Dermatology

High-Risk Human Papillomavirus Infection in Bowen's Disease of the Nail Unit: Report of Three Cases and Review of the Literature

Natalie Grundmeier[a]   Henning Hamm[a]   Benedikt Weissbrich[b]   Sabrina Christine Lang[a]
Eva-Bettina Bröcker[a]   Andreas Kerstan[a]

[a]Department of Dermatology, Venereology and Allergology, University Hospital of Würzburg, and [b]Institute for Virology, University of Würzburg, Würzburg, Germany

Abstract

Background: Bowen's disease (BD) of the nail unit is associated with human papillomavirus (HPV) infection. **Objective:** This study aimed to investigate the frequency of high-risk HPV infection, gender, age and digital distribution in this condition. **Methods:** Biopsy specimens of 3 consecutive cases with periungual BD were investigated for the presence of HPV DNA by in situ hybridization and by polymerase chain reaction (PCR). Furthermore, 74 cases of ungual BD conducted with HPV genotyping as reported in the literature were reviewed. **Results:** PCR of biopsy specimens revealed in 2 cases infection with HPV-16 and in 1 case with HPV-73. Additionally, in 1 HPV-16-positive case HPV-31/33 was detected by in situ hybridization. In line, review of the literature demonstrated a clear association of HPV-positive BD with high-risk HPV types. Interestingly, age at diagnosis was significantly lower in women. Whereas in both genders the second to fourth fingers on both hands were commonly diseased, only in men the thumbs were also prominently affected. **Conclusions:** Infection with high-risk HPV types is common in BD of the nail unit suggesting the aetiological cause. Therefore, patients and partners should be closely followed up for digital and genital HPV-associated lesions.

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Introduction

Bowen's disease (BD) is a squamous cell carcinoma (SCC) in situ of the skin. In 3–5% of cases, it progresses to invasive carcinoma with the capability to develop metastasis.

BD of the nail apparatus differs from BD of the skin since it may mimic various benign conditions such as chronic eczema, verruca vulgaris, paronychia or onychomycosis. Therefore, it is frequently misdiagnosed for many years. Furthermore, it appears to be associated with human papillomavirus (HPV) infections whereas this is a rather rare event in BD of the exposed skin [1, 2]. However, the precise aetiology of BD of the nail unit is unclear. Chronic traumatization, exposure to arsenic or ultraviolet and ionizing radiation have been discussed. In addition, HPV infections are thought to be a causative factor [3, 4]. To follow this line, we studied biopsy specimens of 3 patients with BD of the nail apparatus for the presence of HPV DNA and reviewed 74 cases reported in the literature. Furthermore, we also focused on the distribution of gender, age at onset of disease as well as the frequency of digit involvement.

Patients and Methods

Patients and Specimens

Biopsy specimens from 3 patients with BD of the nail apparatus, treated in our facility between June 2006 and September 2009, were used for this investigation. Tissue specimens fixed in 10% neutralized buffered formalin and embedded in paraffin wax were subjected to routine histopathological examination. Informed consent was obtained from all subjects.

PCR Amplification with Consensus Sequence Primers and Sequence Analysis

Formalin-fixed and paraffin-embedded samples were cut into 10-μm sections. After deparaffinization of the sections with Roticlear (Carl Roth, Karlsruhe, Germany), DNA was extracted from the samples using the Qiagen DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplifi-

Andreas Kerstan, MD
Department of Dermatology
University Hospital of Würzburg
DE–97080 Würzburg (Germany)
Tel. +49 931 2012 6710, E-Mail kerstan_a@klinik.uni-wuerzburg.de

cation of HPV DNA and typing by sequence analysis were performed as previously described [5]. Briefly, polymerase chain reaction (PCR) was carried out using the Qiagen Hot Star polymerase kit and the primers MY09, MY11 and HMB01 [6]. After amplification, PCR products were visualized by staining with ethidium bromide on agarose gels. To confirm the sequence specificity and to determine the HPV type, PCR products of the expected size were sequenced completely in both directions using Big Dye terminator chemistry and the ABI Prism 3100 instrument (Applied Biosystems, Darmstadt, Germany). The obtained sequences were blasted against viral sequences in GenBank to determine the HPV type. General laboratory procedures to prevent PCR contamination were strictly adhered to and negative controls were extracted with each tissue sample.

In situ Hybridization

HPV DNA integration was determined by using the Zytofast chromogenic in situ hybridization implementation kit (Zytomed, Berlin, Germany), according to the manufacturer's instructions. Briefly, 4-µm tissue sections were mounted on silanized slides, deparaffinized, immersed in 100% ethanol for 2 min and air-dried at room temperature, followed by digestion with pepsin (Zytomed) for 40 min at 37°C. After dehydration and air-drying the slides, DNA was heat-denatured at 90°C for 5 min in hybridization solution mixed with a biotinylated HPV probe cocktail containing high-risk HPV types 16/18 and low-risk types 6/11 as well as type 31/33 DNA (Zytomed) and subsequently incubated at 37°C for 1 h. The slides were washed with Tris-buffered saline and exposed to alkaline phosphatase-conjugated streptavidin. The sections were then incubated with nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate chromogen substrate, followed by haematoxylin counterstaining.

