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*automated and standardized (N=134) and (N=185), respectively

† Setting of medication administration (clinic vs. self-administration) was not factored into this analysis.

‡ Additional data provided at Annual Meeting of the American Academy of Dermatology 2015, San Francisco, CA, USA.
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Department of Dermatovenerology, Zaloška 2, SI-1525 Ljubljana, Slovenia. Tel: +386 1 522 41 58, Fax: +386 1 522 43 33; E-mail: office@acta-apa.org

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NOVO

LA ROCHE-POSAY. PREDAN DERMATOLOGIJI.
The first 25 years of Acta Dermatovenerologica Alpina, Pannonica et Adriatica

Jovan Miljković, Editor in Chief

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

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

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

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

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

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

Although Acta Dermatovenerol APA is a “small journal from a small country,” it has significantly improved its quality and international profile over the last 25 years. When celebrating the 25th anniversary of Acta Dermatovenerol APA, we return once again to the vision of its founding editor, Aleksandar Kansky, and to the people that translated his vision into reality. The best recognition of their efforts is certainly the news that Thomson Reuters accepted Acta Dermatovenerol APA this year for coverage in the new Thomson Reuters Web of Science Core Collection index called the Emerging Sources Citation Index (ESCI).
Cosentyx was administered concomitantly with methotrexate (MTX) and/or corticosteroids in arthritis studies (including in patients with psoriatic arthritis and ankylosing spondylitis).

Dosage:

Posology: Cosentyx is intended for use under the guidance and supervision of a physician experienced in the diagnosis and treatment of conditions for which Cosentyx is indicated.

Recommended dose: Patients receiving Cosentyx should be followed closely. Hypersensitivity reactions: In clinical studies, rare cases of anaphylactic reactions have been observed in patients receiving Cosentyx. If an anaphylactic or other serious allergic reaction occurs, administration of Cosentyx should be discontinued immediately and appropriate therapy initiated. Latex sensitive individuals: The removable cap of the Cosentyx prefilled pen contains a derivative of natural rubber latex. No natural rubber latex has to date been detected in the removable cap. Nevertheless, the use of Cosentyx pre-filled pens in latex sensitive individuals has not been studied and there is therefore a potential risk for hypersensitivity reactions which cannot be completely ruled out. Vaccinations: Live vaccines should not be given concurrently with Cosentyx. Patients receiving Cosentyx may receive concurrent inactivated or non-live vaccinations. In a study, after meningooccal and inactivated influenza vaccinations, a similar proportion of healthy volunteers treated with 150 mg of secukinumab and those treated with placebo developed an adequate immune response of at least a 4 fold increase in antibody titres to meningooccal and influenza vaccines. The data suggest that Cosentyx does not suppress the humoral immune response to the active substance or to any of the exipients listed. Clinically important, active infection (e.g. active tuberculosis). Contraindications: Severe hypersensitivity reactions to the active substance or to any of the excipients listed. Clinically important, active infection (e.g. active tuberculosis). Special warnings and precautions for use: Infections: Cosentyx has the potential to increase the risk of infections. In clinical studies infections have been observed most of these were mild or moderate upper respiratory tract infections such as nasopharyngitis, and did not require treatment discontinuation. Related to the mechanism of action of Cosentyx, non serious mucocutaneous candida infections were more frequently reported for secukinumab than placebo in the psoriasis clinical studies (3.55 per 100 patient years for secukinumab 300 mg versus 1.00 per 100 patient years for placebo). Caution should be exercised when considering the use of Cosentyx in patients with a chronic infection or a history of recurrent infection. Patients should be instructed to seek medical advice if signs or symptoms suggestive of an infection occur. If a patient develops a serious infection, the patient should be closely monitored and Cosentyx should not be administered until the infection resolves. No increased susceptibility to tuberculosis was reported from clinical studies. However, Cosentyx should not be given to patients with active tuberculosis. Anti-tuberculosis therapy should be considered prior to initiation of Cosentyx in patients with latent tuberculosis. Crohn’s disease: Caution should be exercised when prescribing Cosentyx to patients with Crohn’s disease as exacerbations of Crohn’s disease, in some cases serious, were observed in clinical studies in both Cosentyx- and placebo-treated groups. Patients who are treated with Cosentyx and have Crohn’s disease should be closely monitored. No dose recommendations can be made.

