the clinical effectiveness of HPV vaccination following surgical treatment of CIN2+ lesions and microinvasive cervical cancer. In women that had undergone conization, the 4vHPV vaccine exhibited 80% clinical effectiveness in disease relapse prevention, with clinical benefits demonstrated up to 4 years post vaccination. Although the 4vHPV vaccine lacked a therapeutic effect on prevalent HPV infection and disease, it may be used as an adjuvant to surgical treatment of cervical disease (10).

Because more than a third of patients with high-grade squamous intraepithelial lesions (HSIL) will experience disease recurrence after initial treatment, a randomized, double-blind, placebo-controlled, proof-of-concept clinical trial has been recently initiated to determine whether the 9vHPV vaccine can reduce the risk of anal and vulvar HSIL recurrence by 50% in previously unvaccinated individuals that recently received treatment for anal or vulvar HSIL. The trial is set to include 345 eligible participants 27 to 69 years old with a history of anal or vulvar HSIL that have not yet received the HPV vaccine. The study is predicted to close in 2022 and, if successful, prophylactic vaccines may become part of the standard treatment of anal and vulvar HSIL (11).

Long-term immunogenicity of HPV vaccines

It has been firmly established in various clinical trials that prophylactic HPV vaccines induce a robust protective immune response; however, real-life data on the long-term stability of vaccine-induced antibodies and the duration of immunity conferred by HPV vaccines are less abundant.

A comparison of long-term antibody responses induced by the 2vHPV and 4vHPV vaccines was evaluated in a cohort of Finnish females that participated in the phase III HPV vaccination trials FUTURE II in 2002 and PATRICIA in 2004, with subsequent serum sample linkage to the Finnish Maternity cohort biobank (12). The study found that, for the majority of vaccinated women, anti-HPV16 and anti-HPV18 antibody levels were higher than the natural infection-related antibody levels and remained stable for
Recent advances in HPV vaccination

up to 12 years post vaccination, confirming long-term protection. Interestingly, whereas the antibody levels induced by the 2vHPV vaccine were high and stable in nearly 100% of women even after 12 years, anti-HPV18 antibody levels declined below the natural infection antibody level in 18% of women that had received the 4vHPV vaccine, suggesting potential differences in immune responses to the two HPV vaccines (12).

To provide data on the duration of immunity induced by different HPV vaccination schedules and the role of a booster dose, a follow-up nonrandomized clinical trial was performed on Mexican girls and women vaccinated with either the 2vHPV or 4vHPV vaccine (13). The geometric mean titers (GMTs) for HPV16 and HPV18 at 5 years post vaccination were shown to be above levels induced by natural infection, confirming non-inferiority of a two-dose schedule. In addition, due to the establishment of robust immune memory, a long-lasting humoral response is induced, suggesting that boosters are unnecessary and unlikely to be profitable (13).

There is evidence that the 2vHPV vaccine induces cross-reactive immunogenicity against HPV31 and HPV45; however, little is known on the durability of this phenomenon. Based on recent data from a subset of subjects previously enrolled in phase I/II and phase IIIb clinical trials of the 2vHPV vaccine, it appears that cross-reactivity is long-lasting with comparable GMTs in girls 9 to 14 years old that had received two doses and women 15 to 25 years old that had received three doses for up to 5 years after the first vaccination. Moreover, specific CD4+ T-cell responses against HPV31 and HPV45 were similar across groups at month 36, with the observed presence of specific memory B cells additionally confirming establishment of cross-protection (14).

More data are becoming available regarding the efficacy of HPV vaccines in HPV-related oropharyngeal cancers. A study evaluating antibody responses in serum samples and oral gargles collected from 150 US men 27 to 45 years old that had received three doses of the 4vHPV vaccine has shown that all participants developed detectable anti-HPV16 and anti-HPV18 antibodies in serum at month 7 with somewhat lower but still high positivity rates in oral gargle samples (93.2% for HPV16 and 72.1% for HPV18). However, the proportion of men with detectable oral antibodies decreased significantly at 18 and 30 months after vaccination (39.8% and 29.6% for anti-HPV16 antibodies, and 10.7% and 4.6% for anti-HPV18 antibodies, respectively). Nevertheless, when detectable, oral gargle anti-HPV antibody levels remained strongly correlated with anti-HPV serum antibody levels (15).

**Dosing schemes**

In 2014, the World Health Organization (WHO) changed the original three-dose HPV vaccine schedule, recommending a two-dose schedule for those starting HPV vaccination at age 14 or younger and a three-dose schedule for individuals that start vaccination at age 15 or older and those that are immunocompromised; this was mostly based on observational studies and immunogenicity trials (16). It was already previously shown that the number of vaccine doses received and the timing between them is crucial for 4vHPV vaccine effectiveness against anogenital warts (17, 18), whereas data on effectiveness against CIN2+ with respect to the age at 4vHPV vaccination and number of doses were lacking. A recent nationwide population-based study on vaccinated and unvaccinated Danish and Swedish women found a reduced risk for CIN2+ for those that had been vaccinated with three doses of 4vHPV vaccine before age 20, whereas women that had had their second dose at least 5 months after the first dose did not have an increased risk of CIN2+ after two doses compared to those receiving three doses (19).

The updated evidence from an Indian follow-up cohort study on the efficacy of less than three doses of the vaccine clearly shows that the protection conferred by a two-dose regimen of 4vHPV vaccine against incident and persistent HPV infection in girls 15 to 18 years old is comparable to that of the three-dose regimen in girls 15 to 18 years old, providing immune-bridging data to allow extension of the target age for HPV vaccination of girls up to 18 years with two doses (20).

Although all three prophylactic HPV vaccines have been in use for several years now, little is known about the use of different HPV vaccines within the same individual. Hence, a randomized clinical trial was conducted to evaluate immune response when administering two different HPV vaccines in varying order. Post first dose of 9vHPV and 2vHPV vaccines, 99.4 to 100% and 100% of vaccinated girls and boys were seropositive to the vaccinated HPV types, respectively, whereas all individuals were seronegative to the HPV types included in the 9vHPV vaccine, regardless of the schedule used. These data show that flexible vaccination schedules can be safely used in the case of a vaccine shortage or change in national immunization program recommendations (21).

Current US guidelines recommend HPV vaccination for girls 11 to 12 years old with a catch-up vaccination for girls/women 13 to 26 years old; however, little is known about the population effectiveness of the existing catch-up vaccination recommendations. Hence, a nested case-control study on 4,357 CIN2+ cases and 21,773 matched controls was conducted, which confirmed that catch-up vaccination confers significant protection against incident CIN2+ and CIN3+ in women receiving at least three doses of the vaccine if they are 14 to 20 years old at the time of the first dose. Conversely, this was not true for women ≥ 21 years old at the first dose. Whereas these findings support current catch-up vaccination guidelines for those that start the series up to age 20, additional research is needed to evaluate the effectiveness of such an approach in women 21 to 26 years old, especially in the light of recent calls to extend HPV vaccination to older women (22).

Results from the Costa Rica Vaccine Trial (23) and trials performed in India (24) implied that even a single dose of the 2vHPV or 4vHPV vaccine can induce sufficient long-lasting immunogenicity and effectiveness against incident HPV infection. Recent large-scale real-world data from Australia have confirmed this hypothesis: compared to unvaccinated women, the adjusted hazard ratios were significantly lower for vaccinated women, regardless of the number of doses received (0.65, 95% CI 0.52–0.81; 0.61, 95% CI 0.52–0.72, and 0.59, 95% CI 0.54–0.65 for one, two, and three doses, respectively) (25). Similar results were also obtained in Denmark, where a one-dose HPV vaccination at age ≤ 16 seemed to ensure similar protection as three doses (26). Because mounting evidence suggests the comparable effectiveness of a single dose to a two/three-dose HPV vaccination schedule in preventing high-grade cervical disease, one-dose HPV vaccination may be a viable strategy in the global elimination of cervical cancer, which would greatly reduce costs and requirements for national HPV vaccination programs (25). The hypothetical future reduction in the number of required HPV vaccine doses to a single dose was shown to be widely supported by immunization program stakeholders in low- and middle-income countries, provided that sufficient evidence on the immunological and clinical efficacy of such
a strategy is obtained and a formal recommendation by the WHO is announced (27).

HPV vaccination in immunocompromised individuals

More data are becoming available on the long-term immunogenicity and safety of HPV vaccines in immunosuppressed populations, who bear the highest burden of HPV-related diseases. In a prospective observational cohort study of 4vHPV-vaccinated youth from the multicenter Pediatric HIV/AIDS Cohort Study Adolescent Master Protocol, HPV antibody titers were compared between perinatally HIV-infected (PHIV) and perinatally HIV-exposed but uninfected (PHEU) youth. Interestingly, in PHIV, antibody titers were shown to be lower for all serotypes compared to PHEU. Higher GMTs were associated with lower HIV RNA viral load and higher CD4 cell count, suggesting that HPV vaccines may be less effective in PHIV children if given at a time of immunosuppression, asserting the need for HPV revaccination when immunocompetence is restored (28). Similar data were acquired from a cohort of perinatally HIV+ infected sexually naive Kenyan boys and girls 9 to 14 years old. The study found that 7 months after receiving three doses of the 4vHPV vaccine, seroconversion to HPV6, HPV11, HPV16, and HPV18 occurred in 99.6%, 97.2%, 98.3%, and 93.3% of the children, respectively. The vaccine was highly immunogenic with very few adverse events (AE) reported. Although HIV+ infected children mounted a robust immune response that was comparable to other vaccinated populations regardless of their immune status, children with higher CD4 cell counts produced higher antibody titers than those with lower CD4 cell counts (29). The immunogenicity of the 4vHPV vaccine was also evaluated in a population of immunocompromised Australian children 5 to 18 years old with different underlying conditions. Following administration of three doses of the 4vHPV vaccine, 86.5%, 89.2%, 89.2%, and 91.9% of children were seropositive at 60 months for HPV6, HPV11, HPV16 and HPV18, respectively, suggesting that a three-dose schedule using the 4vHPV vaccine remains immunogenic in this population for at least 5 years post vaccination (30).

It has been unclear whether HPV vaccines in HIV-infected adults are capable of establishing long-term immunity. Ellsworth et al. provide crucial data from a cohort of HIV-infected 4vHPV-vaccinated adult males from the US, clearly showing that antibody titers peak 4 weeks after a third dose, followed by a gradual decrease in antibody titer levels. However, a delayed administration of the fourth dose of the 4vHPV vaccine induces a rapid increase in antibody titers, suggesting that standard three-dose series are capable of inducing immune memory and conferring long-term protection in HIV-infected men (31).

Extension of indications for HPV vaccination: what is cost-effective?

In light of the recent extension of vaccine indications, a literature review investigated the cost-effectiveness of vaccinating alternative populations. Whereas studies on men who have sex with men (MSM) consistently reported cost-effectiveness, HPV vaccination of heterosexual men would only be cost-effective if 4vHPV vaccine prices were reduced. In contrast, targeted vaccination of women over 26 is unlikely to be cost-effective (32). Authors have suggested that following the establishment of solid girls-only vaccination programs, targeted HPV vaccination of MSM should be the next priority in HPV prevention. However, these economic evaluations have important limitations when it comes to African countries. Given the disproportionate burden of HPV-related cancers in Africa and the time it could take to achieve herd immunity through girls-only HPV vaccination programs, there has also been a call for the introduction of gender-neutral HPV vaccination for national immunization programs in Africa (33).

Real-life efficacy of HPV vaccines

A decade has passed since the implementation of HPV vaccination, and post-licensing data from real-life settings are becoming available for increasing number of countries and populations. To date, more than 100 studies have evaluated the effectiveness and impact of HPV vaccines on preventing HPV infection and related disease (3). The largest systematic review and meta-analysis, which includes data from 60 million individuals and up to 8 years of follow-up after vaccination, summarizes the evidence and demonstrates the remarkable population-level impact of HPV vaccination on HPV infections, anogenital warts, and development of CIN2+ in women in real-world settings (34). The prevalence of HPV16 and HPV18 was found to decrease by 83% in girls 13 to 19 years old (RR 0.17, 95% CI 0.11–0.25) to 6 years after vaccination, and by 66% (RR 0.34, 95% CI 0.23–0.49) in young women 20 to 24 years old. Substantial reductions in the incidence of anogenital warts were also observed: a 67% reduction (RR 0.33, 95% CI 0.24–0.46) in girls, a 54% reduction in young women (RR 0.46, 95% CI 0.36–0.60), a 48% reduction in boys (RR 0.2, 95% CI 0.37–0.75), and a 32% reduction (RR 0.68, 95% CI 0.47–0.98) in young men (34). Within less than a decade since HPV vaccination implementation, we are already noticing a 51% decrease in CIN2+ cases (RR 0.49, 95% CI 0.42–0.58) in girls and a 31% decrease in young women (RR 0.69, 95% CI 0.57–0.84). The results of this meta-analysis provide substantial evidence of the impact of HPV vaccination and provide data on the real-world benefits of HPV vaccines in the prevention of cervical cancer with significant declines in high-risk (hr) HPV infection and CIN2+ (34). Although most studies on the real-world effect of HPV vaccination published in recent years have already been covered by the review by Drolet et al. (34), we additionally present some of the most intriguing data from individual countries.

Decreasing incidence and prevalence of HPV types

Australia was among the first countries to adopt primary HPV-based cervical screening, which for the first time made possible precise monitoring of HPV prevalence at a population level—an approach to HPV monitoring that used to be beyond the reach and budget of research studies (35). Data after the first 7 months of implementation showed 9.25% (95% CI: 9.09–9.42%) prevalence of hrHPV among 116,052 primary screening samples, of which 2.14% (95% CI: 2.05–2.22%) were HPV16 or HPV18-positive. A peak in HPV prevalence was observed at 25 to 29 years of age, but this was due to non-vaccine HPV types. Across all ages, the prevalence of HPV16 or HPV18 remained low (35).