Statistical Analysis

Box-and-whisker plots indicate the minimum value, lower quartile, median, upper quartile and maximum value. Data was subjected to a D'Agostino and Pearson omnibus normality test, followed by an unpaired t test (Graph Pad Prism 5.0; Graph Pad Software Inc., Calif., USA); p values < 0.05 were considered significant.

Results

Case 1

A 46-year-old right-handed male patient presented with a non-healing, sharply demarcated, erythematous hyperkeratotic plaque at the distal phalanx adjacent to the proximal nail fold of his right index finger for several years' duration (fig. 1a). The painful lesion handicapped the patient in his work as a machine fitter. Due to the detection of *Pseudomonas aeruginosa* in the bacterial smear, he had previously been treated for paronychia with topical and systemic antibiotics as well as topical antimycotics, which showed only a temporary effect. Histopathological examination demonstrated BD with numerous atypical keratinocytes disorderly arranged throughout the acanthotic epidermis admixed with atypical mitotic figures (fig. 1d). HPV type 16/18 DNA was detected by in situ hybridization, and PCR confirmed infection with HPV-16. Removal by micrographic surgery showed incipient invasive growth, and even by re-excision no tumour-free margins could be achieved. Consequently, the patient was referred to the hand surgeons of our University Hospital for amputation of the terminal phalanx. Staging examinations including ultrasound of the axillary and cervical lymph nodes and chest X-ray were inconspicuous.

Case 2

A 50-year-old left-handed man attended our clinic with a 2 × 1 cm ill-defined erythematous lesion on the tip of his left thumb spreading to the nail bed and destroying the distal nail plate (fig. 1b). The lesion had previously been treated as a recalcitrant verruca vulgaris with several topical antiwart agents without clinical improvement. Examination of a biopsy specimen of the lesion revealed BD. In situ hybridization for HPV types 16/18 and 31/33 was positive (fig. 2a, b) but only HPV-16 DNA could be characterized by PCR. Micrographic excision of the entire nail unit demonstrated tumour-free margins, but koilocytes scattered in the stratum granulosum of the surgical margins were still present (fig. 2c). Since koilocytes are indicative of HPV infection [7], the lateral margins were adjuvantly treated with imiquimod cream 3–4 times a week for 6 weeks after skin transplantation. No recurrence of the tumour was seen in the 18 months of continuous follow-up.

Case 3

A 67-year-old right-handed man had a 2-year history of a progressively growing hyperkeratotic plaque reaching the proximal nail fold of his right index finger (fig. 1c). Topical treatment with corticosteroid, antifungal and antibiotic creams had not been successful. Histopathology of a biopsy specimen of the lesion revealed BD, which was negative for HPV DNA probe 6/11, 16/18 and 31/33 using in situ hybridization. HPV DNA analysis by PCR yielded HPV-73. Tangential excision was performed, followed by erbium:YAG laser therapy. Recurrence of BD 2 months later was treated by shave excision. One year later the patient showed an erosive plaque in the nail bed, and histological examination again confirmed relapse of BD. Therefore, the entire nail apparatus was excised and the defect covered with a full-thickness skin graft. There was no evidence of recurrence in 24 months of follow-up, but in the meantime HIV infection was diagnosed.

None of our patients showed other verrucous lesions on the hands, feet or in the anogenital region.

BD of the Nail Is Highly Associated with High-Risk HPV Infections

Case reports in which HPV genotyping of ungual BD either by immunohistochemistry, in situ hybridization and/or PCR was performed, including the cases presented here, were evaluated (n = 77, mean age ± SD 50.9 ± 15.0 years, range 22–83; table 1). Studies which did not specify the location on the digit were excluded from the review. The periungual regions of the left hand were affected in 46.9% of cases and those of the right hand in 44.9%, whereas the toes were involved in 8.2% of cases. As shown in table 2, the third and fourth fingers were the most commonly affected digits (27.2 and 26.1%, respectively), followed by the thumb (20.5%), index finger (20.5%), and fifth finger (5.7%). Moreover, gender distribution revealed that in men the thumb was involved in 26.2% of cases, followed by the third (24.6%), second (21.3%) and fourth fingers (21.3%), whereas the predilection sites in women were the fourth (37.1%) and third (33.3%) fingers, followed by the index finger (18.2%) and thumb (7.4%). Interestingly, in women the second to fourth digits on both hands (100% on right hand, 81.3% on left hand) but not the thumbs (0% on right hand, 12.5% on left hand) were mostly affected, whereas in men the right thumb