Interaction with other medicinal products and other forms of interaction:

No interaction studies have been conducted. There is no direct evidence for the role of MTX in the expression of CYP450 enzymes. The formation of some CYP450 enzymes is suppressed by increased levels of cytokines during chronic inflammation. Thus, anti-inflammatory treatments, such as with the IL-17A inhibitor secukinumab, may result in normalisation of CYP450 levels with accompanying lower exposure of CYP450 metabolised co-medications. Therefore, a clinically relevant effect on CYP450 substrates with a narrow therapeutic index, where the dose is individually adjusted (e.g. warfarin) cannot be excluded. On initiation of secukinumab therapy in patients being treated with these types of medicinal products, therapeutic monitoring should be considered. No interaction was seen when Cosentyx was administered concomitantly with methotrexate (MTX) and/or corticosteroids in arthritis studies (including in patients with psoriatic arthritis and ankylosing spondylitis).


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

Lea Hošnjak1, Kristina Fujs Komloš1, Boštjan J. Kocjan1, Katja Seme1, Mario Poljak1

Abstract

Introduction: The present study describes the development and evaluation of the first multiplex type-specific quantitative real-time PCR (RT-PCR), enabling simple, rapid, sensitive, and specific concurrent detection and differentiation of human papillomavirus (HPV) types HPV2, 27, and 57 in a single PCR reaction.

Results: The HPV2/27/57 multiplex RT-PCR with a dynamic range of seven orders of magnitude (discriminating 10 to 10⁸ viral genome equivalents/reaction) has an analytical sensitivity of at least 10 viral copies of each targeted HPV type/reaction, and no cross-reactivities were observed among the included targets. All three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of 10⁴, 10⁵, 500, 100, and 10 copies of non-targeted viral DNA/reaction, and the performance of the HPV2/27/57 multiplex RT-PCR was additionally not affected by the presence of background human genomic DNA. When testing DNA isolates obtained from fresh-frozen tissue specimens of various children’s warts, the results of the HPV2/27/57 multiplex RT-PCR were completely in line with the results of the conventional Low-risk Alpha-PV PCR.

Conclusion: The newly developed HPV2/27/57 multiplex RT-PCR is an appropriate test for use in routine clinical laboratory settings and for studies focusing on the molecular epidemiology, pathogenesis, and natural history of HPV2/27/57-related lesions.

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

Introduction

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

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

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

Materials and methods

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

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

Table 1: Nucleotide sequences of primers and hybridization probes designed for amplification of partial L2 genes of HPV2, HPV27, and HPV57.

<table>
<thead>
<tr>
<th>Primer/probe</th>
<th>Nucleotide sequence (5’–3’)</th>
<th>Nucleotide positiona</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–27F(59.8)b</td>
<td>TACCGGCCCCCCGAGACATT</td>
<td>HPV2(4,386–4,404), HPV27(4,359–4,377)</td>
<td>HPV2(144-bp)</td>
</tr>
<tr>
<td>2R(59.2)</td>
<td>GGGCTTTCCTCCGCTAGGCTC</td>
<td>HPV2(4,529–4,550)</td>
<td>HPV27(145-bp)</td>
</tr>
<tr>
<td>2F(57.6)</td>
<td>AGAATATACCCGGTACGTCC</td>
<td>HPV2(4,503–4,508)</td>
<td>HPV27(145-bp)</td>
</tr>
<tr>
<td>57F(57.8)</td>
<td>GCACAGGCGTGCAAGC</td>
<td>HPV57(4,327–4,343)</td>
<td>HPV57(157-bp)</td>
</tr>
<tr>
<td>57R(57.9)</td>
<td>GGTATGACGCTGTTGCTG</td>
<td>HPV57(4,483–4,496)</td>
<td></td>
</tr>
<tr>
<td>HPV2-Po(68.25)</td>
<td>TEX-CCCAAGAGTGAGACACACATTTAGCA-BQQ</td>
<td>HPV2(4,407–4,434)</td>
<td></td>
</tr>
<tr>
<td>HPV27-Po(68.55)</td>
<td>YAK-CTAGGGCTCCTCTTTGGGCTGTG-BQQ</td>
<td>HPV27(4,432–4,456)</td>
<td></td>
</tr>
<tr>
<td>HPV57-Po(65.34)</td>
<td>FAM-TGCGCCCTGATCTGACTTGG-</td>
<td>HPV57(4,425–4,448)</td>
<td></td>
</tr>
</tbody>
</table>