A decade since HPV vaccination introduction into their program, significant changes in cervical HPV prevalence have also been observed in Sweden. Although the HPV prevalence in women visiting a youth clinic in Stockholm, Sweden, in 2017–2018 was high (72.1% for all HPV types and 65.1% for hrHPV), the prevalence of 27 hrHPV was lower in vaccinated women (60.1 vs. 86.73%, p
Recent advances in HPV vaccination

Recent advances in HPV vaccination

Decreasing incidence and prevalence of anogenital warts

Because anogenital warts usually develop 2 to 3 months after infection, the impact of HPV vaccines on the incidence of anogenital warts provides early evidence of vaccination efficacy. In a recent systematic review from the United States, the authors assessed the real-life impact of HPV vaccines on anogenital warts. Consistent declines in diagnoses of anogenital warts were observed in females 25 and younger following vaccination implementation in 2006 in the United States. In males, the same trend was observed—albeit to a lesser degree, most likely due to herd immunity—until 2011, when male vaccination began, with declines becoming even more evident. In contrast, no trends were observed among people over 25 (40). These data differ somewhat from the results obtained by Mann et al. (41), who analyzed data from sexually transmitted disease clinics in the United States. Declining temporal trends in the prevalence of anogenital warts from 2010 to 2016 were observed not only in women under 40, but also in men who have sex with women only and are younger than 40, and MSM in all age categories (41). Interestingly, a similar declining trend was also observed in older age groups, which is unlikely to be a result of HPV vaccination. Similar data on declining trends in the prevalence of anogenital warts among patients attending sexual health clinics have been reported from England, where the 2vHPV vaccine was introduced in 2008, followed by the introduction of the 4vHPV vaccine in 2012. An 82.3% decline was observed among females 15 to 17 years old and a 67.7% decline in men of the same age between 2014 and 2017 (42).

Decreasing rates of precancerous lesions

Currently, the most proximal outcome to cervical cancer is precancerous lesions (CIN2+), which typically develop a few years after primary HPV infection. More real-life data are becoming available on the impact of HPV vaccination on the development of CIN2+, confirming early findings of randomized controlled trials showing the efficacy of HPV vaccines for preventing cervical dysplasia (43, 44). One of the most dramatic reductions in preneoplastic cervical lesions was observed in Scotland, which has had consistently high (above 85%) 2vHPV vaccine coverage rates among routinely immunized cohorts during the last 10 years. This led to an 89% reduction (95% CI, 81–94%; from 0.59% [95% CI, 0.48–0.71%] to 0.06% [95% CI, 0.04–0.11%]) in prevalent CIN3+ and an 88% reduction (95% CI, 83–92%; from 0.69% [95% CI, 0.58–0.65%] to 0.15% [95% CI, 0.10–0.21%]) in CIN2+ among vaccinated women born in 1995 and 1996, compared to unvaccinated women born in 1988. Moreover, there was evidence of clinically relevant herd protection in unvaccinated women (45).

In New Zealand, the 4vHPV vaccine has been available since 2008. The proportion of newly diagnosed CIN2 attributable to HPV16/18 infection was observed over time, using partial genotyping of samples from women 17 to 24 years old, with biopsynoned CIN2 enrolled in a prospective CIN2 observational management trial (PRINcess). From 2013 to 2016, the proportion of women whose liquid-based cytology samples were HPV16- or HPV18-positive decreased from 43% to 13%. Although vaccinated women had low rates of HPV16- and HPV18-positive CIN2 lesions in the time period observed, a substantial decrease from 66% to 17% in HPV16/18 positivity was documented in unvaccinated women, likely due to a herd effect (46).

In the US, the 4vHPV vaccine was most commonly administered from 2006 to 2015, followed by the 9vHPV vaccine, which is the only available HPV vaccine in the country since 2017. Using archived specimens from women 18 to 39 years old diagnosed with CIN2+ and data from a population-based surveillance system (the HPV Vaccine Impact Monitoring Project), the proportion of CIN2+ by HPV type was assessed over time. The proportion of HPV16- or HPV18-positive CIN2+ declined from 52.7% in 2008 to 44.1% in 2014 (47). Substantial declines in the proportion of HPV16- and HPV18-positive CIN2+ were recorded among women that were vaccinated (55.2–33.3%). Furthermore, a decline was also observed in unvaccinated women (51.0–47.3%), again suggesting the establishment of herd protection against CIN2+ (47).

No evidence of HPV type replacement following HPV vaccination

Despite initial fears that HPV type replacement would occur following HPV vaccination implementation, similar to what was observed with the pneumococcal vaccine, there is a lack of evidence of such a phenomenon with respect to HPV (1). Moreover, an 11-year follow-up on US women showed a 45.8% decrease in the prevalence of HPV types genetically related to HPV16, demonstrating evidence of cross-protection following 4vHPV vaccination, whereas no statistically significant changes in the prevalence of HPV types that are genetically unrelated to 4vHPV vaccine types was observed, suggesting that HPV type replacement has not occurred (48). However, as shown by Gray et al. (49), some HPV types may become more common in groups exhibiting sexual risk-taking beh-
havior, suggesting that continued post-vaccination surveillance (especially for HPV51 and HPV52) may be warranted.

**HPV vaccine safety data: an update**

Despite being labeled extremely safe by the WHO, HPV vaccines have been falsely and unfairly associated with numerous AEs and dubious syndromes. Heightened reporting by the media has significantly impacted already established immunization programs in various countries, including but not limited to Denmark, Japan, and Colombia. A retrospective observational study analyzing clusters of AE reports in Denmark identified four clusters, with fatigue, dizziness, and headache as the most common symptoms reported. These symptoms were mainly non-specific and tended to occur commonly in the targeted population. Moreover, there was no evidence that these clusters represented chronic fatigue syndrome (CFS) or any novel autonomic dysfunction syndrome.

Nevertheless, an anomalous spike in submissions of AE reports (mostly cluster 2) seemed to result from stimulated reporting on symptoms of CFS and postural orthostatic tachycardia syndrome (POTS), further highlighting the influence of media reporting on HPV vaccination coverage. Worryingly, an overview of reports of long-lasting fatigue following 2vHPV vaccination published in 2013 and updated in 2015 that was picked up by the Dutch media inevitably led to further reports on this syndrome. Hence, a retrospective cohort study on 69,429 12- to 16-year-old Dutch girls was conducted to investigate these allegations. The study found that long-lasting fatigue is common among adolescent girls, whereas CFS is rarely diagnosed with no statistically significant increase in incidence rates during the post-vaccination period compared to the pre-vaccination period.

In response to lingering public concerns that HPV vaccine causes autoimmune diseases, a retrospective cohort study evaluating the association between HPV vaccination and diabetes mellitus type 1 on 911,648 individuals was conducted. No increased risk of diabetes mellitus type 1 correlated with HPV vaccination (hazard ratio 1.21, 95% CI 0.94–1.57) was found over the 10 years of the study period (52). Furthermore, a recent systematic review and meta-analysis on more than 169,000 autoimmune disorder events further confirmed that HPV vaccination is not associated with an increased risk for subsequent autoimmune diseases (odds ratio [OR] 1.00, 95% CI 0.95–1.06), particularly among individuals with preexisting autoimmune conditions (OR 0.82, 95% CI 0.70–0.96) (53).

Similar to previously published data, an analysis of reports to the US Vaccine Adverse Event Reporting System (VAERS) of AE following 2vHPV vaccination between 2009 and 2017 found no new or unexpected safety concerns with the use of the 2vHPV vaccine (54).

Because there are no vaccine efficacy or safety data to clearly show that HPV vaccines work in a Japanese population, results from several trials on Japanese men and women were recently published to bridge the findings from international trials on immunogenicity, safety, and efficacy (55–57). Thus, the 4vHPV vaccine was found to be immunogenic, safe, and well tolerated in Japanese boys and men (57), whereas the 2vHPV vaccine was shown to be highly effective against targeted HPV types and conferred cross-protection against HPV31, HPV45, and HPV52 up to 6 years post vaccination (55). Hopefully, these data will finally convince Japanese politicians to reinstate proactive recommendations for HPV vaccination, which could to some degree revert the catastrophic decline in HPV vaccination rates following suspension of these recommendations in 2013 (55).

**No change in sexual behavior following HPV vaccination**

Although some groups expressed concern that HPV vaccination will result in an increase in sexual risk-taking behaviors among adolescents, there is no evidence that HPV vaccination leads to increased promiscuity or unsafe sexual practices. This has been additionally confirmed on a large scale evaluating population-level changes in sexual behaviors prior to and after implementation of school-based HPV vaccination program in British Columbia, Canada. Data on 298,265 heterosexual girls showed that sexual risk behaviors reported by adolescent girls following implementation of HPV vaccination either stayed the same (e.g., number of sexual partners reported) or declined. Thus, significant declines in the proportion of girls reporting ever having sexual intercourse (OR 0.79), having sexual intercourse before age 14 (OR 0.76), and reported substance use before intercourse (OR 0.69) were found in the period from 2003 to 2013 (58). Similar trends were also shown for college-age men and women from the US (59) and Danish pupils (60). Nevertheless, HPV-vaccinated adolescents from Denmark were less likely to use a condom despite their higher sexually transmitted infections (STI) awareness; however, this trend is probably unrelated to HPV vaccination itself (60).

**Novel prophylactic HPV vaccines and innovative technologies for HPV vaccine production**

Despite being highly effective in preventing HPV infections, the 2vHPV and 4vHPV vaccines confer protection against a limited array of HPV types and are associated with high production and delivery costs. To address these issues, research is focusing on finding alternative and/or innovative methods to produce and deliver novel HPV vaccines. Whereas the 9vHPV vaccine may be considered the first of a new HPV vaccine generation, several other second-generation HPV vaccines are currently undergoing preclinical and clinical evaluations.

Instead of using eukaryotic systems, which are expensive to manufacture, HPV L1 virus-like particles (VLPs) can be produced at much lower costs using bacteria. Two Escherichia coli–based prophylactic HPV vaccines against HPV16/18 and HPV6/11 are currently in phase III and phase I clinical trials, respectively (61). To decrease the production costs, expression systems of the methylotrophic yeasts Pichia pastoris and Hansenula polymorpha are also being used to produce HPV6, HPV11, HPV16, and HPV18 VLPs. These types of vaccines are undergoing phase I trials (61). Other advanced systems not presented in detail due to the scope of this review include transgenic plants and attenuated bacteria (e.g., mutant Salmonella enterica or Shigella) that are capable of producing L1 VLPs, and recombinant viral vaccines (e.g., recombinant baculovirus) containing the HPV16 L1 gene (62).

Due to the fact that the L2 protein induces broad cross-neutralizing antibodies, it was thought to be the ideal candidate for generating a so-called pan-HPV vaccine. However, the suboptimal immunogenicity and limited neutralizing spectrum of L2 seemed to be the major caveat in developing efficient prophylactic L2-based HPV vaccines. This might potentially change because a recent study showed that rationally designed flagellin-L2 fusion peptides (Fla-5PcL2) were capable of inducing robust broad-
Recent advances in HPV vaccination

The pentameric subunits or capsomeres of the L1 protein are especially attractive for development of affordable second-generation HPV vaccines. In contrast to VLPs that are composed of 360 copies of the L1, the capsomeres only contain five L1 monomers, expressing all essential neutralizing epitopes capable of inducing immune responses. They are easily produced in E. coli and, because capsomeres can be lyophilized and consequently shipped as well as stored without refrigeration (61, 62), this could significantly improve the availability of HPV vaccine in developing countries. Chinese researchers recently evaluated a novel HPV16 L1 pentamer-loaded hybrid particles vaccine system, showing the importance of particle size when developing novel HPV vaccines, with HPV L1 capsomer particle size of 1 µm generating the strongest immune reactions in BALB/c mice (65).

In addition to its cost, the requirement for continuous refrigeration (between 2 and 8 °C) sets another barrier that hinders widespread availability of the HPV vaccine, especially in remote regions of the world. Data on immunogenicity and efficacy of spray-dried 9vHPV vaccines are encouraging, showing considerable protection of such thermostable vaccine against HPV infection in mice following storage at temperatures up to 40 °C for 3 months. Moreover, it even seems that the 9vHPV vaccine, as currently marketed, could be stored and transported at temperatures above 8 °C without losing efficacy, which would be especially convenient in low and middle-income countries (64).

Whereas vaccines are mostly injected intramuscularly, intradermal administration can be an alternative route of vaccine delivery with several advantages: for example, the antigens can be applied at a lower dosage and may be capable of inducing an immune response in intramuscularly vaccinated non-responders. Results from the first evaluation of a high-density microprojection vaccine patch (Nanopatch™) coated with unadjuvanted HPV vaccine showed that this type of HPV vaccine remained stable during cold shipping, was delivered effectively, and was capable of generating an immune response in rhesus macaques (66).

The future of HPV vaccination

The impact of HPV vaccines has by far exceeded our initial expectations; however, their potential is still largely unexploited. Unfortunately, despite having access to HPV vaccination, many women are still being diagnosed with high-grade cervical lesions, among them also vaccine-eligible women that were not vaccinated. Surprisingly, the three most common self-reported reasons for not receiving the vaccine among vaccine-eligible women diagnosed with CIN2+ from the US were age (e.g., being too old to receive the vaccine; 31.3%), lack of provider recommendation for vaccination (19.9%), and previous diagnosis of HPV infection (17.5%). These results suggest the need to dispel myths about HPV vaccine eligibility—older age and previous diagnosis of HPV infection are not contraindications for HPV vaccination—and to highlight the importance of providers in promotion of vaccination for all eligible patients (67).

An example of an innovative intervention toward increased HPV vaccination coverage is the FASTER strategy, which offers HPV vaccination to all women between 25 and 45 years old attending cervical cancer screening (68). Encouraging data are already available for Mexico, where almost all eligible women (3.282/3.414; 93%) accepted HPV vaccination as part of a combined screening and HPV vaccination program (69).

The idea of combinatorial vaccines that would simultaneously protect against HPV and other diseases also became an attractive approach toward improving vaccination coverage rates. Recently, a proof-of-concept combinatorial vaccine using a VLP displaying an HPV R9 epitope and VAR2CSA placental malaria antigen proved to be able to reduce HPV infection while inducing anti-VAR2CSA IgG antibodies in mice. Such a vaccine with dual specificity would be especially convenient in Sub-Saharan Africa, where the target population for vaccination against both HPV and placental malaria (e.g., adolescent girls) basically overlaps (70). To achieve elimination of rubella while potentially increasing HPV vaccine uptake in developing countries, a combinatorial HPV16/18 and rubella vaccine was designed. Interestingly, immunized mice developed specific antibodies against both HPV and rubella that were even higher than in mice immunized with only rubella or HPV vaccine (71). Moreover, results from a randomized controlled trial showed feasibility of concomitantly administering the 4vHPV vaccine and tetanus, diphtheria, pertussis (Tdap) vaccine with quadrivalent meningococcal CRM197-conjugate vaccine (MenACWY-CRM) in adolescents. Such concomitant administration of different vaccines could maximize the opportunity for improving community protection and compliance with scheduled vaccination (72).