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Fig. 1. BD of the nail unit, clinical appearance and histopathology. **a** Case 1: erythematous, hyperkeratotic plaque on the lateral and proximal nail fold and nail bed with onycholysis of the right index finger. **b** Case 2: verrucous plaque involving the tip, radial nail fold and nail bed of the left thumb. **c** Case 3: circumscribed erythematous, scaly lesion on the distal phalanx reaching the proximal nail fold of the right index finger. **d** Histology of case 1: atypical keratinocytes involving the full thickness of the acanthotic epidermis with mitoses and dyskeratoses. HE. ×200.
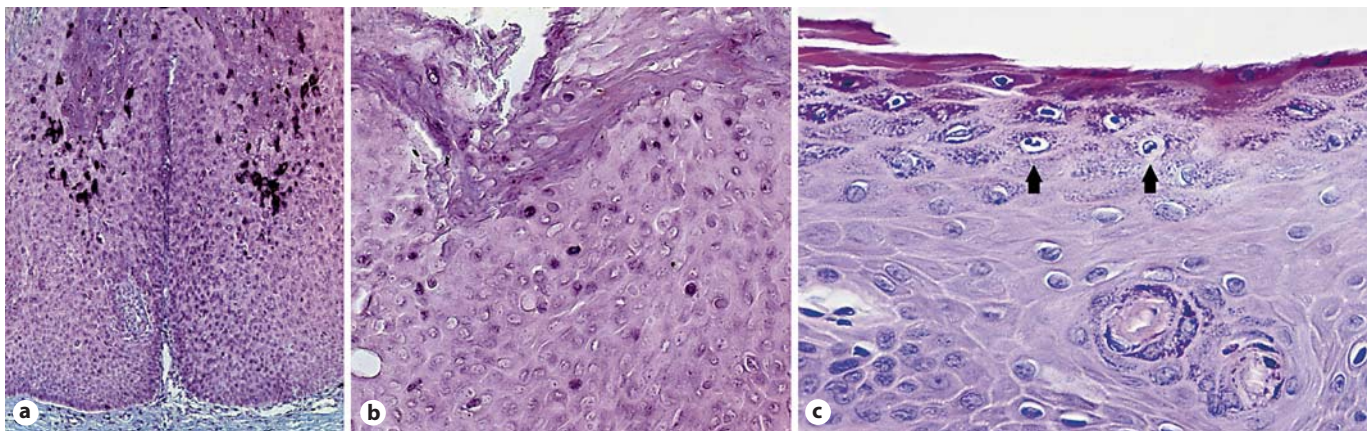
Fig. 2. Case 2: in situ hybridization and surgical excision margins. **a** Probes for HPV type 16/18 DNA revealing uniform nuclear staining of scattered cells in the upper malpighian layer. ×200. **b** Detection of HPV type 31/33. ×200. **c** Koilocytes (arrows) in the stratum granulosum at the excision margins of the nail unit. HE. ×400.

was predominantly diseased (32.3%), in contrast to the left thumb (20%). Besides the right fifth digit of men (12.9%), the fifth digit of either gender was hardly involved.

Single infection with high-risk HPV-16 is most prevalent in BD of the nail apparatus (n = 23, 32.8%), which became even more evident when HPV-16-related types were taken into account (n = 35, 50.0%;

fig. 3a, table 1). However, as in case 3 of our patient series other high-risk HPV types, e.g. 26, 34, 51, 56 and 73, can also be detected alone in lesions (n = 13, 18.6%; fig. 3a), whereas infections with only HPV-18-related genotypes are extremely rare (n = 2, 2.9%). Furthermore, few cases have been reported harbouring multiple HPV genotypes, with at least one of them representing a high-risk type (n = 15, 21.4%;

fig. 3a). Of note, there has been no infection with low-risk HPV species (types 5, 8, 9, 11, 17, 21, 36, 49, 81) alone reported in BD of the nail unit. These HPV types always coexisted with a high-risk type (n = 5, 7.1%). In-depth analysis revealed that in all reported ungual BD cases, HPV-16 was found in 35.1%, HPV-16-related genotypes (31, 33, 35, 52, 58) in 23.4%, HPV-56 in 11.7%, HPV-73 in 9.1%, HPV-33 in 7.8%