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

Figure 1: Schematic diagram showing hybridization of HPV2, HPV27, and HPV57 type-specific hybridization probes to respective L2 gene sequences. The figure was obtained from a multiple sequence alignment of type-specific hybridization probes and complete genome sequences of respective HPV types that were retrieved from the GenBank database (GenBank accession numbers are provided next to all full genome sequences included). Dots show the nucleotide positions of hybridization probes identical to the targeted regions of HPV2, HPV27, and HPV57.
fied using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and cloned into plasmid vectors with the TOPO XL PCR Cloning Kit (Thermo Fisher Scientific). Plasmid HPV2/27/57 DNA was purified using the QIAprep Spin Miniprep Kit (Qiagen), verified by direct Sanger sequencing and quantified at 260 nm using a NanoDrop ND-2000c spectrophotometer (NanoDrop Technologies, Oxfordshire, UK). The quantified plasmids contained 1.31 × 10⁸, 5.30 × 10⁵, and 2.59 × 10⁴ copies of HPV2, 27, and 57 DNA per µl, respectively, and were subsequently serially diluted, as described previously (38). All commercially available reagents were used according to the manufacturers’ instructions.

The HPV2/27/57 multiplex RT-PCR was performed in a 96-well plate on a LightCycler 480 Instrument II using a LightCycler 480 Probes Master Kit (Roche Diagnostics, Mannheim, Germany). The RT-PCR protocol was designed following the manufacturer’s instructions and adjusted to (i) characteristics of targeted nucleotide sequences and synthesized primers/probes and (ii) estimated length of RT-PCR amplicons. The thoroughly optimized reaction mixture consisted of 10 µl of 2 × LightCycler 480 Probes Master (Roche Diagnostics), 0.5 µM of each RT-PCR primer (Table 1), with the exception of the 2-27F(59.8) primer, which was used in a concentration of 1 µM, 0.1 µM of each probe, 5 µl of template DNA (50–100 ng of DNA extracted from clinical samples and 1 × 10⁻¹ to 1 × 10⁶ DNA copies/reaction of plasmid standards), and PCR-grade water up to the final reaction volume of 20 µl. The amplification of targeted nucleotide sequences was performed as follows: (i) initial denaturation of template DNA at 95 °C for 10 min (temperature transition rate of 4.4 °C/s), (ii) followed by 40 amplification cycles consisting of three incubation steps: 95 °C for 10 s (4.4 °C/s), 60 °C for 30 s (2.2 °C/s), and 72 °C for 1 s (4.4 °C/s; fluorescent signal acquisition), and (iii) a final cooling step at 2.2 °C/s to 40 °C with a 30 s hold. Since type-specific hydrolysis probes were labeled with three different 5’ fluorophores (TEX, YAK, and FAM; Table 1), real-time monitoring of the fluorescent signal was performed on 610, 560, and 530 nm channels, indicating amplification of HPV2, HPV27, and HPV57, respectively. In addition, due to the slight overlap of the emission spectra of the dyes, the software’s color compensation function was applied during the analysis of all RT-PCR experiments. Moreover, the specificity of all HPV2/27/57 RT-PCR amplicons was further confirmed by direct Sanger sequencing with the same primers as used for the RT-PCR, as described previously (39).