A large study of 18,247 formalin-fixed paraffin-embedded specimens from 50 countries highlighted the potential impact of the 9vHPV vaccine in preventing HPV-related disease, suggesting that the addition of the five high-risk HPV types (HPV31, HPV33, HPV45, HPV52, HPV58) to HPV6, HPV11, HPV16, and HPV18 in the 9vHPV vaccine could potentially prevent approximately 90% of cervical cancer cases and globally reduce 50% of all HPV-related cancer cases (73). Moreover, a meta-analysis by Drolet et al. (34) that evaluated data from 65 studies on more than 60 million HPV-vaccinated individuals with up to an 8-year follow-up provided the most up-to-date information on HPV vaccine impact at a population level. Most importantly, Drolet et al. have shown that, compared to countries with single-cohort HPV vaccination and/or low coverage rates, countries that adopted multiple age cohorts for HPV vaccination schedules tend to observe faster and higher-impact results, including benefits due to herd effect (34).

The success of HPV vaccines has drastically reframed our initial goals, moving from cervical cancer prevention to cervical cancer elimination. However, multi-level interventions based on a combination of HPV vaccination, screening, and treatment of cervical disease will have to be tightly coordinated and supported by national and/or regional healthcare officials and authorities in order to approach the cervical cancer elimination threshold. Australia, a pioneer in introducing a successful national HPV vaccination program with high and consistent coverage rates across both sexes, will mostly likely be one of the first countries to reach elimination thresholds; the estimates predict that the age-standardized annual incidence of cervical cancer will drop below six new cases per 100,000 women by 2020, and fewer than four new cases per 100,000 women by 2028. With a combination of regular cervical cancer screening, treatment of preneoplastic lesions, and high HPV vaccination coverage rates, cervical cancer will probably be eliminated as a public health problem in Australia within the next 20 years (74). A modeling study performed by Simms et
al. (75) further elucidates the requirements for elimination of cervical cancer. In the absence of further interventions, an estimated 44.4 million cervical cancer cases would be diagnosed at the global level over the period from 2020 to 2069, with almost two-thirds of cases occurring in low- and middle-income countries. However, if widespread high-coverage HPV vaccination in combination with regular cervical cancer screening is adopted from 2020 onward, we could also achieve elimination thresholds of fewer than four cases of cervical cancer per 100,000 women per year in low-income countries by the end of the century, and this effect would be achieved a few decades earlier in high-income countries (by 2065–2069) and in middle-income countries (by 2070–2079) (75).

Conclusions

Additional evidence contributes to the already overwhelming data on HPV vaccine safety, durability of immune protection, and real-life effectiveness. Unfortunately, HPV vaccines will inevitably experience obstacles despite their substantial success, with the most threatening issue currently being the HPV vaccine shortage. Potential barriers that prevent worldwide implementation of gender-neutral HPV vaccination should be addressed and acted upon immediately in order to exploit the incredible potential of prophylactic HPV vaccines in time.
Recent advances in HPV vaccination


_Spremenite zaščito v preventivo._
Non-ablative radio frequency for the treatment of androgenetic alopecia

Yimei Tan1, Liu Wei2, Yiyi Zhang1, Andy Goren3,4, John McCoy3, Andrija Stanimirovic5, Torello Lotti4, Maja Kovacevic6

Abstract

Introduction: Medical treatment of androgenetic alopecia (AGA) is mainly limited to pharmacological and surgical interventions. Patients’ desire for noninvasive and non-systemic treatments has accelerated research into medical devices that can promote hair growth. Low-level laser therapy (LLLT) was the first such device. However, its success has been limited by contradictory and often controversial efficacy claims. Work previously performed in animal models of AGA has demonstrated the viability of the wound repair mechanism as a potential treatment modality. This study therefore explores the use of a non-ablative radio frequency (RF) device in the treatment of AGA.

Methods: A single blinded study compared a non-ablative RF device versus a sham device in 24 men with AGA. Each subject received four treatments over the 12-week study.

Results: In this preliminary study of 24 AGA patients treated with a novel RF device, we demonstrated that 54% showed a clinical response. Furthermore, among patients that underwent four or more treatment sessions, 40% experienced a 30% or more increase in hair counts compared to baseline.

Conclusions: If validated in a larger cohort, non-ablative RF may prove to be an important clinical tool in the treatment of AGA.

Keywords: radio frequency, androgenetic alopecia, treatment

Introduction

Androgenetic alopecia (AGA) affects up to 50% of the population by age 60 (1). To date, the US Food and Drug Administration (FDA) has only approved two drugs for the treatment of AGA: topical minoxidil and oral finasteride. In addition to pharmacological interventions and surgical hair restoration, low-level laser therapy (LLLT) devices for the treatment of AGA have been introduced to the market. The efficacy of these devices has been subject to controversy due to a lack of quality evidence (2), and so there is a large unmet medical need for a non-invasive treatment for AGA.

Several researchers have independently shown the role of wound healing in hair regrowth. Chuong reported that wound healing induces new hair growth in mice (3). Ansell et al. reported acceleration of wound healing during the hair anagen phase (4). Sun et al. reported a case of new hair growth on a bald scalp following wound repair (5). In addition, Dhurat et al. demonstrated that the use of microneedling in combination with minoxidil was superior to minoxidil monotherapy for the treatment of AGA (6). Although it is not clear whether the microneedling enhanced minoxidil permeability or sulfotransferase enzyme induction, it nevertheless serves as additional human evidence for hair follicle regeneration through injury (6).

Non-ablative RF is a common mode of treatment for skin wrinkles and laxity (7). The treatment induces dermal injury by heat generated under the skin (8). We hypothesized that using a specially adapted non-ablative RF device to deliver treatment to the scalp will be an effective treatment for AGA.

Methods

Twenty-four otherwise healthy male subjects were recruited from a dermatology clinic. Subjects were diagnosed at baseline with Norwood stage 3 or above. The average age of the subjects was 29.5 years. The study was approved by the hospital ethics committee (Hairmore Hospital Group, Beijing, China) and all subjects provided informed consent prior to beginning the trial. A specially adapted fractional non-ablative RF device (Innogen, Tel Aviv, Israel) was used for application of the treatment. The device generates a RF of 460 KHz with a power output of 12 W and 62 mJ/pin.

The study design was a single-blinded placebo-controlled study. Five subjects were assigned to the placebo (sham device) group and 19 to the active group. The placebo group received the same treatment with the RF device except that the device was powered off. Subjects received an average of four treatment sessions administered every 3 weeks. Each session lasted for approximately 5 minutes at high power settings. The placebo group was intentionally small due to the small size of the study and the known placebo response of hair growth to placebo drug trials; that is, less than a 13.7% increase in hair counts for the topical minoxidil vehicle (9). An independent two-sided samples t-test (MedCalc v18.2.1) was used to determine the significance of average increase in hair counts from baseline in the treatment group versus the placebo group. At baseline, a 1 cm² target area at the vertex of the scalp was shaved on each subject. A tattoo was administered at the center of the target area. A photo-trichogram of the target area was taken (FotoFinder, Germany) and at the end of the study, a photo-trichogram of the target area was repeated (FotoFinder, Germany). Hair counts were performed manually by a medical resident.

Results

Subjects completed the study with an average of 3.63 sessions. The average increase in hair counts from baseline in the treatment group was higher than the placebo group 22.8% vs 9.6% (p = 0.08).
The results are tabulated in Table 1 and shown in Fig. 1. Although the significance did not reach the $p = 0.05$ level, we believe the effect is of clinical importance because only one of the five subjects in the placebo group had a clinical response; that is, an increase in hair counts greater than 13.7% compared to baseline (9). None of the subjects reported treatment-related adverse events; however, the smell from tissue damage during therapy was of concern to most (circa 75%) of the subjects in the treatment arm.

Discussion

Previous studies on AGA preformed using animal models demonstrated the viability of the wound repair mechanism as a potential treatment modality for AGA (3–5). Moreover, a pilot study on 100 cases of mild to moderate AGA comparing the effectiveness of 5% minoxidil lotion only and 5% minoxidil lotion combined with microneedling treatment demonstrated that microneedling with minoxidil was superior to minoxidil monotherapy for the treatment of AGA (6). Non-ablative RF can similarly induce dermal injury by heat generated under the skin (8). To that end, we explored the use of a non-ablative RF device to induce scalp tissue wound healing mechanisms for hair regrowth. Non-ablative RF is appropriate for all skin types and hair colors, and we are not aware of any potential contradictions to the procedure. In our study, we observed significant hair regrowth in the treatment arm compared to the placebo arm.

We chose a similar protocol used for skin rejuvenation, four sessions in total performed 3 weeks apart. One limitation of this study was that not all the patients in the treatment arm received the minimum recommended treatments; for example, Subject 3 only received two treatments total. Nevertheless, we observed a striking difference between the treatment arm and the sham device. In addition, all the patients in the placebo arm received the recommended minimum number of treatments. Although the sample size of the study was small, it demonstrates that non-ablative RF could potentially provide a non-invasive method to stimulate hair regrowth as an additional treatment modality for alopecia. A larger study is needed to confirm these results.

Table 1 | Percent change in target area hair counts (TAHC) at baseline versus after treatment with non-ablative radio frequency device. Data are tabulated first according to treatment arm and then by percent increase in TAHC.

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Sessions</th>
<th>Arm</th>
<th>Baseline hair count</th>
<th>Post-treatment hair count</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>4</td>
<td>Treatment</td>
<td>19</td>
<td>31</td>
<td>63.16</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>6</td>
<td>Treatment</td>
<td>16</td>
<td>26</td>
<td>62.50</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>2</td>
<td>Treatment</td>
<td>16</td>
<td>23</td>
<td>43.75</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>3</td>
<td>Treatment</td>
<td>18</td>
<td>25</td>
<td>38.89</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4</td>
<td>Treatment</td>
<td>12</td>
<td>16</td>
<td>33.33</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>6</td>
<td>Treatment</td>
<td>6</td>
<td>8</td>
<td>33.33</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>3</td>
<td>Treatment</td>
<td>18</td>
<td>23</td>
<td>27.78</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>3</td>
<td>Treatment</td>
<td>20</td>
<td>25</td>
<td>25.00</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>2</td>
<td>Treatment</td>
<td>22</td>
<td>27</td>
<td>22.73</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>4</td>
<td>Treatment</td>
<td>21</td>
<td>25</td>
<td>19.05</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>2</td>
<td>Treatment</td>
<td>16</td>
<td>19</td>
<td>18.75</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>4</td>
<td>Treatment</td>
<td>19</td>
<td>22</td>
<td>15.79</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>3</td>
<td>Treatment</td>
<td>22</td>
<td>25</td>
<td>13.64</td>
</tr>
<tr>
<td>14</td>
<td>32</td>
<td>4</td>
<td>Treatment</td>
<td>23</td>
<td>25</td>
<td>8.70</td>
</tr>
<tr>
<td>15</td>
<td>28</td>
<td>7</td>
<td>Treatment</td>
<td>23</td>
<td>25</td>
<td>8.70</td>
</tr>
<tr>
<td>16</td>
<td>34</td>
<td>2</td>
<td>Treatment</td>
<td>19</td>
<td>20</td>
<td>5.26</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
<td>2</td>
<td>Treatment</td>
<td>20</td>
<td>21</td>
<td>5.00</td>
</tr>
<tr>
<td>18</td>
<td>24</td>
<td>2</td>
<td>Treatment</td>
<td>23</td>
<td>24</td>
<td>4.35</td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td>6</td>
<td>Treatment</td>
<td>24</td>
<td>20</td>
<td>-16.67</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>4</td>
<td>Placebo</td>
<td>20</td>
<td>23</td>
<td>15.00</td>
</tr>
<tr>
<td>21</td>
<td>26</td>
<td>4</td>
<td>Placebo</td>
<td>21</td>
<td>23</td>
<td>9.52</td>
</tr>
<tr>
<td>22</td>
<td>24</td>
<td>4</td>
<td>Placebo</td>
<td>22</td>
<td>24</td>
<td>9.09</td>
</tr>
<tr>
<td>23</td>
<td>50</td>
<td>4</td>
<td>Placebo</td>
<td>23</td>
<td>25</td>
<td>8.70</td>
</tr>
<tr>
<td>24</td>
<td>29</td>
<td>4</td>
<td>Placebo</td>
<td>22</td>
<td>23</td>
<td>4.55</td>
</tr>
</tbody>
</table>

Figure 1 | Average percent change in target area hair counts (TAHC) after treatment with non-ablative radio frequency device.

Conclusions

Wound healing is associated with hair regrowth in various animal models. Microneedling of scalp tissue presents promising evidence for a similar mechanism for inducing hair regrowth in humans. Here we demonstrate that a non-ablative RF device can be an effective treatment for AGA.

References

Prvi zaviralec IL-23 sedaj dostopen v obliki napolnjenega injekcijskega peresnika

Tremfya (guselkumab)

SKRAJŠAN PVOZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Vzgojenim, ki so zmerne do hude psoriaze s plaki pri odrašcih, ki so primerni za sistemsko zdravljenje. Primo injiciranje je treba narediti v tednih 0 in 4. Sledi vzdrževalno odmerjanje vsakih 8 tednov. Pri bolnikih, pri katerih po 16 tednih zdravljenja ni odziva, je treba razmisliti o prenehanju zdravljenja.

Interakcije: Interakcije med guselkumabom in različnimi substrati CYP (CYP3A4, CYP2C9, CYP2C19, CYP2D6 in CYP1A2) niso verjetne. Pri sočasnem odmerjanju guselkumaba in substratov CYP450 odmerka ni treba prilagajati.

Varnosti in učinkovitosti zdravila Tremfya v kombinaciji z imunosupresivami, vključno z biološkimi zdravili ali fototerapijo, niso ocenili.