Table 1. BD of the nail unit as reported in the literature including HPV typing

Reference, year	Sex/age (years)	Finger or toe	HPV type	Localization/ clinical presentation	Cases positive for HPV	Specifics
Kawashima et al. [33], 1986	F/69	Lf3	34	Periungual/ verrucous plaque	1/1	Warts on contralateral palm and index finger
Ostrow et al. [34], 1987	M/37	Rf1	16	Periungual/ verrucous nodule	1/1	Diagnosis of epidermodysplasia verruciformis
Moy et al. [35], 1989	M/47 M/78 M/55 M/67 F/22	Lf3 Lf4 Rf2 Lf4 Rf4	16 – –[1] – 16	Periungual/ verrucous lesion in most patients	2/5	[1]Samples that contained DNA sequences related to but distinct from HPV types 6/11, 16, 18 or 51
Rüdlinger et al. [25], 1989	F/42	Rf4	35	Periungual/ verrucous lesion	1/1	Also bowenoid papulosis, positive for HPV-35
Guitart et al. [14], 1990	M/66 M/57 M/44 F/63 F/50 F/69	Rf1 Lf1 Lf2 Rf4 Lf1, 3, 4, 5 Lf3	– – – – – 16/18	Nail bed verrucous lesion in most patients	1/6	Warts on other fingers (once) Polydactylous disease (once) History of recurrence on different fingers (once) History of radiodermatitis (twice) Uterine carcinoma, positive for HPV-16/18 (once)
Eliezri et al. [36], 1990	n.d./n.d.	n.d.	16 or HPV-16-related	Periungual/n.d.	7/12	Series of periungual BD
Ashinoff et al. [30], 1991	M/49 M/73 M/26 M/73 M/75 M/46 M/29 F/41	Lf2 Rf3 Rf1 Rf3 Rf5 Lf2 Lf4 Rf4	– n.t. 16 n.t. 16 – 16 n.t.	Nail bed, periungual/ verrucous lesion, paronychia	3/8	
McGrae et al. [15], 1993	M/42	Rf2, 3, 4	16	Nail fold/ verrucous plaque	1/1	Polydactylous disease (3/3 lesions HPV-positive), genital wart, positive for HPV-6, flat wart on hand
Sau et al. [2], 1994	M/55 M/64 M/63 M/61 M/39 M/48 F/42	Lf3 Rf3 Lf2 Lf1 Lf3 Lf4 Lf3	16 – – 16 – 16 16	Nail bed, periungual/ verrucous lesion, nail dystrophy, onycholysis, paronychia	4/7	Additionally vulval intra-epithelial neoplasia, positive for HPV types 21 and 34
Nordin et al. [26], 1994	F/31	Rf3	16	Subungual/tumour	1/1	Vulvar and cervical dysplasia in the past and wart on the same finger positive for HPV-16
Mitsuishi et al. [37], 1997	M/53 F/24 F/34	Rf3 Rf4 Lf3	16 – 73	Periungual/ verrucous lesion pigmented plaque	2/3	
Forslund et al. [27], 1997	F/57	Lf4	16	Periungual/ onycholysis, yellowish lesion	1/1	History of cervical intra-epithelial neoplasia (HPV-16-positive) and perianal condylomata acuminata
Sass et al. [38], 1998	M/67	Lf4	16	Nail bed/LM	1/1	
Theunis et al. [39], 1999	M/67 M/78 M/83	Lf4 Rf1 Rf1	16 16 16	Subungual/LM, subungual hyperkeratosis	3/3	
Ota et al. [23], 2002	M/80	Lf2	18	Periungual/ erythematous plaques	1/1	Formerly gynaecologist
Lambiase et al. [40], 2003	M/25	Rf3	56	Nail bed/LM	1/1	

Table 1 (continued)

Reference, year	Sex/age (years)	Finger or toe	HPV type	Localization/ clinical presentation	Cases positive for HPV	Specifics
Hara et al. [16], 2004	F/46	Rf2, 3 Lf1, 2, 3, 4 Lt1, 2, 4, 5	58	Periungual/hyperkeratotic and verrucous plaques, nail dystrophy	1/1	Polydactylous, additionally vulva and cervical carcinomas in the past, positive for HPV-58
Sato et al. [41], 2004	M/55	Lf4	11, 16	Periungual/verrucous lesion	1/1	
Weisenseel et al. [42], 2006	M/49	Lf3	73	Paraungual/ erythematous plaque	1/1	
Ekeowa-Anderson et al. [28], 2007	F/23	Lf2	21, 34, 49, 58	Periungual/hyperkeratotic, pigmented plaque, nail dystrophy	1/1	Concurrent vulvar intra-epithelial neoplasia, positive for HPV types 21, 31 and 34
Shimizu et al. [43], 2008	M/34 M/68 M/63 F/29 F/51	Lt2 Rf1 Rt4 Lt1 Lt2	56 56 – 56 –	Nail apparatus LM LM Ulcer LM Nail deformity	3/5	
Guldbakke et al. [44], 2008	M/32	Rf1, Lf2	73	Periungual/ verrucous lesion	1/1	Polydactylous disease, Hodgkin's lymphoma 18 years previously, successfully treated by chemotherapy
Inokuma et al. [45], 2009	M/41	Rf2	56	Nail matrix/LM	1/1	
Kreuter et al. [21], 2009	M/62 M/52 M/59 M/71 F/62	Rf2 Rf1 Lf2 Lf1 Rf2	26 73, 81 33 56, 9, 17, 36 16	Periungual/verrucous plaque, hyperpigmented flat plaque	5/5	1 HIV-positive patient (HPV-26-positive); periungual warts (thrice), anogenital warts (once), penile intra-epithelial neoplasia (once)
Turowski et al. [17], 2009	M/42 M/44	Lf1, Rf4, 5 Rf3	16 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 70[2]	Periungual/verrucous plaque	2/2	2 HIV-positive patients [2]In situ hybridization with high-risk HPV probe
Nakajima et al. [46], 2010	M/56	Lf3	8, 16, 58	Periungual/n.d.	1/1	
Gormley et al. [22], 2011	M/47 M/44 M/42 M/44 F/44 F/50 F/43	Rf4 Lf1, Rf4, 5 Rf1 Rf3 Lf2, 4 Rf4 Rf3	33, 51 16 33, 73 n.t. 33, 51, 73 33, 51 n.t.	Periungual/hyperpigmented plaque, verrucous plaque	5/7	4 HIV-positive patients, 2 of them with HPV-73-positive ungual BD; 4 cases with either genital warts or abnormal Papanicolaou smear finding in the past
Ohishi et al. [47], 2011	M/50 M/36 M/41 M/32 M/43 M/66 M/41	Lf4 Lf4 Lf3 Lf3 Rf1 Lf3 Rf5	56 16 59 56 56 52 33	Nail apparatus/ LM, hyperkeratosis of nail bed, onycholysis	7/7	
Grundmeier et al. (this report), 2011	M/46 M/50 M/67	Rf2 Lf1 Rf2	16 16, 31/33 73	Nail apparatus/ hyperkeratotic plaque	3/3	1 HIV-positive patient (HPV-73-positive)

R = Right; L = left; f = finger; t = toe; LM = longitudinal melanonychia; – = negative; n.t. = not tested; n.d. = not described.