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

The tissue samples were first processed for total DNA extraction with a QIAamp DNA Mini Kit (Qiagen) and spectrophotometric analysis of eluted DNA, as described previously (40). Up to 100 ng of extracted DNA was used for downstream PCR analyses. The integrity of the extracted DNA was determined by the quantitation RT-PCR, enabling amplification of the 150-bp fragment of human beta-globin gene. Briefly, the beta-globin RT-PCR was performed on a LightCycler 2.0 Instrument (Roche Diagnostics) using a Quantitect SYBR Green PCR + UNG Kit (Qiagen). The reaction mixture consisted of 12.5 µl of 2 × Quantitect SYBR Green PCR Master mix, 0.5 µM of each primer (41), 5 µl of extracted DNA, and PCR-grade water up to the final reaction volume of 25 µl. The amplification of human DNA was performed as follows: (i) initial denaturation of template DNA at 95 °C for 15 min (temperature transition rate of 20 °C/s), (ii) followed by 45 amplification cycles consisting of three incubation steps: 94 °C for 15 s (20 °C/s), 60 °C for 20 s (20 °C/s), and 72 °C for 20 s (2 °C/s; fluorescent signal acquisition at 530 nm), (iii) a melting curve analysis, consisting of three incubation steps: 95 °C for 0 s (20 °C/s), 50 °C for 30 s (20 °C/s), and 95 °C for 0 s (0.1 °C/s), and (iv) a final cooling step at 20 °C/s to 40 °C with a 30 s hold. Testing triplicates of 10-fold serially diluted standards of commercially available human DNA (Human Genomic DNA; Promega, Madison, WI), spanning from 100 ng to 1 pg of DNA per reaction, showed that the beta-globin RT-PCR had a sensitivity of at least 10 pg of human DNA per reaction. The correlation coefficient (R²) of the standard curve estimated from amplification of human DNA standards over six orders of magnitude and the efficiency of human DNA amplification (E) were estimated at 0.996 and 91.4%, respectively. Only beta-globin-positive DNA isolates (melting peaks between 80.5 and 81.5 °C) were considered adequate for further analyses.

To detect low-risk Alpha-PVs associated with various mucosal and cutaneous warts, a PCR protocol targeting an approximately 190-bp fragment of the E1 gene of HPV2, HPV3, HPV6, HPV7, HPV10, HPV11, HPV13, HPV17, HPV28, HPV29, HPV32, HPV39, HPV40, HPV42, HPV43, HPV44, HPV57, HPV74, HPV77, HPV78, HPV91, HPV94, HPV95, and HPV125 was performed, as described elsewhere (31), and HPV types were subsequently determined by direct Sanger sequencing of all eligible PCR products, as described previously (39). Furthermore, a FRET-based HPV6/11 RT-PCR (40), enabling reliable detection and differentiation of 25.3, 42.9, and 43.4 DNA copies of HPV11 and prototypic and non-prototypic HPV6 genotypic variants, respectively, was additionally used to determine the causal agents of condylomata acuminata.

**Results**

Testing replicates of 10-fold serially diluted plasmids containing targeted fragments of HPV2, HPV27, and HPV57 in concentrations spanning from 1 × 10⁴ to 1 × 10¹ DNA copies per reaction, in a background of 100 ng of Human Genomic DNA, showed that the HPV2/27/57 multiplex RT-PCR is able to detect at least 10 viral copies of each targeted HPV type per a single reaction (Fig. 2; A1, B1, C1). The dynamic range of HPV2/27/57 multiplex RT-PCR was seven orders of magnitude for all targeted HPV types, enabling reliable discrimination of 10 to 10¹ viral genome equivalents per a single reaction. The correlation coefficients (R²) of standard curves estimated from amplification of plasmid standards containing fragments of HPV2, HPV27, and HPV57 were 0.999, 0.999, and 0.998, respectively. The amplification efficiencies (E) were estimated at 95.2, 92.0, and 92.2% for HPV2, HPV27, and HPV57, respectively, and the performance of the HPV2/27/57 multiplex RT-PCR was not affected by the presence of background human genomic DNA. In addition, as shown in Fig. 2 (A2, B2, and C2), no cross-reactivities of HPV27/HPV57, HPV2/HPV57, and HPV2/HPV27 were observed when using primer/probe combinations targeting HPV2, HPV27, and HPV57, respectively. Moreover, all three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of 1 × 10⁴, 1 × 10³, 500, 100, and 10 copies of non-targeted viral DNA per reaction (Fig. 2; A3, B3, C3).