Neželeni učinki: Ozužbe zgornjih dihal, gastroenteritis, okužbe z virusom Herpes simplex, dermatofitije, preobčutljivost, glavobol, diareja, urtikarija, izpuščaj, artralgija, eritem in bolečina na mestu injiciranja (vsi NU so opisani v povzetku glavnih značilnosti zdravila).

SAMO ZA STROKOVNO JAVNOST

Janssen, farmacevtski del Johnson & Johnson d.o.o., Šmartinska cesta 53, 1000 Ljubljana, tel: 01 401 18 00 e-mail: info@janssen-slovenia.si

Pemphigus vulgaris possibly associated with application of a tissue expander in a patient with Crohn’s disease and primary sclerosing cholangitis

George Badavanis1, Efstathia Pasmatzi2, Nikiforos Kapranos1, Alexandra Monastirli3,4, Pavlos Constantinou4, George Psaras5, Panayiota Protopapa6,7, Dionysios Tsambaos1,2

Abstract

Pemphigus vulgaris (PV) is an autoimmune disease of the skin and mucous membranes characterized by suprabasal acantholysis and formation of blisters and erosions due to generation of IgG autoantibodies directed against desmosomal proteins. Tissue expanders are devices that, through controlled mechanical overstretch, are capable of generating new skin that is used to cover wounds or extended surgical defects. We report the case of a 13-year-old girl suffering from Crohn’s disease (CD) and primary sclerosing cholangitis (PSC) who developed PV after application of a tissue expander for surgical removal of a giant congenital melanocytic nevus (GCMN). To the best of our knowledge, the case presented here is the first report of PV possibly associated with the application of a tissue expander and also the first report of coexistence of PV with either PSC or with PSC and CD in the same patient. Triggering or acute exacerbation of PV may be considered a possible side-effect of tissue expander application, especially in patients with a genetic predisposition for pemphigus and/or other autoimmune diseases. In view of the increasing use of tissue expanders in clinical practice, physicians should be aware of this rare side-effect in order to promptly diagnose it.

Keywords: pemphigus vulgaris, tissue expander, Crohn’s disease, primary sclerosing cholangitis, giant congenital melanocytic nevus

Introduction

The term pemphigus is derived from the Greek word pémphix ‘blist’, which is used to describe a group of potentially life-threatening autoimmune mucocutaneous blistering disorders. The main pathogenetic feature of pemphigus is the generation of circulating mostly IgG autoantibodies directed against desmosomal adhesion protein molecules, which leads to acantholysis (loss of coherence between keratinocytes) and intraepithelial blister formation.

Pemphigus vulgaris (PV) is the most common type of pemphigus, and it accounts for over 80% of cases with the highest incidence in the 5th and 6th decades of life. It affects both sexes equally and is rarely observed in children, being classified as childhood PV when it occurs in patients < 12 years old and as juvenile PV in patients 12 to 18 years old (2). The primary lesion of PV is characterized by a flaccid blister or bulla containing clear fluid that mostly arises on apparently normal skin or mucosa lining the oral cavity, esophagus, nose, eyes, and anogenital region. Rupture of the intraepithelial blisters/bullae usually leads to the formation of painful erosions. PV is caused by circulating IgG autoantibodies primarily directed against desmoglein 3 (Dsg3) and/or desmoglein 1 (Dsg1), and against large numbers of other non-desmoglein proteins known to regulate the adhesion and survival of keratinocytes (3). Systemic corticosteroids (with or without concomitant administration of immunosuppressants) represent the first-line treatment of PV (4).

Giant congenital melanocytic nevus (GCMN) is a pigmented melanocytic lesion present at birth or developing soon thereafter (incidence < 1:20,000 newborns), which will reach a diameter of at least 20 to 40 cm in adult life. It is due to mutations in the BRAF or NRAS genes, which result in defects in the differentiation, mitotic activity, and migratory potential of melanoblasts, or melanocyte precursor cells (5). It may affect any region of the body, but it is usually observed on the trunk and the extremities, revealing variable shades of black and diverse clinical characteristics of its surface (hairy or not, smooth, coarse, raised, or flat). Apart from the tremendous negative psychosocial impact on patients and their families, GCMN has two serious complications: neurocutaneous melanosis and malignant transformation. However, the rate and incidence of the latter still remain controversial.

Excision-based plastic surgery is the mainstay of therapy for GCMN, although it provides no guarantee of protection against malignancy because about half of melanomas found in patients with GCMN occur elsewhere on the body (6, 7). The surgical removal of GCMN has always been a challenge for surgeons, with full-thickness replacement of the skin being the procedure of choice. The technique of tissue expansion, which was introduced more than 60 years ago (8), has revolutionized the management of GCMN, permitting the safe excision of sizeable lesions with favorable cosmetic results (9).

We report the case of a 13-year-old girl suffering from Crohn’s disease (CD) and primary sclerosing cholangitis (PSC) who developed PV after application of a tissue expander for surgical removal of a GCMN. To the best of our knowledge, this case is the first report of coexistence of PV with either PSC or with PSC and CD in the same patient.

1Center for Dermatologic Diseases, Limassol, Cyprus. 2Department of Dermatology, School of Medicine, University of Patras, Patras, Greece. 3Laboratory of Molecular Histopathology, Athens, Greece. 4Histopathology & Cytology Laboratory, Nicosia, Cyprus. 5Plastic Surgery Department, University of the Witwatersrand, Johannesburg, South Africa. 6Paediatric Liver, GI, and Nutrition Centre, Archbishop Makarios III Hospital, Nicosia, Cyprus. 7King’s College Hospital, London, United Kingdom. ✉ Corresponding author: pasmatzi@med.upatras.gr, pasmatzi@otenet.gr

Received: 3 August 2019 | Returned for modification: 26 August 2019 | Accepted: 9 September 2019
Case report

A 13-year-old Caucasian girl presented in December 2018 to the Center for Dermatologic Diseases in Limassol, Cyprus with an 11-month history of multiple pruritic blisters on the trunk and the extremities. They had appeared 3 weeks after partial surgical removal of a GCMN on her right tibia subsequent to a 12-month application of a tissue expander made of silicone, which was removed a few minutes prior to surgery. Interestingly, these lesions had begun in the region of the expanded skin (Fig. 1a) and subsequently spread to involve the trunk and the extremities. The past medical history of the patient was significant for CD and PSC, which were diagnosed in 2007 and presently remain in remission. Systemic treatment for these disorders at the time of her presentation included azathioprine (50 mg/day), sulfasalazine (1.5 g/day), and ursodeoxycholic acid (1.0 g/day). She is being closely monitored by our colleagues at the Paediatric Liver, GI, and Nutrition Centre of Archbishop Makarios III Hospital in Nicosia, Cyprus and at King’s College Hospital in London.

Clinical examination revealed multiple annular bullous lesions scattered all over the trunk and extremities (Figs. 1b & c), which revealed a positive Nikolsky’s sign. The results of histopathological examination of biopsies obtained from the lesional skin (Fig. 2a) and of direct immunofluorescence of biopsies obtained from the perilesional skin (Fig. 2b) were consistent with PV. Enzyme-linked immunosorbent assay (ELISA) revealed no circulating autoantibodies against Dsg1 and/or Dsg-3. For technical reasons, circulating antibodies against other desmosomal adhesion protein molecules could not be determined. The results of routine hematological and biochemical investigations were within normal limits.

In view of the coexisting CD and PSC, the treatment of PV in our patient was undertaken by a multidisciplinary team (including dermatologists, pediatricians, gastroenterologists, and hepatologists) according to the guidelines of the European Dermatology Forum (EDF) and the European Academy of Dermatology and Venereology (EADV) (4). Initial treatment for PV consisted of oral prednisolone 20 mg daily (0.5 mg/kg/day), and oral omeprazole (20 mg/day) was given for gastric ulcer prophylaxis. A complete clinical remission was achieved within 9 days. According to the above guidelines, prednisolone should be tapered by a 25% reduction in biweekly steps (at < 20 mg/day more slowly). During steroid tapering by a 25% reduction every 3 weeks, the patient experienced a massive relapse upon initiation of prednisolone 10 mg/day. Then, in view of the devastating psychological impact of this relapse on the patient and in an attempt to achieve a rapid resolution, it was decided to increase the prednisolone dosage to 25 mg/day, which led to a complete remission within 5 days. Nevertheless, 3 days after reduction of the dosage to 20 mg/day a new relapse occurred, which was initially unexplained to us until we were informed by the patient’s parents that in the previous few days she had experienced excessive emotional stress and had been exposed to sunlight for many hours. Because both emotional stress and sunlight exposure are well-known major triggering/aggravating factors in patients with PV (10, 11), the relapse of the patient was attributed to these factors and it was decided to go back to the dosage of 25 mg/day and to increase the azathioprine dosage of 50 mg/day (which she has received in the last 6 years for treatment of her CD) to 100 mg/day in an attempt to take advantage of the steroid-sparing effect of this agent.

A complete remission was achieved after 12 days of combined treatment. The patient is currently receiving prednisolone 15 mg/day and azathioprine 100 mg/day, and she remains lesion-free. Further careful steroid tapering will follow.

Discussion

CD is a relapsing inflammatory bowel disease with increasing incidence and prevalence worldwide. Although it mainly affects the gastrointestinal tract, extraintestinal systemic manifestations and...

Figure 1 | a) Occurrence of bullae in the area of expanded skin, 3 weeks after partial surgical removal of a GCMN on the patient’s right tibia subsequent to application of a tissue expander. b & c) On clinical examination, multiple polycyclic annular bullous lesions were seen on the trunk and extremities.

Figure 2 | a) On light microscopy of the lesional skin, suprabasal acantholysis with clefting, blister formation, and a single row of keratinocytes attached to the basement membrane were observed. There was a minimal perivascular lymphohistiocytic infiltrate with several eosinophils (H&E ×40). b) On direct immunofluorescence of the perilesional skin, intraepidermal intercellular IgG deposits were seen along the epithelial cell surfaces (×20).
associated immune disorders are quite common. Its typical clinical picture is characterized by abdominal pain, fever, diarrhea (occasionally containing blood and/or mucus), and clinical signs of bowel obstruction (12). It is thought that environmental factors trigger susceptibility loci, resulting in a disturbed innate and adaptive immune response toward a diminished diversity of commensal microbiota (12).

PSC is an idiopathic, slowly progressive cholestatic liver disease characterized by inflammation and fibrosis of the intra- and extrahepatic ducts, which may progress to cholangiocarcinoma, cirrhosis, and end-stage liver disease (13, 14). The association between PSC and inflammatory bowel disease is well established because about 60 to 80% of PSC patients are expected to suffer from concurrent ulcerative colitis or CD (13, 14).

Although the coexistence of CD and PV has previously been reported in many patients, a possible association between the two conditions still remains obscure (15). In a case-control study in a sizeable cohort of hospitalized patients, Husu et al. (16) found no significant association between the two entities. Kridin et al. (17), using a database of 4.5 million patients, performed a cross-sectional study of the prevalence of CD comparing PV patients with age-, sex-, and ethnicity-matched controls. They found no association between PV and CD in the general population because the prevalence of CD in patients with PV was comparable to that found in controls; however, a significant association was observed between PV and CD in patients younger than 40 years. To the best of our knowledge, there are no published reports of the coexistence of PV and PSC or of PV with concomitant PSC and CD in the same patient.

Various factors are capable of triggering or exacerbating pemphigus in susceptible individuals, including drugs, vaccines, infections, malignant neoplasms, other autoimmune diseases, dietary factors, radiotherapy, pregnancy, emotional stress, UV-radiation, burning, and pesticides (11). Case reports on the occurrence of PV over surgical scars due to Koebner phenomenon (i.e., the appearance, after a lag time, of typical lesions of a disease following trauma) are sparse, and trauma is not considered a major triggering factor for PV (18–20).

Tissue expanders are devices that use controlled mechanical overstretch to generate new skin, which is used to cover wounds or extended surgical defects. In its typical form, the method consists of the surgical insertion of a biomimetic inflatable balloon beneath the skin that exerts progressively increased stretch, achieved by the periodic injection of saline solution into it. Infections and tumor or scar formation are the most significant side effects of the method (21, 22). Prolonged skin stretching by expander gives rise to multiple and complex morphological and functional changes at the molecular and cellular level and leads to the production and release of growth factors such as epidermal growth factor (EGF), transforming growth factor β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and interleukins (IL), which play a pivotal role in the regulation of stress-induced cell growth. Moreover, mechanical stretching of the skin is capable of activating various signaling pathways that can promote cell proliferation or induce apoptosis (22, 23).

The pathogenetic mechanisms underlying the occurrence of PV subsequent to overstretching of apparently normal skin through tissue expanders are unknown. It seems possible, however, that skin injury through overstretching may lead to overexpression and enhanced presentation of epithelial pemphigus antigens, particularly in genetically predisposed individuals (24–26), and to an early nonspecific release of proinflammatory cytokines (IL-1, IL-6, and tumor necrosis factor [TNF]), which results in blister formation through non-antigen-specific mechanisms (by modulating C3, plasminogen activator, and plasminogen activator inhibitor expression), whereas later development of blisters/bulles may be induced by recruitment of antigen-specific B and T cells (27, 28). In view of this, it seems reasonable to suggest that application of the tissue expander in our patient, together with the significant pathophysiological alterations caused by tissue stretching, possibly contributed to triggering PV in an individual with a strong genetic predisposition to autoimmune diseases.

The question of whether silicone, which the tissue expander was made of, might have been involved in the pathogenetic mechanisms of PV remains to be elucidated. This polymeric compound is widely used in medicine for multiple purposes and is not a biologically inert material, as previously thought, but exerts distinct immunological effects, and according to recent studies it seems to be associated with a higher likelihood of autoimmune/rheumatic disorder diagnosis (29–31).