Table 2. Digital distribution (numbers/percentages) of ungual BD including polydactylous cases

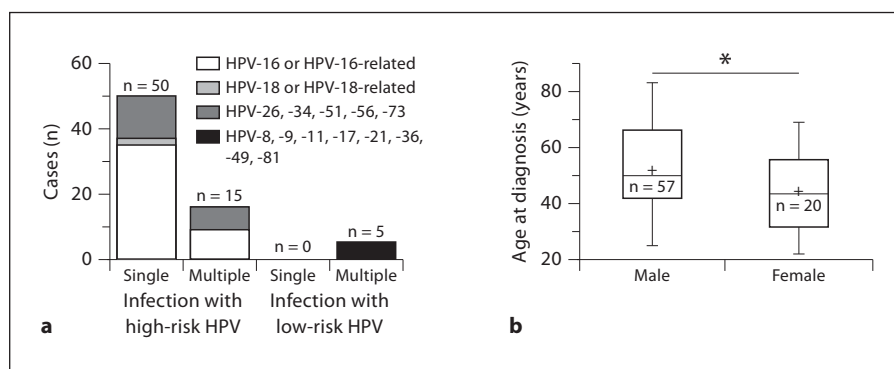| Digit	Males			Females			Male/female, total
	left hand	right hand	total, %	left hand	right hand	total, %	
1	6/20	10/32.3	26.2	2/12.5	0/0	7.4	18/20.5
2	7/23.3	6/19.3	21.3	3/18.8	2/18.2	18.5	18/20.5
3	8/26.7 } 80%	7/22.6 } 54.8%	24.6	6/37.5 } 81.3%	3/27.3 } 100%	33.3	24/27.2
4	9/30	4/12.9	21.3	4/25	6/54.5	37.1	23/26.1
5	0/0	4/12.9	6.6	1/6.2	0/0	3.7	5/5.7
Sum	30/100	31/100	100	16/100	11/100	100	88/100

and HPV-51 in 3.9%, followed by HPV types 34, 26, 18, 59, 11, 8, 21, 9, 17 as well as 49 which were each detected at a frequency between 1.3 and 2.6%. Taken together, analysis of the HPV-positive cases reported in the literature demonstrates that BD of the nail apparatus is commonly associated with high-risk HPV infections.

It has been reported that BD of the nail unit predominantly affects males at the age of 50–70 years [1, 2]. Since then a rather large number of women has also been reported (n = 20; table 1). Based on the reviewed data including the patients reported here, we therefore analysed age and gender distribution of ungual BD. Interestingly, age at diagnosis was significantly lower in women than in men (mean age ± SEM, women 44.6 ± 3.3 vs. men 53.1 ± 1.9 years; fig. 3b). Men were affected more often than women, by a ratio of 2.85:1.

Fig. 3. HPV genotypes and age distribution in patients with BD of the nail unit. **a** HPV genotypes described from 1986 to 2011. HPV-16-related types: 16, 31, 33, 35, 52, 58; HPV-18-related types: 18, 39, 45, 59, 68, 70. The HPV-16/18-positive cases reported by Guitart et al. [14] and Turowski et al. [17] were omitted since they were not further specified. **b** Sex distribution of affected patients in relation to the age at diagnosis. Analysis covers the data shown in table 1 excluding the case series by Eliezri et al. [36] where age was not determined. * p = 0.029; + indicates the mean.

Discussion

While BD is commonly found on the trunk, face, fingers and genitalia, the occurrence in the nail apparatus is rare. In this location, involvement of the nail fold typically presents as either an erythematous plaque or as a scaly, verrucous or crusted lesion with periungual swelling. Nail bed involvement may cause onycholysis, subungual hyperkeratosis and dyschromia, whereas nail matrix involvement may lead to longitudinal erythronychia, melanonychia or nail plate dystrophy (table 1) [8, 9].

Various studies have attributed an aetiological role of HPV in the development of BD of the nail tissues, because its detection is a common finding (table 1). HPV is a double-stranded DNA virus of the Papillomaviridae family and the species are phylogenetically classified into the genera α, β, γ and μ/ν [10]. α-HPV species can be further divided into high-risk and low-risk HPV, according to their oncogenic potential. The high-risk group consists of α-HPV types 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 73 and 82 [10], which are often detected in intra-epithelial neoplasia and/or invasive epithelial cancer. There is convincing evidence that α-HPV type 16 and related mucosal types play a crucial role in the development of cervical and anogenital carcinomas [11]. The role of HPV in skin cancer was first demonstrated in patients with the rare hereditary skin disease epidermodysplasia verruciformis [12]. However, involvement of HPV in the development of SCC in non-genital skin is still controversial but it seems that β-HPV species (type 2) may be involved in the progression of cutaneous SCC [13]. As shown here by analysis of the literature, BD of the nail unit is highly associated with high-risk α-HPV infection. Therefore, initiation or progression of ungual BD by HPV is very likely.