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

**Figure 2** | Evaluation of the performance of HPV2/27/57 multiplex RT-PCR based on the amplification of plasmid standards containing targeted nucleotide sequences of HPV2, HPV27, and HPV57. (A1, B1, and C1) RT-PCR amplification plots of replicates of 10-fold serially diluted plasmids containing targeted fragments of HPV2 (A1), 27 (B1), and 57 (C1) in concentrations spanning from $1 \times 10^8$ to $1 \times 10^4$ DNA copies per reaction, in a background of 100 ng of commercially available human DNA (Human Genomic DNA; Promega, Madison, WI), showing that the HPV2/27/57 multiplex RT-PCR is able to detect at least 10 viral copies of each targeted HPV type per a single reaction. (A2, B2, and C2) No amplification of HPV27/HPV57 (A2), HPV2/HPV57 (B2), and HPV2/HPV27 (C2) was observed when using primer/probe combinations targeting HPV2, HPV27, and HPV57, respectively. (A3, B3, and C3) RT-PCR amplification plots showing that all three primer/probe combinations are efficient in amplifying 500 copies of HPV2 (A3), HPV27 (B3), and HPV57 (C3) in a background of $1 \times 10^8$, $1 \times 10^7$, 500, 100, and 10 viral copies of HPV27/HPV57, HPV2/HPV57, and HPV2/HPV27 per reaction, respectively.
Discussion

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

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

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

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Beta-globin RT-PCR</th>
<th>Low-risk Alpha-PV PCR</th>
<th>HPV6/11 RT-PCR</th>
<th>HPV2/27/57 multiplex RT-PCR</th>
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Legend/abbreviations: aThe integrity of the extracted DNA was determined by the quantitative RT-PCR, enabling amplification of the 150-bp fragment of human beta-globin gene. A previously published Low-risk Alpha-PV PCR (31) was used to detect Alpha-PVs that are most frequently associated with various mucosal and cutaneous variants, respectively, was used to determine the causal agents of condylomata acuminata. Tissue samples obtained from patients nos. 8–10 and 31–35 were additionally tested for the presence of Gamma/Mu-PVs and/or MCV (data not shown).
taneous and mucosal wart-associated Alpha-PV types (31). In contrast, because more than 90% of warts identified in the anogenital region of adult patients are etiologically associated with sexually transmitted HPV6 and HPV11 (11, 12, 16–18, 43), when testing this patient population the method of choice in our laboratory is the HPV6/11 RT-PCR (40), followed in the case of a negative result by HPV2/27/57 multiplex RT-PCR and Low-risk Alpha-PV-PCR (31). Alpha-PV-negative wart tissue samples are additionally tested in our laboratory for research purposes only for the presence of several Gamma- and Mu-PVs that cause sporadic cutaneous warts (7, 9, 11, 12, 26, 44, 45). All HPV-negative warts identified in the anogenital region of patients of all ages and both sexes are additionally tested in our laboratory for the presence of molluscum contagiosum virus (MCV) using the MCV FRET RT-PCR (45) because, due to the similar clinical appearance of lesions, up to 10% of molluscum contagiosum lesions can be misdiagnosed as condylomata acuminata or verrucae vulgares and vice versa (45–48).

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

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

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

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Conflicts of interest

The authors have no conflicts of interest to declare.

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References


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Celočen položaj za neželeni učinki:

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Neželeni učinki:

- Pri bolnikih, ki so pod vplivom immunomodulacijskih zdravil ali druge zdravil, ki so podvzletali neželenih učinkov, je bil odziv na Enbrel® nezadosten.

Neželeni učinki:

- Previdnost je potrebna pri bolnikih, ki so pod vplivom immunomodulacijskih zdravil ali druge zdravil, ki so podvzletali neželenih učinkov, je bil odziv na Enbrel® nezadosten.

Celočen položaj za neželeni učinki:

Previdnost je potrebna pri bolnikih, ki imajo trudno alergijo ali večalo, hkrati z otrokom, ki je pod vplivom immunomodulacijskih zdravil ali ki je pod vplivom druge zdravila, ki so podvzpletali neželenih učinkov.
Microbiological characteristics of perianal streptococcal dermatitis: a retrospective study of 105 patients in a 10-year period

Anja Šterbenz1, Katja Seme1, Liza Lea Lah1, Olga Točkova2, Tina Kamhi Trop3, Nataša Švent-Kučina1, Mateja Pirš1

Abstract

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

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

Introduction

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

Results

In the 10-year study period, we identified a total of 105 BHS isolates from rectal or perianal skin swabs that were submitted to the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, between January 2006 and December 2015. Only the first BHS isolate from the perianal region of each patient was included in the study. Sampling sites included the rectum, perianal skin, and perineum. For each patient with a positive BHS culture, data were collected on patient age and sex, streptococcal species, and antimicrobial susceptibility. Identification was confirmed to the species level by colony morphology, catalase test, and a commercial latex agglutination test (PathoDxXtra Strep Grouping Kit, Thermo Fisher Scientific, Waltham, MA, USA) or matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility was determined using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines until April 2014 and afterwards the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (15, 16).