Interestingly, in the case presented here, no circulating autoantibodies against Dsg3 and/or Dsg1 could be detected. Proteomic studies have revealed that numerous non-Dsg autoantibodies are present in the sera of patients with PV, which are directed against autoantigens involved in the physiology and cell adhesion of keratinocytes, such as autoantibodies against desmocollins, cholinergic receptors, mitochondrial proteins, thyroid peroxidase, plakophilin 3, E-cadherin, plakoglobin, and numerous other protein antigens (3, 32). In view of the negative results of ELISA for Dsg autoantibodies in the case presented, it would be interesting to know whether this young patient had developed autoantibodies against these proteins. However, detection and quantification of these autoantibodies are not among the routinely performed tests and cannot be carried out in our laboratory because they require techniques that are available only in specialized research units. These non-Dsg autoantibodies might be of importance in the pathogenesis of PV. Indeed, Chernyavsky et al. (33) found that non-Dsg autoantibodies in the sera of patients with Dsg1/3-negative acute PV are pathogenic because IgGs from these individuals induced skin blistering in neonatal mice subsequent to suprabasal acantholysis. Moreover, serum levels of autoantibodies to desmocollin 3 (Dsc3), M3 muscarinic acetylcholine receptor (M3AR), and secretory pathway Ca2+/Mn2+-ATPase isoform 1 (SPCA1) correlated with the disease stage of PV, whereas absorption of these autoantibodies on recombinant Dsc3, M3AR, or SPCA1 prevented skin blistering after passive transfer to BALB/c mice.

To the best of our knowledge, the case presented here is the first report of PV possibly associated with the application of a tissue expander. The possibility that the occurrence of PV in our patient was unrelated to the placement of the expander cannot be definitely ruled out; however, it seems extremely unlikely in view of the close temporal relationship of the development of the lesions and the application of the device as well as the occurrence of the lesions initially on the expanded skin of the ipsilateral limb before spreading to other areas.

In conclusion, triggering or worsening of PV may be regarded as a possible side-effect of tissue expander application, with patients having a history of pemphigus and/or other autoimmune disorders being at higher risk. In view of the increasing use of tissue expanders in clinical practice, physicians should be aware of this rare side-effect and able to promptly diagnose it.
Primary cutaneous plasmacytosis: a case report

Adelina Costin1✉, João Alves1, Diogo Cerejeira1

1Department of Dermatology and Venereology, Garcia de Orta Hospital, Almada, Portugal. ✉ Corresponding author: adelina_costin@hotmail.com

Abstract

Primary cutaneous and systemic plasmacytosis is a rare disorder characterized by infiltration of the skin by polyclonal plasma cells of unknown etiology, frequently accompanied by polyclonal hypergammaglobulinemia and diffuse superficial lymphadenopathy. It primarily arises in patients of Japanese descent, and it is exceedingly rare in Caucasians. We describe a 36-year-old Portuguese male who presented with disseminated reddish-brown plaques over the trunk, neck, and upper limbs with normal gammaglobulinemia consistent with a diagnosis of primary cutaneous plasmacytosis.

Keywords: cutaneous plasmacytosis, plasma cell dyscrasia

Introduction

Primary cutaneous plasmacytosis is a rare reactive plasmacytic disorder characterized by infiltration of the skin by mature polyclonal plasma cells. Initially thought to represent an exclusively cutaneous process, it was renamed primary cutaneous and systemic plasmacytosis due to the observation of frequent extracutaneous involvement (1). It primarily arises in patients of Japanese descent, with fewer than a dozen reports in Caucasians (2). The pathogenesis is unclear and is thought to be related to an unspecified reactive inflammatory response.

Case report

An otherwise healthy 36-year-old Caucasian male presented to our outpatient clinic with a history of 10 years' duration of progressive development of slightly pruritic hyperpigmented lesions that began on his upper trunk and gradually spread to the entire trunk, neck, and upper limbs. He denied fever, malaise, night sweats, or other constitutional symptoms and he did not have a relevant family history of hematologic disorders. On physical examination he presented with multiple reddish-brown ovoid thin plaques, up to 2 cm in size on his back, chest, neck, and upper limbs (Figs. 1–3). Neither lymphadenopathy nor hepatosplenomegaly was appreciated clinically.

Figure 1 | Erythematous to brownish plaques on the posterior neck.

Figure 2 | Erythematous to brownish macules and plaques on the anterior chest.

Figure 3 | Detail of erythematous to brownish plaques on the trunk.

Serum and urine electrophoresis were within normal range, with a normal IgG value (637 mg/dl). Complete blood count, metabolic panel, antinuclear antibodies, and erythrocyte sedimentation rate revealed no alterations, with normal beta-2 microglobulin and interleukin-6 (IL-6) levels (< 2.0 pg/ml). Serum and urine immunofixation studies found no monoclonal immunoglobulins. The serological examination for syphilis, hepatotropic viruses, HIV, and Borrelia burgdorferi was negative.
Chest computed tomography and abdominal ultrasound were performed, with no changes disclosed. Bone marrow aspiration revealed normocellularity (< 5% of plasma cells).

A punch biopsy was obtained from a cutaneous lesion. The histological examination revealed a dense perivascular inflammatory cell infiltrate in superficial and deep dermis, with predominance of mature plasma cells intermingled with some lymphocytes, histiocytes, and eosinophils (Fig. 4). The plasma cells displayed no atypia. Immunohistochemical study showed coexistence of both kappa and lambda-chain positive cells, thus confirming the polyclonal nature of plasma cells.

Neither topical corticosteroids nor topical tacrolimus was effective. The patient declined a psoralen and ultraviolet A (PUVA) therapy trial, remaining in clinical follow-up since then.

**Discussion**

The clinical presentation of primary cutaneous plasmacytosis is characteristic, with gradual development of multiple reddish-brown plaques and nodules predominantly on the trunk (3).

It mainly affects middle-aged patients of Asian descent, without a clear sex predilection (4). Less frequently it can present as a solitary lesion, not usually associated with extracutaneous manifestations and occurring predominantly in children (5).

Idiopathic infiltration of mature polyclonal plasma cells has been reported not only in the skin but also in other organs, such as the lungs, lymph nodes, liver, and spleen, in which case it is referred to as primary cutaneous and systemic plasmacytosis (6). The most frequent extracutaneous manifestations are superficial lymphadenopathy (up to 58%) and hypergammaglobulinemia (up to 93%) (1).

There are several secondary causes of mature plasmacytic infiltrates that should be excluded, such as collagen vascular disease and chronic infections. A main differential diagnosis is generalized cutaneous B cell pseudolymphoma due to their striking histological similarities. Extracutaneous involvement such as lymphadenopathies, hypergammaglobulinemia, and no previous history of culprit drug intake are important clues for establishing the diagnosis.

Whereas the exclusively cutaneous forms follow a benign and chronic course without spontaneous remission, in some cases with systemic involvement it can follow an aggressive course, with cases of lymphoid interstitial pneumonia and mesangial proliferative glomerulonephritis and renal insufficiency having been reported (4). The potential for malignant transformation of cutaneous plasmacytosis is not known. The few reports of lymphoma do not establish a correlation, and the relationship between lymphoma and plasmacytosis is not established (7).

Due to its rarity and most of the literature consisting of case reports and case series, the etiology and therapeutic approaches remain poorly established. Although the exact pathogenesis is unknown, increased serum levels of IL-6 have been reported in these patients, similarly to multicentric Castleman disease. However, none of the previous studies identified human herpesvirus 8 in cutaneous lesions of primary systemic plasmacytosis (8). Hyperproduction of IL-6 could be an important factor in the pathogenesis of cutaneous plasmacytosis because it induces B-cell proliferation and terminal differentiation.

Several treatments have been attempted, including topical and systemic corticosteroids, systemic antibiotics, and combined chemotherapies using agents such as melphalan, vincristine, cyclophosphamide, azathioprine, or even anti-CD20 antibody therapy, with poor clinical responses. On the other hand, topical tacrolimus and PUVA therapy have shown some improvement of skin lesions (9).

**Conclusions**

This exceedingly rare case highlights the unique features of a poorly understood plasmacytic disorder, supporting the concept that primary cutaneous plasmacytosis is a unique clinical entity.

**References**

Papular eruption on UV-exposed skin in a 7-year-old boy caused by Enterobius vermicularis infection

Mateja Starbek Zorko1,2, Anja Trajber Horvat3

1Department of Dermatovenereology, Ljubljana University Medical Center, Ljubljana, Slovenia. 2Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. 3Department of Dermatovenereology, Maribor University Medical Center, Maribor, Slovenia.

✉Corresponding author: mateja.starbekzorko@kclj.si

Abstract

Enterobiasis is the most common parasite infestation in children; it is often asymptomatic and may rarely be a cause of skin eruption. We present the case of a 7-year-old boy with sudden onset of pruritic erythematous-squamous confluent papules and plaques on UV-exposed skin, caused by proven enterobiasis. To our understanding, this is the first case of photodermatosis-like dermatitis caused by enterobiasis reported in the literature.

Keywords: child, Enterobius vermicularis, dermatitis, UV-exposed skin

Case report

We present the case of a 7-year-old boy with sudden onset of pruritic, well-circumscribed erythematous-squamous macules and papules on his face that progressed to his neck and extremities in a few days. Due to suspicion of bacterial infection, his pediatrician prescribed local gentamicin ointment and systemic antibiotic with penicillin. After 3 days no improvement was seen, and he was sent to a specialist in infectious diseases, who excluded bacterial infection, discontinued antibiotic therapy, and prescribed him antihistamines. Because of a persistent itchy rash, after 1 week he was sent to our outpatient clinic, where, in addition to peroral antihistamines, topical corticosteroid therapy was started. Because of a burning sensation following the application of local corticosteroid cream, the parents discontinued the therapy. After 1 week no improvement was seen, and so he was hospitalized at Department of Dermatovenereology for further diagnostics and treatment.

He had no previous skin problems and was otherwise a healthy child. He was not taking any medication and had no known allergies. There were no skin diseases in his family, but his mother had a known pollen allergy.

At the time of hospitalization confluent, well-demarcated erythematous-squamous papules and plaques on UV-exposed skin (the face, neck, and extremities) and dry, scaly lips were observed (Figs. 1-2). The skin of other parts of the body was unaffected, and his general somatic status was unremarkable.

Apart from eosinophilia (7.1%, normal range 1–6%), other laboratory exams—complete blood count, liver enzymes and creatinine, electrolytes, creatine kinase, lactate dehydrogenase, and complete urine analysis—were all within normal limits. Immunoserology (ANA, ENA, and anti-ds DNA) and porphyrins in erythrocytes and urine were all within normal range. Due to the presence of eosinophilia, we suspected either parasitosis or an atopy.

A perianal cellophane swab was positive for Enterobius vermicularis. There was a highly elevated complete IgE (626 kU/l, normal range for a child of 7 years up to 71 kU/l) and later positive specific IgEs to wheat, barley, buckwheat, soy, house dust mites, and mixture of grass, tree, and weed pollen were found.

The patient was treated with a single 100 mg dose of the antiparasitic mebendazole, and he continued taking antihistamines systemically because of the pruritus. Once daily we applied wet saline dressings followed by topical methylprednisolone acetate cream on the affected skin. After 1 week of therapy, his skin lesions regressed completely over the following days (Figs. 3-4) and did not repeat after playing outside or after sun exposure, later that year, and not even the next year.

Discussion

Enterobius vermicularis is an intestinal parasite that is usually transmitted in humans by direct fecal-oral transfer by the hands.

Figure 1 | Erythematous-squamous papules and plaques on the face and neck.
The pinworm lives in the small intestine and migrates to the perianal area at night, where it deposits eggs. It is the most common nematode infection in school-aged children and due to insufficient hygiene can quickly spread to other family members (1). In adults it can also be transmitted by inhaling or ingesting dust with airborne eggs while changing bedding or handling infested children’s clothes (1).

The highest prevalence of enterobiasis (up to 50%) has been recorded in the United States and northwestern parts of Europe, in various institutions and at summer camps (1, 2). The exact prevalence of the infestation in Slovenia is not known, presumably because of inaccurately reported cases, but the results of the last few years show that the prevalence is increasing (3).

Up to one-third of infested patients can be asymptomatic, and others usually present with perianal or perineal pruritus and eosinophilia. In children other non-specific signs such as irritability, restlessness, insomnia, enuresis, anorexia, and weight loss have been reported (2). Infestation with *Enterobius vermicularis* can also
cause extra-cutaneous signs, including gastrointestinal symptoms such as diarrhea and rarely appendicitis, diverticulitis, granuloma of the colon, perianal abscess, and ileum perforation (2, 4). Due to nocturnal migration of the parasite from the lumen of the gastrointestinal tract, some helminths can invade the genitourinary tract and cause vulvovaginitis, salpingitis, epididymitis, and urinary tract infections (1, 4). Enterobius vermicularis infection has rarely been reported as the cause of generalized pruritic dermatitis or even urticaria (1, 2, 5, 6), but none of these reports described eruption only on photo-exposed skin, as seen in our case. A study in Israel showed that Enterobius vermicularis-induced symptoms were more common in children with underlying dermatoses (e.g., atopic dermatitis, neurodermatitis, contact dermatitis, seborrheic dermatitis, or psoriasis) (2), and a study by Wördemann et al. showed that enterobiosis in Cuban children is a risk factor for allergic rhinoconjunctivitis or atopic dermatitis (7). However, studies investigating the association between Enterobius vermicularis and allergic conditions have shown conflicting results (8).

Laboratory findings of eosinophilia and raised IgE suggest invasive disease, whereas infestation limited to the gut mostly does not cause eosinophilia (9). In our patient no perianal or perineal pruritus was noticed, but eosinophilia and raised IgE levels suggested parasitosis. Specific IgE levels confirmed hypersensitivity to several allergens, but up until then our patient had no skin disease and no symptoms suggesting allergic disease. Enterobius vermicularis infection is usually confirmed by an adhesive tape test showing parasite eggs. Stool analysis for parasites or ova is positive only in 5 to 15% (2, 5, 6). As light yellowish threadlike worms, helminths can be found on toilet paper or in the stool (1). In complicated cases, the diagnosis can be made with endoscopy, where pinworms in the distal part of colon can be seen (5).

Although histopathological findings of skin lesions caused by parasitosis are nonspecific (5), we suggested performing a skin biopsy due to the unusual presentation of the skin lesions in our case, but the parents did not agree to this.