It appears that periungual skin is predisposed to HPV infection due to microtrauma. This idea is supported by the notion that BD usually arises at the lateral or proximal nail fold or hyponychium and invades the nail unit by extension. In support of this idea, the right thumb of men, which is predominantly exposed to traumata or abrasion, followed by the second, third and fourth fingers in either gender are affected (table 2). Furthermore, cases of polydactylous disease have been reported, supporting a role of an infectious agent in periungual BD [14–17]. However, given

the very low incidence of BD of the nail apparatus, additional predisposing factors may be needed for virus entry, e.g. chronic traumatization, chronic paronychia, exposure to X-rays, ingestion of arsenic, and immunosuppression [18]. HIV infection might account for immunosuppression. Interestingly, in case 3 of our patient series periungual BD was positive for the rare α-HPV type 73, and advanced HIV disease (CDC B3) was diagnosed 18 months after onset of BD of the nail unit. Based on the limited number of reported HIV-positive patients with ungual BD, it is striking that α-HPV type 73 DNA could be detected in 3 out of 7 HPV-73-positive cases and the rare α-HPV type 26 in 3 out of 3 HPV-26-positive cases with BD/SCC of the nail apparatus [19–22]. Therefore, these data support the hypothesis that HIV-related immune suppression might predispose for the acquisition of rare HPV types [21, 22]. It is tempting to speculate that the handedness might determine the location of ungual BD. In our patients the hand affected by BD of the nail unit correlated with the preference of hand usage. While this could be an explanation for the preferred occurrence of ungual BD on the right thumb of men (table 2), the second to fourth fingers on both hands in either gender were among the most commonly affected digits. However, data on the handedness in these patients is largely missing to further strengthen this hypothesis.

Detection of the same HPV types in anogenital lesions and BD of the nail unit suggests the possibility of auto-inoculation of the finger from the genital area to the finger or vice versa as it has been exemplified by several cases showing the same HPV genotype found in lesions of BD of the nail and genital epithelial dysplasia/neoplasia (table 1). In line, one interesting

paper described an 80-year-old gynaecologist who developed periungual BD on the left index finger, which was positive for α-HPV type 18 DNA by PCR typing [23]. Since α-HPV type 18 DNA is usually not detected in BD of the nail unit (table 1, fig. 3a), it is very likely that infection occurred during carrying out his profession.

Of note, our review of published cases of BD of the nail unit revealed a significant earlier age of manifestation in females (fig. 3b). Since the average age of α-HPV-16-positive women with cervical intraepithelial neoplasia (CIN) grade 3 or invasive cancer is significantly lower as compared with HPV-16-negative groups [24], auto-inoculation of the finger from the genital area is an obvious explanation for the early onset of disease. In line, in 6 (30%) cases of affected women, the HPV type detected in ungual BD correlated with the type detected in vulvar intra-epithelial dysplasia (VIN) and/or CIN or even carcinomas [14, 16, 25–28]. However, the frequency of women affected by both BD of the nail unit and HPV-positive VIN/CIN is most likely higher, since data on the latter was largely missing in the reviewed literature. Of note, diagnosis of VIN/CIN preceded that of BD of the nail unit. It is conceivable that vice versa in cases of unknown genital HPV status, BD of the nail unit might hint to a manifestation of VIN/CIN. Therefore, diagnosis of ungual BD in women should always include a gynaecological examination.

The detection of high-risk α-HPV species in BD of the nail apparatus might affect clinical management. The safest treatment is micrographic surgery, but many less invasive and non-surgical treatments such as laser ablation, cryotherapy, photodynamic therapy, topical 5-fluorouracil and topical imiquimod have been used in selected cases

[4, 29–31]. However, given the risk of recurrence and in favour of our observation that koilocytes indicative of persistent HPV infection might remain at the margins in micrographic surgery (fig. 2c), topical treatment with imiquimod in the vicinity of the surgical defect may be considered as a local adjuvant treatment (see case 3) [32].

Up to now, there has been no evidence that HPV-positive BD of the nail apparatus predisposes to metastasis. However, Kreuter et al. [21] demonstrated that expression of Ki67 and p16^{INK4a}, both immunohistological markers for cell proliferation, were significantly higher in HPV-positive periungual lesions as compared to HPV-negative, non-periungual BD. Therefore, the high proliferative activity might be one of the factors explaining the high local recurrence rate of HPV-positive ungual BD [21]. Due to the increased risk of relapse, which can lead to metastasis, long-term follow-up, including palpation and ultrasound of the regional lymph nodes, is mandatory.

In summary, biopsy and histopathological examination are required for diagnosis and differentiation from other ungual and periungual dermatoses. The strong association of high-risk HPV infection and BD of the nail unit as reviewed in the literature and described in our case series supports a causal role of the virus and should prompt for a follow-up of digital and genital HPV-associated lesions in patients and partners.