Materials and methods

We retrospectively reviewed microbiology laboratory records and searched for BHS isolates in the perianal area. BHS isolates from rectal or perianal skin swabs that were submitted to the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, between January 2006 and December 2015 were included in the study. Only the first BHS isolate from the perianal region of each patient was included in the study. Sampling sites included the rectum, perianal skin, and perineum. For each patient with a positive BHS culture, data were collected on patient age and sex, streptococcal species, and antimicrobial susceptibility. Identification was confirmed to the species level by colony morphology, catalase test, and a commercial latex agglutination test (PathoDxXtra Strep Grouping Kit, Thermo Fisher Scientific, Waltham, MA, USA) or matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility was determined using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines until April 2014 and afterwards the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (15, 16).
is presented in Figure 2. The majority of streptococcal isolates were obtained from children younger than 15 (89/105; 84.7%). The median age of patients was 5 (average 11.8 years, age range 1 to 84 years) and 73 out of 105 (69.5%) were male (Fig. 3).

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

**Discussion**

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

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

Our study evaluated a 10-year distribution of BHS in swabs from the rectum and perianal skin that were submitted to our laboratory for bacterial culture. As shown in Figure 1, the majority of isolates were GABHS, followed by GBBHS and non-group A or B beta-hemolytic streptococci (groups C and G). The seasonal distribution of all GABHS, GBBHS, and non-group A or B BHS perianal cultures is presented in Figure 4. Almost half (46.6%) of the GABHS perianal cultures were obtained during the spring (between March and June), and the second peak was observed in December. Similar to GABHS, the number of GBBHS perianal cultures peaked in the spring and winter months. Only a small number of non-group A or B BHS belonging to groups C and G were isolated. All GABHS isolates were susceptible to penicillin and clindamycin, whereas the rate of erythromycin resistance was 1.4%. All GBBHS isolates were susceptible to penicillin, whereas 14.8% and 7.4% were resistant to erythromycin and clindamycin, respectively. The number of non-group A or B BHS isolates was too low to reliably assess antimicrobial susceptibility; nonetheless, all of the isolates were susceptible to penicillin.
streptococcal cultures were obtained from children under 15. Our results are in accordance with previous studies, suggesting that, although PSD predominantly occurs in young children, it should not be considered an exclusively pediatric disease (1, 3, 6). More than 66% of BHS perianal isolates obtained in our study were from male patients, which is in agreement with previous observations suggesting male predilection of PSD (Fig. 3) (2, 3, 20).

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

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

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

Early initiation of antibiotic treatment provides rapid improvement of symptoms (8). Our study has shown that susceptibility of BHS to penicillin remains excellent. GABHS isolates are rarely resistant to erythromycin or clindamycin, whereas higher resistance rates for both antibiotics were observed in GBBHS isolates. A 7- to 10-day course of oral penicillin V (50,000 to 100,000 IU/kg) is considered to be the initial treatment of choice for pediatric GABHS-associated PSD (2, 3, 13, 14, 26, 25). However, recurrence of the disease may occur in up to 39% of children treated and a repeated course of antibiotics is necessary, whereas some advocate prolonged treatment (e.g., 14–21 days) (26–30). Unfortunately, studies comparing the optimal duration of antibiotic therapy are lacking. Alternatively, children can be treated with oral amoxicillin (50 mg/kg/day) and, if compliance is an issue, one dose of penicillin G 1.2 M IU im can be used in children weighing > 27 kg, and one dose of penicillin G 600,000 IU im in children weighing < 27 kg (24, 25). In children with penicillin allergy, midacemycin (40 mg/kg/day), clarithromycin (15 mg/kg/day), or clindamycin (30 mg/kg/day) can be used (24, 25), although data regarding their efficacy rely solely on a subset of treated children (2, 4, 14, 20, 31).