Benzimidazole compounds such as mebendazole and albendazole, given orally, are the most effective treatment for enterobiasis (2, 9). Due to mebendazole malabsorption, in genitourinary infection the suggested treatment is ivermectin rather than mebendazole (1, 3). If a child has enterobiosis, it is recommended that other family members also be treated because they may have an asymptomatic infection. It is also very important to increase personal hygiene, especially hand washing (2, 9). Our patient was treated with a single dose of mebendazole, repeated after 2 weeks, as suggested in literature (9). After systemic therapy for infestation and symptomatic therapy for dermatitis, we observed complete regression of the dermatitis, which did not reappear after being outside, exposure to known allergens, or exposure to sun, and so we concluded that Enterobius vermicularis infection was the main cause of the sudden onset of papular eruption on UV-exposed skin.

Conclusions

Based on our case, we suggest that enterobiosis should be considered in the differential diagnosis of persistent pruritic dermatitis on UV-exposed skin in children with elevated IgE and/or eosinophilia. The diagnosis of infection is easily confirmed by microscopic examination of an adhesive tape test. Treatment with anti-parasite drugs is recommended. One must keep in mind that reinfection is quite frequent, which is why it is very important to treat all family members in order to minimize the possibilities of reinfection. In a child with eruption on UV-exposed skin, one should bear in mind enterobiasis as a possible cause of the rash. With a positive adhesive tape test and the right treatment, it is possible to avoid additional laboratory tests and their costs in order to exclude other causes of photodermatosis.

References

Zdravilo SKYRIZI je indicirano za zdravljenje zmerne do hude psoriaze v plakih pri odraslih, ki so kandidati za sistemsko zdravljenje. Sestava: Ena napolnjena injekcijska brizga vsebuje 75 mg risankizumaba v 0,83 ml raztopini. Risankizumab je humaniziran monoklonalni IgG1, monoklonsko proizveden, selektirano za beljakovino interlevkin (IL)-23. Vzorec brizga vsebuje 6,08 mg serumbeta, ki se dodaja na 150-mg odmerek. Terapeutski indikaciji: zdravljenje zmerne do hude psoriaze. Odmerjanje in način uporabe: Zdravilo SKYRIZI je namenjeno za uporabo pod vodstvom in nadzorom zdravnika, ki ima izkušnje z diagnostičnimi in zdravilnimi posamezniki. Risanizumab je treba administrirati razen v primerih zdravljenja psoriaze z drugimi zdravilnimi učinkovinami ali zdravili, ki so namenjeni za zdravljenje opažanj zmenja kože in/ali družbene točke. Zdravilo SKYRIZI je namenjeno prehodnemu ali konsolidacijskemu zdravljenju psoriaze. Poslovna in profesionalna zgodovina: vrednostni odredbi in oceni vključujeta: podplutbe na mestu injiciranja, eritem, hematom, krvavitev, draženje, bolečine, srbenje, reakcijo, ipo. Sestava: Risanizumab je selektiven inhibitor IL-23/p19, ki je monoklonalko proizveden iz izoliranih heparinotrakulnih (HLA) blastlikeh lakticnih živčnikov. Ustreznost: Predvidevane učinke: možnost, da Nič ne ostane na koži: v študijah UltIMMa-1 in UltIMMa-2 je 75% bolnikov po 16 tednih zdravljenja dosego PASI 90 in ≥56% bolnikov je po 52 tednih dosego PASI 100. Risanizumab je treba uporabiti, če je bolnik prejel živo cepivo in potrebuje bolniki za zdravljenje, se izboljša z nadaljevanjem zdravljenja več kot 16 mg injekciji v subkutan injekciji. Uredbe: Pred uporabo zdravilnih učinkovin ali zdravil, ki se nanašajo na zdravljenje psoriaze, se v rednem okviru treba dosledno potrditi, da so zdravila v navodilih, da se uporabljajo za tobolnik prejel živo cepivo ali neko gospodarstvo anamneza okužb, ki vključujejo: tinea versicolor, tinea manuum, onhamomikozo; reakcije na mestu injiciranja vključujejo podplutbe na mestu injiciranja, temer hematom, krvavitev, draženje, bolečine. Zdravila, imetnik dovoljenja za promet kažeta, vrednostni odredbi bolnikov, ki so se zdravilo z risanizumobom in treba namreč, naj poslužijo za razvidno pomnožijo, če se je pojavit preobčutljivostna reakcija, je treba uporabo risanizumaba premaknuti. Zdravilo SKYRIZI je namenjeno prehodnemu ali konsolidacijskemu zdravljenju psoriaze. Pred uporabo zdravilnih učinkovin ali zdravil, ki se nanašajo na zdravljenje psoriaze, se v rednem okviru treba dosledno potrditi, da so zdravila v navodilih, da se uporabljajo za to. V primeru izpuščenja odmerka je treba odmerek uporabiti čim prej. Zdravilo SKYRIZI je namenjeno za uporabo pod vodstvom in nadzorom zdravnika, ki ima izkušnje z diagnostičnimi in zdravilnimi posamezniki. Risanizumab je selektiven inhibitor IL-23/p19, ki je monoklonalko proizveden iz izoliranih heparinotrakulnih (HLA) blastlikeh lakticnih živčnikov. Ustreznost: Predvidevane učinke: možnost, da Nič ne ostane na koži: v študijah UltIMMa-1 in UltIMMa-2 je 75% bolnikov po 16 tednih zdravljenja dosego PASI 90 in ≥56% bolnikov je po 52 tednih dosego PASI 100. Risanizumab je treba uporabiti, če je bolnik prejel živo cepivo in potrebuje bolniki za zdravljenje, se izboljša z nadaljevanjem zdravljenja več kot 16 mg injekciji v subkutan injekciji. Uredbe: Pred uporabo zdravilnih učinkovin ali zdravil, ki se nanašajo na zdravljenje psoriaze, se v rednem okviru treba dosledno potrditi, da so zdravila v navodilih, da se uporabljajo za to.
Asymptomatic reactivation of hepatitis B virus after prolonged treatment with etanercept

Nuno Gomes1✉, Beatriz Leão2, Cândida Abreu2, Filomena Azevedo1, Sofia Magina1,3

Abstract

Etanercept is an anti-tumor necrosis factor α (anti-TNFα) drug used for treating immunemediated inflammatory diseases. It is least associated with hepatitis B virus (HBV) reactivation. We present a 71-year-old man with psoriasis refractory to phototherapy and acitretin, which led to etanercept monotherapy. Before anti-TNFα treatment, past contact with HBV was elicited; antibodies to Hbc and Hbs were positive whereas HbsAg was negative. Seven years after treatment initiation, while the patient was completely asymptomatic, a transaminase elevation was found and a reactivation of HBV was documented, with a high viral load of the virus. He started entecavir therapy and, after a 14-month follow-up, the viral load is still detectable at a low level, as well as HbsAg. We emphasize the late and asymptomatic reactivation of HBV associated with soluble anti-TNFα monotherapy. This case reinforces the importance of current recommendations for periodic monitoring of viral load and HBV markers in all patients that have had prior contact with HBV (positive anti-HBc), with or without indication for treatment of HBV (HbsAg and HBV-DNA negative).

Keywords: hepatitis B virus, hepatitis B virus reactivation, etanercept, anti-TNFα, psoriasis

Table 1 | Evolution of hepatitis B virus (HBV) serological status.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HbsAg (NR &lt; 0.9 – R &gt; 1.1)</td>
<td>0.37</td>
<td>0.20</td>
<td>217.40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Hbc (NR &lt; 0.9 – R &gt; 1.1)</td>
<td>7.27</td>
<td>9.40</td>
<td>8.70</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Hbs (IU/l)</td>
<td>29.30</td>
<td>2.92</td>
<td>79.99</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.44</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Hbe (NR &lt; 0.9 – R &gt; 1.1)</td>
<td>–</td>
<td>–</td>
<td>0.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HbeAg (NR &lt; 0.9 – R &gt; 1.1)</td>
<td>–</td>
<td>–</td>
<td>982.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HBV-DNA</td>
<td>–</td>
<td>–</td>
<td>16,000,000 IU/ml</td>
<td>11,871 IU/ml</td>
<td>626 IU/ml</td>
<td>262 IU/ml</td>
<td>315 IU/ml</td>
<td>157 IU/ml</td>
<td>258 IU/ml</td>
</tr>
</tbody>
</table>

NR = non-reactive, R = reactive, IU = international units.

Introduction

Hepatitis B virus (HBV) reactivation associated with anti-tumor necrosis factor α (anti-TNFα) drugs for the treatment of immunemediated inflammatory diseases is rarely reported in the absence of surface hepatitis B antigen (HbsAg) (1). Etanercept, a soluble anti-TNFα, is the least associated with HBV reactivation (2). Clinical hepatitis reactivation has a wide spectrum, ranging from asymptomatic cases to potentially fatal fulminant hepatic failure (3, 4). In 2015, the European Centre for Disease Prevention and Control (ECDC) estimated the prevalence of HBV infection as 0.9%; in Portugal, it is estimated that 0.4 to 1.0% of the population is positive for HbsAg (5). The highest published reported incidence of HBV reactivation in patients with past HBV infection is 5%, including one fatal case due to liver failure (6). Therefore it is considered safe, and the European Association for the Study of the Liver (EASL) (7) recommends that patients with past HBV infection (anti-HBc positive and anti-Hbs positive) in the absence of HbsAg (and negative for HBV-DNA) should start anti-TNFα therapy with clinical and analytical monitoring and serial HBV-DNA testing (3, 8).

Case report

We present the case of a 71-year-old man followed at the Dermatology Outpatient Clinic since 2002 due to plaque psoriasis. He started on phototherapy and acitretin with insufficient response (Psoriasis Area and Severity Index [PASI] 11.4), and so etanercept 50 mg per week was prescribed in 2010, with PASI 0 at 20 weeks' follow-up. Before anti-TNFα treatment, past contact with HBV was elicited: antibodies to Hbc and Hbs (quantified titer: 29.30 IU/l) were positive with HbsAg being negative; anti-HIV and anti-HCV were both negative (Table 1). He has been on etanercept continuously since 2010, treatment was interrupted for 2 months in 2015, with recurrence of psoriatic lesions, and he resumed treatment again with efficacy. In October 2015, HBV screening elicited the same serological markers.

In September 2017, asymptomatic transaminase elevation was found (alanine aminotransferase [ALT] 99 IU/l, aspartate aminotransferase [AST] 66 IU/l), and etanercept was immediately stopped (PASI 0 at this date) on suspicion of toxicity. On reevaluation in November 2017, transaminases were still on the rise (ALT 431 IU/l, AST 220 IU/l), HbsAg became positive, and a high viral load of HBV was found (16,000,000 IU/ml). He was sent to the Infectious Diseases Clinic in December 2017 and on the same day started entecavir. The patient remained completely asymptomatic, without hyperbilirubinemia or coagulopathy. The HBV was genotype A, without detected resistances.

After 1 month of entecavir therapy, the viral load was 11,871 IU/ml (a decrease of three logarithms) and transaminases were...
within normal values. At 3 months of therapy, he had HBV-DNA of 626 IU/ml and abdominal ultrasonography was irrelevant except for steatosis. At 14 months of therapy, viral load is still detectable at a low level as well as HBsAg. Consequently, the patient will maintain entecavir even without immunosuppression. He remained asymptomatic during the entire follow-up period. At 17 months’ follow-up since etanercept was stopped, he is only being treated with topical medications for psoriasis and has a PASI score of 2.

Discussion

We emphasize the late and asymptomatic reactivation of HBV associated with soluble anti-TNFα monotherapy in a 71-year-old patient suffering from psoriasis. In our specific case, unfortunately, the HBV viral load prior to the start of etanercept was not measured.

The majority of publications in this field are in the scope of rheumatological diseases. Tamori et al., for example, designed a prospective study to clarify the prevalence of HBV reactivation in rheumatoid arthritis patients receiving long-term immunosuppressive therapy. Among 42 HBsAg-negative and anti-HBc-positive patients that received anti-TNFα therapy, no HBV reactivation occurred (9). More recently, Lee et al. published an extensive review of 269 cases of HBsAg-negative and anti-HBc-positive patients with rheumatic diseases treated with etanercept, which resulted in seven HBV reactivations (2.6%), but the majority of patients were also treated with a disease-modifying anti-rheumatic drug besides etanercept (10). To our knowledge, this is the first report of HBV reactivation in a patient receiving etanercept monotherapy for treatment of plaque psoriasis with a negative HBsAg prior to treatment (1, 6, 11, 12).

This case reinforces the importance of current recommendations for periodic monitoring of viral load and HBV markers in all patients that had prior contact with HBV (positive anti-HBc), with or without indication for treatment of HBV (HBsAg and HBV-DNA negative). A patient with a positive viral load followed by a rise in transaminases may avoid clinical hepatitis if appropriately treated. This monitoring is essential for the early recognition of viral reactivation, which may be absolutely asymptomatic or severe enough to cause death (3, 4, 7, 13). After reactivation of HBV, the therapy for psoriasis may be compromised.

References

Targetoid erythema surrounding multiple seborrheic keratoses induced by chemotherapy with gemcitabine

Chiara Retrosi1, Roberta Vezzoni1, Claudio Conforti1✉, Paola Corneli1, Giovanni Magaton-Rizzi1, Iris Zalaudek1, Nicola Di Meo1

Abstract

The cutaneous adverse effects of gemcitabine include allergic skin rash frequently associated with pruritus, alopecia, sweating, dermatitis with boils, and ulcerations. We report the case of a patient that developed inflammation of seborrheic keratoses after gemcitabine treatment.

Keywords: gemcitabine, seborrheic keratoses, drug adverse reaction

Received: 8 July 2019 | Returned for modification: 22 August 2019 | Accepted: 13 November 2019

Introduction

Seborrheic keratosis is the most common benign skin tumor of middle-aged and elderly adults. Seborrheic verrucae are benign skin lesions, usually brownish, that are mainly located on the chest but can develop in any skin region (1).

Chemotherapy of internal neoplasms or eczemas may lead to the onset of seborrheic keratoses or promote their inflammation (2). The literature includes numerous adverse effects of chemotherapy drugs on the skin, and in particular several cases of nucleoside analogues (such as gemcitabine) known to cause inflammation of seborrheic keratoses (3–6).

The authors report a case of inflammation of seborrheic keratoses after administration of gemcitabine.