Disclosure Statement

All authors state that they do not have any commercial or other interest that might have influenced the drawing up and the results of this paper.

References

1 Mikhail GR: Bowen disease and squamous cell carcinoma of the nail bed. Arch Dermatol 1974;110:267–270.
2 Sau P, McMarlin SL, Sperling LC, Katz R: Bowen's disease of the nail bed and periungual area. A clinicopathologic analysis of seven cases. Arch Dermatol 1994;130:204–209.
3 Koch A, Schonlebe J, Haroske G, Kostler E, Wollina U: Polydactylous Bowen's disease. J Eur Acad Dermatol Venereol 2003;17:213–215.

4 Gong HS, Cho JH, Roh YH, Chung MS, Baek GH: Bone invasion by squamous cell carcinoma in situ (Bowen's disease) of the finger during treatment with imiquimod 5% cream: case report. J Hand Surg Am 2010;35:999–1002.
5 Weyandt GH, Tollmann F, Kristen P, Weissbrich B: Low risk of contamination with human papilloma virus during treatment of condylomata acuminata with multilayer argon plasma coagulation and CO$_2$ laser ablation. Arch Dermatol Res 2011;303:141–144.

6 Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, Scott DR, Rush BB, Lawler P, Sherman ME: Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235–240.
7 Hajdu SI: The link between koilocytes and human papillomaviruses. Ann Clin Lab Sci 2006;36:485–487.
8 Mirza B, Muir JB: Bowen's disease of the nail bed. Australas J Dermatol 2004;45:232–233.

9 Zabawski EJ Jr, Washak RV, Cohen JB, Cockerell CJ, Brown SM: Squamous cell carcinoma of the nail bed: is finger predominance another clue to etiology? A report of 5 cases. Cutis 2001;67:59–64.

10 De Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H: Classification of papillomaviruses. Virology 2004;324:17–27.

11 Zur Hausen H: Papillomaviruses in the causation of human cancers – a brief historical account. Virology 2009;384:260–265.

12 Pfister H, Ter Schegget J: Role of HPV in cutaneous premalignant and malignant tumors. Clin Dermatol 1997;15:335–347.

13 Asgari MM, Kiviat NB, Critchlow CW, Stern JE, Argenyi ZB, Raugi GJ, Berg D, Odland PB, Hawes SE, de Villiers EM: Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. J Invest Dermatol 2008;128:1409–1417.

14 Guitart J, Bergfeld WF, Tuthill RJ, Tubbs RR, Zienowicz R, Fleegler EJ: Squamous cell carcinoma of the nail bed: a clinicopathological study of 12 cases. Br J Dermatol 1990;123: 215–222.

15 McGrae JD Jr, Greer CE, Manos MM: Multiple Bowen's disease of the fingers associated with human papilloma virus type 16. Int J Dermatol 1993;32:104–107.

16 Hara H, Honda A, Suzuki H, Sata T, Matsukura T: Detection of human papillomavirus type 58 in polydactylous Bowen's disease on the fingers and toes of a woman – concurrent occurrence of invasive vulval and cervical carcinomas. Dermatology 2004;209:218–222.

17 Turowski CB, Ross AS, Cusack CA: Human papillomavirus-associated squamous cell carcinoma of the nail bed in African-American patients. Int J Dermatol 2009;48:117–120.

18 Neubert T, Lehmann P: Bowen's disease – a review of newer treatment options. Ther Clin Risk Manag 2008;4:1085–1095.

19 High WA, Tyring SK, Taylor RS: Rapidly enlarging growth of the proximal nail fold. Dermatol Surg 2003;29:984–986.

20 Handisurya A, Rieger A, Bankier A, Koller A, Salat A, Stingl G, Kirnbauer R: Human papillomavirus type 26 infection causing multiple invasive squamous cell carcinomas of the fingernails in an AIDS patient under highly active antiretroviral therapy. Br J Dermatol 2007;157:788–794.

21 Kreuter A, Gambichler T, Pfister H, Wieland U: Diversity of human papillomavirus types in periungual squamous cell carcinoma. Br J Dermatol 2009;161:1262–1269.

22 Gormley RH, Groft CM, Miller CJ, Kovarik CL: Digital squamous cell carcinoma and association with diverse high-risk human papillomavirus types. J Am Acad Dermatol 2011;64:981–985.

23 Ota M, Kawashima M, Mitsuishi T: Multiple Bowen's disease of the fingers. Eur J Dermatol 2002;12:275–277.

24 Karube A, Sasaki M, Tanaka H, Nakagome O, Dahiya R, Fujimoto S, Tanaka T: Human papilloma virus type 16 infection and the early onset of cervical cancer. Biochem Biophys Res Commun 2004;323:621–624.

25 Rüdlinger R, Grob R, Yu YX, Schnyder UW: Human papillomavirus-35-positive bowenoid papulosis of the anogenital area and concurrent human papillomavirus-35-positive verruca with bowenoid dysplasia of the periungual area. Arch Dermatol 1989;125:655–659.

26 Nordin P, Stenquist B, Hansson BG: Joint occurrence of human papillomavirus type 16 DNA in Bowen's disease on a finger and in dysplasia of the vulva and the uterine cervix. Br J Dermatol 1994;131:740.