To date, cefuroxime is the only alternative antibiotic in treatment of PSD that has been assessed in a randomized controlled trial (13). In comparison to penicillin, an increased efficacy of a 7-day course of cefuroxime (20 mg/kg/day) was observed, with shorter duration of symptoms and faster bacterial eradication (13, 14). However, the study was not blinded and, because no follow-up was performed after the end of the treatment, optimal duration of antibiotic therapy with cefuroxime could not be evaluated (13). Furthermore, usage of cephalosporins is not recommended for treatment of BHS due to their broad spectrum of activity, which can lead to the development of antibiotic resistance in other bacteria (24). In addition to oral therapy, patients can also receive topical treatment with antiseptics (e.g., chlorhexidine) or antibiotics (e.g., bacitracin, mupirocin, fusidic acid, erythromycin, and gentamicin), although their usefulness remains uncertain (2, 3, 14, 19, 30). Unfortunately, no controlled trials were conducted to evaluate the efficacy of antimicrobial therapy for non-GABHS-associated PSD. In adults with predominantly GBBHS-induced PSD, a 7- to 10-day treatment with oral penicillin V (1–1.5 M IU/day) is considered standard therapy (1, 6, 24, 25). Alternatively, patients can receive one dose of penicillin G 1.2 M IU/day im or, when penicillin allergy is suspected, oral midecamycin (400 mg tid), clarithromycin (250–500 mg bid), and azithromycin (500 mg 1st day, 250 mg 2nd–5th day) (25). However, physicians should be aware of important differences between pediatric and adult cases of PSD. Only 42% of adult patients with PSD are successfully treated with the first course of oral antibiotics, possibly due to the higher minimal inhibitory concentration for penicillin in GBBHS compared to GABHS, hence a higher dosage of the same antibiotic might be necessary, whereas some advocate prolonged treatment (6, 32). Kahlke et al. (6) have clearly shown that the presence of concomitant dermatological and/or anorectal conditions that have not yet developed in children (e.g., hemorrhoids, skin tags, anogenital warts, and anal cancer) contribute to reduced rates of successfully treated infections in adults (1, 6). In addition, these conditions can present with symptoms that are otherwise observed in PSD (e.g., perianal erythema in patients with hemorrhoids) and may be the reason for frequent misdiagnosis in adults.
(6). Although complications of PSD are rare, a urine analysis may be performed to screen for possible post-streptococcal glomerulonephritis (19, 30).

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

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

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

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

Funda Tamer1 ✉

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

Introduction

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

Vitiligo is characterized by depigmented macules and patches that are widely and symmetrically distributed. Autoimmune mechanisms play an important role in the etiology. Physical and emotional stress can trigger vitiligo in genetically predisposed patients. However, coexistence of erythema dyschromicum perstans and vitiligo is extremely rare, and similar immune mechanisms have been implicated in the pathogenesis of these cutaneous pigmentary disorders.

Keywords: erythema dyschromicum perstans, vitiligo

Abstract

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

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

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

Coexistence of erythema dyschromicum perstans and vitiligo

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

Wood’s lamp examination showed discrete depigmentation with sharp borders. The patient admitted that the depigmented lesion had been present for the last 6 months. Moreover, the lesion appeared as a small macule and it gradually increased in size. It was asymptomatic and there were no other depigmented macules elsewhere. Thus, the diagnosis of vitiligo was made based on the clinical features and Wood’s lamp examination. Furthermore, the patient admitted that the hyperpigmented lesions first appeared on the back and had extended to the occipital region and chest over the last 5 years. He had used topical corticosteroids previously, but no clinical response had been achieved. The past medical history was unremarkable. He denied taking any medication. The skin biopsy was taken from the hyperpigmented lesions on the middle of the back. Histopathological examination revealed vacuolar degeneration on the basal layer, melanophages, and...
lymphocytic infiltration in the upper dermis. Periodic acid–Schiff, crystal violet, and Congo red stains were performed and the specimen did not show any amyloid deposits or metachromasia (Fig. 2). Therefore we confirmed the diagnosis of erythema dyschromicum perstans. The patient was put on topical steroid and oral antihistaminic treatment.