Case report

A 78-year-old woman with right lung adenocarcinoma (Stage IV), diagnosed in April 2018, came to our attention for a newly emerging erythematous purple-colored skin rash spread all over the back. Clinically numerous preexisting seborrheic keratoses were clearly evident on the patient’s back, and the rash spared the perilesional skin around these. She denied having taken new drugs in the last month with the exception of chemotherapy; in fact, she received six cycles of chemotherapy with gemcitabine and cisplatin from September until October 2018, and subsequently she developed intensive itching, erythema, edema, and scaling in multiple preexisting seborrheic keratoses.

On physical examination, an erythematous rash was observed on the back, especially on some seborrheic keratoses on which targetoid lesions with saving of the skin surrounding keratosis were observed (Fig. 1a). The patient did not have a fever, but only asthenia and generalized itching.

A dermoscopic examination (20×, Dermatoscope DermLite 3Gen) was carried out and showed dotted and linear vessels and a purplish-erythematous background surrounding seborrheic keratosis. The numerous seborrheic keratoses were dermoscopically confirmed based on evidence of classic criteria: milia-like cysts, comedo-like openings, gyri and sulci, and network-like structures (Fig. 1b).

In our case we did not consider it appropriate to resort to a histological examination for diagnosis because today dermoscopy is a diagnostic tool with very high sensitivity and specificity for most skin lesions. Therefore, evaluating the history, as well as the clinical and dermoscopic examination of the skin manifestation, we considered it unnecessary to perform a biopsy.

Diagnosis of seborrheic keratosis with targetoid inflammation related to chemotherapy was made, and a cycle of methylprednisolone 40 mg to scale for 10 days was started, resulting in complete resolution of the clinical cutaneous manifestations.

Discussion

Many adverse skin events to chemotherapy drugs are described in the literature and, in particular, nucleoside analog drugs such as gemcitabine are known to cause inflammation of seborrheic keratoses. Based on the scientific evidence and the absence in the literature of a correlation between cisplatin and targetoid reactions, we considered the skin reaction described to have most likely been caused by gemcitabine.

Inflammation of seborrheic keratoses is a rarely described cutaneous reaction of chemotherapy (7), and in the literature there...
is only one previous case report mentioning this skin reaction (8), in which a patient with pancreatic cancer received chemotherapy with gemcitabine and developed an inflammatory reaction on preexisting seborrheic keratoses.

Cases of inflammation of seborrheic keratoses induced by other chemotherapy drugs such as cytarabine, 5-fluorouracil, docetaxel, vincristine, and doxorubicin are described (3–6), but as reported in the literature these are predominantly nucleoside analogues.

The majority of systemic adverse effects of chemotherapy are most often predictable, such as nausea, vomiting, diarrhea, decreased white blood cells, decreased immune defenses, and anemia, as well as common side effects on the skin and annexes: alopecia, erosive stomatitis, or aphthous ulcers. There are also other side effects such as inflammation of benign skin tumors, which is more rarely observed and less commonly diagnosed than side effects of chemotherapy (9).

The mechanism of action causing this side effect is not known; it has been hypothesized that cytotoxic damage directed against keratinocytes may be at work, whereas in other studies an infiltrate of lymphocytes into the dermis appears to be the cause of these skin lesions (7).

It is possible that chemotherapy treatment may involve changes in dosage or the method of administration (e.g., a cycle may be postponed for a week) precisely because of the toxic effects.

**Conclusions**

In the case we describe, it was not necessary to interrupt the chemotherapy because, although the clinical presentation was very extensive, it responded well to systemic corticosteroid therapy. For the dermatologist it is essential to recognize at an early stage the adverse effects caused by chemotherapy agents for early drug treatment without the need to stop the oncological therapy.

**References**

To the Editor:

We read with great interest the article by Singh et al. on the relationship between environmental factors and pityriasis rosea (PR), and between PR and dengue virus (DENV) infection (1), which allows us to make some considerations.

In their retrospective study, the authors found temporal clusters of PR in the autumn and winter months, although not in all the years analyzed and with a statistically significant but weak correlation.

A higher prevalence of PR in cold seasons has been claimed (2, 3), but controversial opinions remain (4), mostly connected with the occurrence of the disease in clusters (5). In our experience, PR develops evenly distributed throughout the year (6). These differences may be due to the geographical areas where the various studies are performed. Notably, Singh et al. (1) examined an environmental factor greatly overlooked in previous studies; namely, the main total rainfall. They found that heavy rainfall is linked to a decreased incidence of PR, suggesting that this happens because the vector mosquitoes of dengue fever decreased during the period of heavy rainfall. Furthermore, in their PR patients they found a positive correlation with the presence of NS1 antigen or antibody positivity against DENV in their serum. Therefore, they suggest that DENV may be one of the etiological factors for PR (1). In this regard, some remarks should be made. The authors state that the average time between dengue fever and the onset of PR was over 78 days, and they mentioned a pair of twins authors state that the average time between dengue fever and the onset of PR was over 78 days, and they mentioned a pair of twins developing PR 5 weeks after dengue fever. However, the incubation period really seems too long to ascribe DENV an etiological role in PR. In fact, exanthem in dengue fever (Herman’s rash) has a maculopapular or macular confluent pattern developing over the face, thorax, and flexor surfaces, with islands of skin sparing that usually appear on the 3rd to 4th day of the fever and last 2 to 3 days. Fever typically abates with the cessation of viremia, usually 3 to 7 days after the onset of symptoms. A second maculopapular exanthem, which lasts 1 to 5 days, may occur within 1 to 2 days after the fall of the fever (7, 8). Such a long incubation time for PR makes a direct relationship unlikely for the DENV. Usually, the virus causes a cutaneous eruption, very different from the PR pattern after a short incubation period of a few days (7, 8). What is most likely is that DENV decreases the host’s immunological response, permitting human herpesvirus 6 (HHV-6) and/or HHV-7 endogenous reactivation. In fact, the association of effective inhibition of type I interferon production and signaling in infected cells and effective modulation of infected cells such as monocytes, macrophages, B cells, and dendritic cells by DENV can decrease innate and adaptive immunity (8, 9). Regarding the possibility of an autoimmune mechanism in PR pathogenesis, suggested by the authors, this is unlikely. No specific autoantigens have ever been identified in PR, whereas immunohistological evidence of cellular immune response and an increased number of antigen-presenting cells in lesional skin have been well demonstrated (9, 10). Data from studies on mediators in the sera of PR patients showed high levels of interferon alpha and gamma, fractalkine, and interleukin 22, in agreement with a cell-mediated immune response against microbial pathogens (11–13). Indeed, several studies have identified HHV-6 and HHV-7 systemic activation in PR patients, HHV-6 and HHV-7 DNA and HHV-6 viral messenger RNA expression by in situ hybridization, and HHV-6 and HHV-7 antigens in PR skin lesions (14, 15) converging fully with a productive viral infection and emphasizing the role of both viruses in the pathogenesis of the disease. Finally, to date, everything suggests a cell-mediated immune response against HHV-6 and/or HHV-7.

References

Lokalno zdravljenje aktiničnih keratoz

Učinkovito zdravljenje in zelo dober kozmetični izid po 2 ciklih zdravljenja

- odkriva subklinične aktinične keratoze v obeh ciklih zdravljenja
- učinkovito in dolgotrajno odstrani subklinične in klinične lezije ne glede na njihovo število
- režim zdravljenja določa premor med dvema cikloma za omilitev kožne reakcije

Skrajšan povzetek glavnih značilnosti zdravila


-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-
Reply to: comment on “Is there a relationship between environmental factors and pityriasis rosea?”

Mehak Singh1, Manoj Pawar2, Antonio Chuh3, Vijay Zawar2

To the Editor:

We read with appreciation the comments and speculations presented by Drago et al. (1). We would like to draw your attention to the fact that this study was carried out in the humid subtropical climate of Guwahati (Köppen climate classification Cwa), which has broad temperature, precipitation, and humidity variations coupled with a kaleidoscope of genetic and ethnic diversity.

In a study by Chuh (2), a large number of autoantibodies were identified in the sera of patients with pityriasis rosea (PR) in contrast to the sera of controls, which forms the basis of the explanation that follows.

Our study highlights the Trojan horse phenomenon (3), or antibody-dependent enhancement seen in the development of a rash that results in a secondary autoimmune response in the form of PR rash, which differs significantly from that of either the acute exanthem in dengue fever (Herman’s rash) or the secondary maculopapular rash in terms of the time frame as well as clinical presentation. According to this, the virus persists sub-clinically and fester, leading to formation of autoantibodies to the virus. We postulate that these autoantibodies may be responsible for the epidermotropic attack on the skin. The development of such autoantibodies is not subject to a specific timeline. However, we do agree with Drago et al. that the histopathological and immunopathological evidence is amiss. Our first instinct was also to attribute this phenomenon to dengue virus (DENV) immunomodulation permitting the development of such a rash, which again was refuted on the same grounds as pointed out by the author. This hypothesis also underscores the reason why secondary dengue infections hit harder the second time and why dengue hemorrhagic fever has been found to be T-cell mediated as well (4).

Viruses affect antigen presentation and modulate the cytokine milieu, which may in turn play a part in initiation or perpetuation of autoimmunity (5), and such modulation can take place in a timeframe ranging from a few weeks to years.

In 1997, Drago et al. (6) first suggested the role of human herpesvirus 7 (HHV 7) in the etiology of PR by detecting HHV 7 in peripheral blood mononuclear cells and plasma of patients with PR and not in controls, thus suggesting a probable causal relationship. However, subsequent studies could not find any association between HHV 7 and PR. These correlations may therefore have an underlying ecological fallacy or a genetic predisposition in a particular geographical area. A reactivation of HHV 6 by HHV 7 has been postulated to be the culprit in the development of PR rash (7), and this may also be the case here, whereby DENV might reactivate the same.

In response to the question regarding T-cell mediation of PR etiopathogenesis, we would like to cite the example of Thelser’s murine encephalomyelitis virus (TMEV)-induced encephalomyelitis (8). According to this model, lymphocyte subtypes play a paradoxical role in the TMEV disease process; that is, they participate in virus clearance during the acute phase of the disease and aggravate the demyelinating process in the chronic phase of the disease (8). In view of these findings, it might be hypothesized that an extensive peripheral priming of polyfunctional cross-reactive T cells during symptomatic primary DENV infection due to high viral loads and continuous restimulation could potentially establish and maintain a distinct repertoire of virus-specific T cells, which might lead to epitope spreading and could create a predisposition to PR development (9). Genetic factors, such as the major histocompatibility complex haplotype, are not only directly responsible for disease susceptibility, but will also profoundly influence the antiviral immune response (9).

In a study by Han et al. (10), a time lag of 4 to 6 months was observed between H1N1 viral infection and onset of narcolepsy. This was attributed to minimum 80% cell loss to have a clinical presentation. Although the development of PR rash is not based on this mechanism, such lags are nonetheless not unheard of and should be investigated further for a different underlying mechanism in addition to the previously described mechanisms. As mentioned by the author, this was an incidental finding and we are currently trying to decipher and speculate on the underlying mechanisms of the rash development as well.

Finally, direct proof of causality for viruses to trigger autoimmunity is a challenge due to a variety of reasons; for example, temporal association. That is, the time since the onset of the clinical disease might be so remote from the trigger factor that it becomes too difficult to establish causality. Furthermore, at the time of diagnosis, easily detectable traces of a causative pathogen might no longer exist in the host’s system (11).

We would like to challenge the statement that the timeline does not coincide with the development of PR rash; instead, it highlights and supports the Trojan horse phenomenon of autoimmunity.

References


SP Dynamis
Vrhunski laserski sistem in inovativna tehnologija

Er:YAG (2940 nm) Do mikrometra natančna hladna in vroča ablacija brez poškodb okoliških tkiv
- Odstanjevanje vseh tipov benignih lezij
- Glajenje kože
- Tretmaji brazgotin in strij
- Smrčanje

Nd:YAG (1064 nm) Dopolnjuje efekt Er:YAG z najbolj varnimi globinskimi tretmaji
- Odstanjevanje varic in vaskularnih lezij
- Akne, roacea, glivice, bradavice
- Odstranjevanje dlak
- Kirurški posegi - krčne žile, laserska lipoiliza, hiperhidroza

Unikatne patentirane kombinacije obeh valovnih dolžin
Brezo uporabe polnil, stimulacija naravnega colagenja, popolnoma naraven videz
- Neinvazivni facelift - Fotona4D®
- Glajenje gub okoli oči - SmoothEye®
- Oblikovanje polnejših ustnic brez uporabne filerjev - LipLase™
- Neinvazivno »topljenje« maščob in zategovanje kože - TightSculpting®

Nudimo vrhunska šolanja in individualne pristope pri uvajanju laserske tehnologije v vašo prakso.

Že 55 let predani izdelavi najkakovostnejših in najzmogljivejših laserjev.

www.fotona.com
Sorel combo mazilo

Sorel combo 50 mikrogramov/500 mikrogramov v 1 g mazilo v tubi s 60 g mazila

Za lokalno zdravljenje stabilne psoriaze vulgaris v plakih pri odraslih.

Sorel combo mazilo vsebuje kombinacijo kalcipotriola in betametazona.

SKRAJŠANI POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Sorel combo 50 mikrogramov/500 mikrogramov v 1 g mazilo

SESTAVA: En gram mazila vsebuje 50 mikrogramov kalcipotriola (v obliki monohidrata) in 0,5 mg betametazona (v obliki dipropionata). Za celoten seznam pomožnih snovi glejte poglavje 6.1 SmPCja.

TERAPEVTSKE INDIKACIJE: Topikalno zdravljenje stabilne psoriaze vulgaris v plakih pri odraslih, kjer je mogoče topikalno zdravljenje.

ODMERJANJE IN NAČIN UPORABE: Odmerjanje: Zdravilo Sorel combo mazilo nanašamo na prizadete predele enkrat dnevno. Priporočeno trajanje zdravljenja je 4 tedne. Obstajajo izkušnje pri ponavljajočem se zdravljenju z zdravilom Sorel combo mazilo do 52 tednov. Če je po 4 tednih potrebno nadaljevati zdravljenje ali ga ponovno uvesti, se lahko zdravljenje nadaljuje po zdravniškem pregledu. Pri uporabi zdravil, ki vsebujejo kalcipotriol, največji dnevni odmerek ne sme presegati 15 g. Zdravil, ki vsebujejo kombinacijo kalcipotriola in betametazona, je kontraindicirano pri bolnikih z znanimi motnjami presnove kalcija.