27 Forslund O, Nordin P, Andersson K, Stenquist B, Hansson BG: DNA analysis indicates patient-specific human papillomavirus type 16 strains in Bowen's disease on fingers and in archival samples from genital dysplasia. Br J Dermatol 1997;136:678–682.

28 Ekeowa-Anderson AL, Harwood CA, Perrett CM, Sahota A, Annan H, Ran H, Leigh IM, Gibbon KL: Vulval intraepithelial neoplasia and periungual Bowen's disease concordant for mucosal (HPV-34) and epidermodysplasia verruciformis (HPV-21) human papillomavirus types. Clin Exp Dermatol 2007;32:304–307.

29 Lee JW, Hur J, Yeo KY, Yu HJ, Kim JS: A Case of pigmented Bowen's disease. Ann Dermatol 2009;21:197–199.

30 Ashinoff R, Li JJ, Jacobson M, Friedman-Kien AE, Geronemus RG: Detection of human papillomavirus DNA in squamous cell carcinoma of the nail bed and finger determined by polymerase chain reaction. Arch Dermatol 1991;127:1813–1818.

31 Usmani N, Stables GI, Telfer NR, Stringer MR: Subungual Bowen's disease treated by topical aminolevulinic acid-photodynamic therapy. J Am Acad Dermatol 2005;53:S273–S276.

32 Alam M, Caldwell JB, Eliezri YD: Human papillomavirus-associated digital squamous cell carcinoma: literature review and report of 21 new cases. J Am Acad Dermatol 2003; 48:385–393.

33 Kawashima M, Favre M, Jablonska S, Obalek S, Orth G: Characterization of a new type of human papillomavirus (HPV) related to HPV5 from a case of actinic keratosis. Virology 1986;154:389–394.

34 Ostrow RS, Manias D, Mitchell AJ, Stawowy L, Faras AJ: Epidermodysplasia verruciformis. A case associated with primary lymphatic dysplasia, depressed cell-mediated immunity, and Bowen's disease containing human papillomavirus 16 DNA. Arch Dermatol 1987;123:1511–1516.

35 Moy RL, Eliezri YD, Nuovo GJ, Zitelli JA, Bennett RG, Silverstein S: Human papillomavirus type 16 DNA in periungual squamous cell carcinomas. JAMA 1989;261: 2669–2673.

36 Eliezri YD, Silverstein SJ, Nuovo GJ: Occurrence of human papillomavirus type 16 DNA in cutaneous squamous and basal cell neoplasms. J Am Acad Dermatol 1990;23:836–842.

37 Mitsuishi T, Sata T, Matsukura T, Iwasaki T, Kawashima M: The presence of mucosal human papillomavirus in Bowen's disease of the hands. Cancer 1997;79:1911–1917.

38 Sass U, Andre J, Stene JJ, Noel JC: Longitudinal melanonychia revealing an intraepidermal carcinoma of the nail apparatus: detection of integrated HPV-16 DNA. J Am Acad Dermatol 1998;39:490–493.

39 Theunis A, Andre J, Noel JC: Evaluation of the role of genital human papillomavirus in the pathogenesis of ungual squamous cell carcinoma. Dermatology 1999;198:206–208.

40 Lambiase MC, Gardner TL, Altman CE, Albertini JG: Bowen disease of the nail bed presenting as longitudinal melanonychia: detection of human papillomavirus type 56 DNA. Cutis 2003;72:305–309.

41 Sato T, Morimoto A, Ishida Y, Matsuo I: Human papillomavirus associated with Bowen's disease of the finger. J Dermatol 2004;31: 927–930.

42 Weisenseel P, Prinz J, Korting H: Treatment of paraungual HPV73-positive Bowen disease with imiquimod cream. Hautarzt 2006; 57:309–310, 312.

43 Shimizu A, Tamura A, Abe M, Motegi S, Nagai Y, Ishikawa O, Nakatani Y, Yamamoto Y, Uezato H, Hoshino H: Detection of human papillomavirus type 56 in Bowen's disease involving the nail matrix. Br J Dermatol 2008;158:1273–1279.

44 Guldbakke KK, Brodsky J, Liang M, Schanbacher CF: Human papillomavirus type 73 in primary and recurrent periungual squamous cell carcinoma. Dermatol Surg 2008; 34:407–413.

45 Inokuma D, Aoyagi S, Saito N, Iitani MM, Homma E, Hamasaka K, Shimizu H: Bowen's disease of the nail matrix presenting as melanonychia: detection of human papillomavirus type 56. Acta Derm Venereol 2009; 89:638–639.

46 Nakajima H, Teraishi M, Tarutani M, Sano S: High prevalence of coinfection with mucosal high-risk type HPV (HR-HPV) and cutaneous HR-HPV in Bowen's disease in the fingers. J Dermatol Sci 2010;60:50–52.

47 Ohishi K, Nakamura Y, Ohishi Y, Yokomizo E, Ohara K, Takasaki M, Ueno T, Kawana S, Mitsuishi T: Bowen's disease of the nail apparatus and association with various high-risk human papillomavirus types. J Dermatol Sci 2011;63:69–72.

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