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

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

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

**Conclusion**

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

**References**

Methotrexate-induced panniculitis in a patient with rheumatoid arthritis

Raghda Al Maashari1 ✉, Mowafak M. Hamodat2

Abstract

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

Keywords: panniculitis, methotrexate, rheumatoid arthritis, nodulosis

Introduction

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

Case history

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

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

At that time, the laboratory data were as follows: white blood cell count 8.4 × 109/l (normal range 4.5–11 × 109/l), hemoglobin 127 g/l (117–155 g/l), platelet count 406 × 109 (normal range 140–450 × 109/l), erythrocyte sedimentation rate 10 mm/hr (normal range 0–20 mm/hr), and a positive antinuclear antibody titer 1:80 speckled pattern. Rheumatoid factor, cyclic citrullinated peptide, and extractable nuclear antigen were all negative.

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

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

Discussion

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

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

The first report that documented the occurrence of MIAN was published in 1986 (3) and since then a number of case reports and systematic studies have reported this phenomenon, which describes the development or acceleration of nodulosis in patients receiving methotrexate therapy for autoimmune conditions. This phenomenon is thought to occur in eight to 10 percent of rheumatoid arthritis patients (1). The time period between the beginning...
An interval of methotrexate administration and the development of the nodules is variable (weeks to years) (4).

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

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

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

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

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

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

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

References

Samo za strokovno javnost.

SI.BCC.11.2012.0042

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

1. korak
Odstranjevanje okuženega nohta

2. korak
Nadaljevanje zdravljenja okuženega dela kože s protiglivično kremo

Zdravljenje v dveh korakih omogoča:
• Hitro in temeljito odstranjevanje okuženega dela nohta
• Dnevno vidno napredek¹
• Enostavno zdravljenje brez bolečin¹
• Globinsko odstranjevanje glivic²

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

Skrajšan povzetek glavnih značilnosti zdravila

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

Samo za strokovno javnost.
Erythema exsudativum multiforme induced by a taurine-containing energy drink

Antigona Mithat Begolli Gerqari¹, Mybera Ferizi², Sadije Halimi², Aferdita Daka², Syzana Hapciu², Ilir Mithat Begolli¹✉, Mirije Begolli¹, Idriz Hysen Gerqari¹

Abstract

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

Keywords: Erythema exsudativum multiforme, taurine, vasculitis

Introduction

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

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

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

Case report

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

Discussion

Erythema exsudativum multiforme is an immunologically mediated skin reaction or a reaction to viruses or bacteria (10), classified in the group of type-IV delayed cell-mediated hypersensitivity. The minor form is localized on the skin and the mucosa are not involved (1, 9). The typical skin sign is herpes iris, or target lesions with a red to reddish-blue color. The localization of the changes occur in photo-exposed areas. The main causes are various drugs, food containing some additives, bacteria, especially streptococcus, and some viruses, such...
as herpes simplex virus and Epstein Barr virus in particular. Patients may experience several recurrences per year. Furthermore, the major form of erythema multiforme is a rare and life-threatening disease that presents with both skin and mucosal involvement (2, 3, 10). In such cases, the lesions begin on the face and develop on the trunk with blisters on macular skin lesions (2, 3). Hence, the same disease appears at different stages and with different severity. Although Stevens–Johnson syndrome mostly appears as a reaction to some medicines, it has been separated from the erythema multiform spectrum and added to toxic epidermal necrolysis (3). Mucosal involvement in a situation in which erythema multiforme is caused by herpes simplex must be taken into consideration when differentiating between erythema multiforme and Stevens–Johnson syndrome (2, 3, 10). The prognosis of the disease varies and depends on the cause and the state of the patient’s immune system. Energy drinks containing taurine have recently been blamed for causing hypersensitivity reactions such as urticaria, in some cases anaphylaxis, and in rare cases even erythema multiforme (8). Taurine, an organic compound found in animal tissues (5), has been studied in the medical and pharmaceutical industry as a food and drink supplement that lowers the risk of cardiovascular disease, mostly via a mechanism that prevents hypertension and decreases blood cholesterol (6). Nevertheless, it is important to emphasize the potential hypersensitivity reaction to synthetic taurine (11).

References

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

Miguel Costa-Silva, Ana Pedrosa, Filomena Azevedo, Alberto Mota

1Department of Dermatology and Venereology, Centro Hospitalar São João, EPE Porto, Portugal. 2Faculty of Medicine, University of Porto, Porto, Portugal.

✉ Corresponding author: miguelcostaesilva.dermato@gmail.com

Abstract

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

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

Introduction

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

Case description

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

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

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

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

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

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

Conclusion

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

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References

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