NEŽELENI UČINKI: Pogosti: pruritis in luščenje kože. Občasni: bakterijske, glivične in virusne okužbe kože, folikulitis, atrofija kože, poškodovanje psoriaze, dermatitis, eritem, izpuščaj, poslabšanje psoriaze, dermatitis, eritem, izpuščaj. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo. Med zdravljenjem je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo. Med zdravljenjem je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo. Med zdravljenjem je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo. Med zdravljenjem je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo. Med zdravljenjem je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože. Če se ne izboljša v tekučem času, je treba izvedeti za zdravilo aatrofi kože.
materinega odmerka certolizumab pegola, ki doseže dojenčka v 24-urnem obdobju, od 0,04 % do 0,30 %. Poleg tega je certolizumab pegol beljakovina, ki se po peroralnem zaužitju razgradi v ob rojstvu pa je bilo 0,09 %. Po 4 in 8 tednih so bile koncentracije pri vseh dojenčkih pod mejo kvantifikacije. Klinični pomen majhnih koncentracij certolizumab pegola pri dojenčkih ni znan.

so bile pri 13 vzorcih pod mejo kvantifikacije (BLQ-nosečnostjo, ni. Uporaba zdravila Cimzia v času nosečnosti lahko zaradi zaviranja TNF vpliva na normalen imunski odziv pri novorojencu. Zdravilo Cimzia se sme uporabljati med nosečnostjo:

Po začetnem odmerku je vzdrževalni odmerek zdravila Cimzia za odrasle bolnike s psoriazo v plakih 200 mg vsaka 2 tedna. Pri bolnikih z nezadostnim odzivom se lahko razmisli
Psoriaza v plakih: Po začetnem odmerku je vzdrževalni odmerek zdravila Cimzia za odrasle bolnike s psoriazo v plakih 200 mg vsaka 2 tedna. Pri bolnikih z nezadostnim odzivom se lahko razmisli o odmerku 400 mg vsaka 2 tedna. Razpoložljivi podatki pri odraslih z psoriazo v plakih kažejo, da je klinični odziv običajno dosežen v 16 tednih zdravljenja. Pri bolnikih, ki so izpustili odmerek, svetujemo, da si naslednji odmerek zdravila Cimzia vbrizgajo takoj, ko je to mogoče, ter nato z aplikacijo nadaljnjih odmerkov nadaljujejo po osnovnih

Ko je klinični odziv potrjen, se lahko uporabi alternativni vzdrževalni odmerek certolizumab pegola 400 mg vsaka 4 tedna. Pri rezemalni nobestnosti je treba razmisliti o nadaljevanju zdravljenja. Pri nekaterih bolnikih z začetnim delnim odzivom se lahko stanje z nadaljevanjem po 16 tednih

Zdravnik se mora z bolnikom pogovoriti, katera oblika injiciranja je najprimernejša. Zdravnik se mora z bolnikom pogovoriti, katera oblika injiciranja je najprimernejša.
DUPIXENT®
dupilumab
DOLGOROČNI NADZOR

PRVO BIOLOŠKO ZDRAVILO za
zdravljenje zmernega do hudega ATOPIJSKEGA DERMATITISA pri odraslih

Hitro in trajno izboljšanje:
obsega in resnosti vnetja kože¹,²
intenzivnosti srbečice¹
kakovosti življenja¹,²

Dokazan dolgoročni varnostni profil²


www.sanofigenzyme.com

SAMO ZA ZDRAVSTVENE DELAVCE
Sanofi-Aventis d.o.o. | Letališka cesta 29a | 1000 Ljubljana | Slovenija | GZHS.DUP.19.11.0580 (12/19)
Dupilumab je mogoče uporabljati skupaj z lokalnimi kortikosteroidi ali brez njih. Uporabiti je mogoče lokalne zaviralke kalcinevrina, vendar mora biti njihova uporaba omejena na predele, ki so težavi za zdravljenje, na primer obraz, vrat, intertriginosni predeli in predel spolov. Pri bolnikih, ki se v 16 tednih zdravljanja atopijske dermatologije ne odzivajo, je treba razmisлитi o prenehanju zdravljenja. Nekaterim bolnim, ki so uvodni omeje značajno bolje od uporabe lokalnih kortikosteroidi, je treba prenehati z njihovim uporabo in prepričati se, da je dovoljno dobro zdravljenje.

**Terapevtske indikacije:** Atopijski dermatitis: Zdravljenje z dupilumabom je priporočljivo pri odraslih in mladostnikih, starih 12 let ali več, ki so kandidati za sistemsko zdravljenje. Astma: Zdravljenje začetnih ali nadaljevalnih simptomov za občutično astmo, ki je zaznana pri pacientih z občutljivostjo na vnetja tipa I-IV.

**Kontraindikacije:** Prirosti s hudo okvaro ledvic, ki so kandidati za sistemske zdravila, astma: dodatno vzdrževalno zdravljenje hude astme z značilno povišanim številom krvnih eozinofilcev in/ali s povišanim deležem izdhanega dušikovega oksida (dino) pri odraslih in mladostnikih, starih 12 let ali več, ki bo lečevo z učinkom, ki je priporočljiva za odmeve ali terapijo nasprotnika, ki je priporočljiva za odmeve ali terapijo nasprotnika.

**Neželeni učinki:**

- **Pogosten:** edem na mestu injiciranja, bolečina na mestu injiciranja, srbenje na mestu injiciranja.
- **Zelo pogosten:** anafilaktična reakcija.
- **Zelo redki:** eritem na zelo pogosten, edem na zelo pogosten.
- **Zelo redki:** anafilaktična reakcija.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.

**Neželeni učinki:**

- **Pogosten:** edem na mestu injiciranja, bolečina na mestu injiciranja, srbenje na mestu injiciranja.
- **Zelo pogosten:** anafilaktična reakcija.
- **Zelo redki:** eritem na zelo pogosten, edem na zelo pogosten.
- **Zelo redki:** anafilaktična reakcija.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.

**Kontraindikacije:** Prirosti s hudo okvaro ledvic, ki so kandidati za sistemske zdravila, astma: dodatno vzdrževalno zdravljenje hude astme z značilno povišanim številom krvnih eozinofilcev in/ali s povišanim deležem izdhanega dušikovega oksida (dino) pri odraslih in mladostnikih, starih 12 let ali več, ki bo lečevo z učinkom, ki je priporočljiva za odmeve ali terapijo nasprotnika, ki je priporočljiva za odmeve ali terapijo nasprotnika.

**Neželeni učinki:**

- **Pogosten:** edem na mestu injiciranja, bolečina na mestu injiciranja, srbenje na mestu injiciranja.
- **Zelo pogosten:** anafilaktična reakcija.
- **Zelo redki:** eritem na zelo pogosten, edem na zelo pogosten.
- **Zelo redki:** anafilaktična reakcija.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.
- **Zelo redki:** pričutek, ki je zelo redki.

**Kontraindikacije:** Prirosti s hudo okvaro ledvic, ki so kandidati za sistemske zdravila, astma: dodatno vzdrževalno zdravljenje hude astme z značilno povišanim številom krvnih eozinofilcev in/ali s povišanim deležem izdhanega dušikovega oksida (dino) pri odraslih in mladostnikih, starih 12 let ali več, ki bo lečevo z učinkom, ki je priporočljiva za odmeve ali terapijo nasprotnika, ki je priporočljiva za odmeve ali terapijo nasprotnika.
The Complete Cosentyx Approach is defined as efficacy in both skin and persistent psoriasis manifestations in nails, scalp, palms, and soles, as well as psoriatic arthritis; controls irreversible structural damage (PASI) and improves quality of life.

**NATIONAL SUCCINCT STATEMENT**

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 human monoclonal antibody produced in Chinese Hamster Ovary (CHO) cells. Therapeutic indications: Psoriasis, moderate to severe plaque psoriasis in adults who are candidates for systemic therapy, Psoriatic arthritis, alone or in combination with methotrexate (MTX) in adults 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. Psoriasis: 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-TNF 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. Based on clinical response the dose can be increased to 300 mg. Ankylosing spondylitis: 150 mg of secukinumab. Based on clinical response, the dose can be increased to 300 mg. Erythema nodosum: 300 mg of secukinumab. No dose adjustment is required. Based on immunogenicity studies, 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. The solution the syringe must not be shaken. 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: Hypersensitivity 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: Pregnancy: In order to improve the traceability of biological medicinal products, the name and the batch number of the administered product should be clearly recorded. Infertility: Secukinumab has the potential to increase the risk of infections. Serious infections have been observed in patients receiving secukinumab in the post-marketing setting. Caution should be exercised when considering the use of secukinumab 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 physician should closely monitor and secukinumab should not be administered until the infection resolves. 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 secukinumab, non-serious mucocutaneous candida infections were more frequently reported for secukinumab than placebo in the psoriasis clinical studies. 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 secukinumab in patients with latent tuberculosis. Inflammatory bowel disease: Cases of new or exacerbations of Crohn's disease and ulcerative colitis have been reported. Caution should be exercised when prescribing secukinumab to patients with inflammatory bowel disease, including Crohn's disease and ulcerative colitis. Patients should be closely monitored. Hypersensitivity reactions: In clinical studies, rare cases of anaphylactic reactions have been observed in patients receiving secukinumab. If an anaphylactic or other severe allergic reactions occur; administration of secukinumab 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 secukinumab. Patients receiving secukinumab may receive concurrent inactivated or non-live vaccinations. Secukinumab does not suppress the humoral immune response to the meningococcal or influenza vaccines. Concomitant immunosuppressive therapy: In psoriasis studies, the safety and efficacy of secukinumab in combination with immunosuppressants, including biologics, or phototherapy have not been evaluated. Secukinumab was administered concomitantly with methotrexate, sulfasalazine and/or corticosteroids in arthritis studies (including in patients with psoriatic arthritis and ankylosing spondylitis). Caution should be exercised when considering concomitant use of other immunosuppressants and secukinumab. Women of childbearing potential: Women of childbearing potential should use an effective method of contraception during treatment and for at least 30 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 secukinumab. In a study in subjects with plaque psoriasis, no interaction was observed between secukinumab and midazolam (Versed®) substrate. No interaction was seen when secukinumab was administered concomitantly with methotrexate 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, Rhinorrhea, Diarhoea. Uncommon: Oral candidiasis, Tinea pedis, Otis externa, Lower respiratory tract infections, Neutropenia, Conjunctivitis, Inflammatory bowel disease, Urticaria. Rare: Anaphylactic reactions, Exfoliative dermatitis. Not known: Mucosal and cutaneous candidiasis (including oropharyngeal candidiasis). Marketing authorisation holder: Novartis EUPharmu-Lembdt, Visto Building, Elm Park, Milton Road, Dublin 4, Ireland. Additional information and literature: Novartis Pharma Services Inc., Branch Office Slovenia, Venda hotel o u 57, 1000 Ljubljana. General classification for supply: Rp/Spec. Please read the summary of product characteristics before prescribing. This text was last revised in October 2019.
LOCOIDON™ lipocrema 1 mg/g krema
1 g krema vsebuje 1 mg hidrokortizonbutirata

Uravnoteženo razmerje med oljno in vodno fazo (posebej primerna za suho kožo, ki se lušči)
Rp P70 NDC € 2,70

Loclean™ (Lipocrema) uporabljamo pri kožnih obolenjih, ki se odzivajo na lokalno zdravljenje s kortikosteroidi:
- kontaktni ekzem (akutni, subakutni, kronični),
- dermatitis: navadni, detritivi, atopijski,
- solarni, lakični, hipoplastični, intertriginižni,
- disekeroločni, med kamentozni, fotodermatitis,
- psoriča,
- lichen ruber planus,
- kronični eritematodes,
- vzdrževalno zdravljenje dermatoz, ki so bile predhodno zdravljene z močnejšimi kortikosteroidi.

Kožna oboljena, ki se odzivajo na lokalno zdravljenje s kortikosteroidi
Močna učinkovitost z varnostnim profilom blagega steroide

SKRAJAN POUŠTEK GLAVNIH ZNAČILNOSTI ZDRAVLJA

IME ZDRAVLJA
Locleen™ lipocrema 1 mg/g krema

Kaksovina in količinska sestava
1 g krema vsebuje 1 mg hidrokortizonbutirata

Farmacevtska utilita
Locleen™ lipocrema je stopenjsko značilna, ko učinkovito zdravlja kožno obolenje.

Farmaceutski indikacije
Kožni oboljenci, ki se odzivajo na lokalno zdravljenje s kortikosteroidi:
- kontaktni ekzem (akutni, subakutni, kronični),
- dermatiti: navadni, detritivi, atopijski, solarni, lakični, hipoplastični, intertriginižni, disekeroločni, med kamentozni, fotodermatitis,
- psoriča,
- lichen ruber planus,
- kronični eritematodes,
- vzdrževalno zdravljenje dermatoz, ki so bile predhodno zdravljene z močnejšimi kortikosteroidi.

Zdravilo Locleen™ lipocrema ima umestno enotno razmerje med oljno in vodno fazo, kar določa učinkovito in roj izbruh. Postopek je primerno za suho kožo, ki se lušči.

Omejilo za uporabo
Prikazani je individualno in oprostno od normalnih umetnih zdravstvenih ustreznosti. Zdravilo vložimo na bolečino, ali na umetni nanos na oblasti.

Pobude opozoravek in proizvajalec
Pobude opozoravek in proizvajalec

NEUBAHN® NEUBAHN® prosteklo.

Razvojne učinkove ali zdravilne uporabe

Razvojne učinkove ali zdravilne uporabe

Kontrola:
Postopek obstaja za reaktivno ali vsakokratno postopek. Zdravilo je protokoliran in je v skladu z zimsko varnostno standardno standardo.

Kontraindikacije
Postopek obstaja za reaktivno ali vsakokratno postopek. Zdravilo je protokoliran in je v skladu z zimsko varnostno standardo standardo.

Neoznane in dejavnosti
Postopek obstaja za reaktivno ali vsakokratno postopek. Zdravilo je protokoliran in je v skladu z zimsko varnostno standardo standardo.