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Summary

Background Cutaneous warts have a cure rate after therapy of no more than approximately 50%. Recently, we developed and validated a standard assessment tool for warts (Cutaneous WARTS diagnostic tool, CWARTS) based on phenotypical characteristics.

Objectives To assess whether patient and morphological wart characteristics predict the human papillomavirus (HPV) type in a specific wart and whether these characteristics as well as the HPV type predict a favourable treatment response.

Methods Photographs were used to score nine morphological wart characteristics using the newly developed CWARTS tool. Genotyping of 23 wart-associated HPV types was performed using the hyperkeratotic skin lesion–polymerase chain reaction/multiplex genotyping assay. The results were correlated with a favourable response to treatment with monochloroacetic acid, cryotherapy or a combination of cryotherapy and salicylic acid. Odds ratios were calculated using logistic regression in a generalized estimating equations model.

Results Black dots (capillary thrombosis) strongly predicted the presence of any HPV type in a wart. From all characteristics tested, the HPV type most strongly predicted the treatment response when the warts were treated with monochloroacetic acid or a combination of cryotherapy and salicylic acid with a significantly decreased treatment response if the warts contained HPVs of the alpha genus (HPV2, HPV27 or HPV57). When cryotherapy alone was used for common warts, HPV type did not play a role, but cryotherapy was less effective in the presence of callus and when the wart was located deeper in the skin.

Conclusions Morphological characteristics of the warts and the HPV genotype influence treatment outcome and thus potentially influence future treatment decisions for common and plantar warts.

What's already known about this topic?

- Cutaneous warts are a major health concern because of their high prevalence.
- Although there are multiple treatment options, cure rate after therapy is no more than approximately 50%.

What does this study add?

- Predictive characteristics of warts are identified that will improve decision making regarding therapeutic options.

Warts are hyperkeratotic papules and plaques induced by human papillomaviruses (HPVs).[1] At present more than 350 HPV genotypes are recognized.[2] Clinically, cutaneous warts are divided into two basic wart types: common warts (all locations except the soles of the feet) and plantar warts (on the soles of the feet).

Cutaneous warts are a common ailment in children and adults with an average prevalence of 3–13%, ranging up to 33% in primary school populations.[3–6] Quality of life can be considerably affected because of physical and psychological distress.[7] A wide variety of treatments are available but are not very effective.[8] Treatment results and HPV distribution differ between common and plantar warts.[8,9] Monochloroacetic acid (MCA) and cryotherapy are the most effective first-line treatments in common warts, whereas for plantar warts MCA is most effective.[10] Nevertheless, only approximately 50% of patients are cured when optimal treatment per wart type is chosen.[8,10,11]

HPV2, HPV27 and HPV57 of the alpha genus and HPV1 of the mu genus are the most frequently found HPV types in cutaneous warts.[12] In the past clinical morphological characteristics have been associated with some HPV genotypes.[13,14] Egawa found that HPV1 DNA sequences could be detected in warts with granular intracytoplasmic inclusion bodies, that punctate keratotic lesions with filamentous intracytoplasmic inclusion bodies were associated with HPV63 (mu genus) and that pigmented warts with homogeneous intracytoplasmic inclusion bodies contained one of the gamma HPVs: HPV4, HPV60 or HPV65.[13] More recently we have found that compared with HPV2-, HPV27- and HPV57-positive warts, HPV1-containing warts usually occur in children, preferentially on a plantar surface and with a short duration before presentation to a physician.[12] However, clinical morphological wart characteristics in relation to HPV type, thus far, have not been systematically investigated.

The HPV type in a wart may influence the treatment response.[15] HPV1-positive warts had a much better response to treatment with salicylic acid, but also disappeared much faster with a wait-and-see policy than HPV2-, HPV27- or HPV57-positive warts.[15] In another study, the response to cryotherapy appeared to be unrelated to HPV genotype.[16] Next to HPV genotype, location and duration of the warts and morphological wart characteristics could also influence treatment response. In a former study, morphological characteristics of warts were defined by a group of dermatologists and general practitioners and validated for inter- and intraobserver agreement. An assessment tool for diagnosing warts – the Cutaneous WARTS diagnostic tool (CWARTS tool) – was developed and was used in this study.[17]

The purposes of this study were (i) to assess whether patient and morphological wart characteristics can predict the HPV type in a specific wart and (ii) to assess whether the patient characteristics and morphological wart characteristics as well as the HPV type causing the wart can predict a favourable treatment response in common and plantar warts. We performed a retrospective secondary analysis using photographs, HPV data and treatment response in a large randomized controlled trial (WARTS-2 trial).[10] Firstly, characteristics, as obtained using the CWARTS tool, as well as patient characteristics were analysed for association with HPV genotype. Then, in the second part of this study we investigated phenotypical characteristics and HPV genotype and related them to treatment response.

Materials and methods

This is a retrospective secondary analysis of a selected subpopulation of the WARTS-2 trial.[10] In brief, the WARTS-2 trial was a multicentre randomized parallel-group superiority trial to compare the effectiveness of MCA with the most effective usual treatments, thus in common warts MCA was compared with cryotherapy and in plantar warts MCA was compared with a combination of cryotherapy and salicylic acid. For the treatment with MCA every 2 weeks a saturated concentration of 76% was used that was applied and subsequently covered with tape by the general practitioner or practice assistant until all warts were cured; for cryotherapy every 2 weeks three subsequent freeze–thaw cycles were applied to common warts and in plantar warts cryotherapy was combined with daily self-administration of petroleum jelly containing 40% salicylic acid.[10] A favourable treatment response was reached when all warts were cured at 13 weeks.[10]

Patients who were immunocompetent with new cutaneous warts (common or plantar) aged 4 years and older were recruited from 41 general practices. Common warts were defined as warts on the hands and all other locations except the soles of the feet, which were defined as plantar warts.[10] More details of the treatment protocol of the WARTS-2 trial and the outcome assessment have been published previously.[10] The study protocol was approved by the medical ethical committee of the Leiden University Medical Centre and conducted according to the principles of the Declaration of Helsinki (version 2008) and the Medical Research Involving Human Subjects Act.

Photographs were taken by a trained research nurse using the Dino-Lite digital microscope (AnMo Electronics Corporation, Hsinchu City, Taiwan), set to a fixed scale of '20' resulting in a scale of 5·7 : 1 and using a standard object that assured a standard camera–wart distance from a selection of the patients who were included in the WARTS-2 trial. At an earlier stage, a clinical assessment tool, the CWARTS tool, had been developed and validated for inter- and intraobserver agreement by respectively 18 and six physicians with a different set of pictures based on the score of nine dichotomized morphological characteristics: (i) arrangement (confluent/multiple or solitary); (ii) level (elevated or skin level); (iii) aspect (rough/lobed or smooth/not lobed); (iv) border (sharply or not sharply circumscribed); (v) colour (lighter/skin colour/yellow or red); (vi) presence of white skin flakes; (vii) presence of black dots (capillary thrombosis); (viii) border

erythema; and (ix) callus.[17] In short, inter- and intraobserver agreement for most characteristics was moderate to strong with average intraclass correlation coefficients of 0·51 and 0·42, respectively. For the current study, the CWARTS tool was used by two researchers (S.C.B. and K.E.H.) to score the photographs of the warts for these nine morphological characteristics and in cases of a different score consensus was reached. Wart size and wart clearance were assessed by trained research nurses during home visits.[10]

In order to assess HPV genotype, swabs were taken and analysed using the hyperkeratotic skin lesion–polymerase chain reaction/multiplex genotyping assay (Labo Biomedical Products BV, Rijswijk, the Netherlands). All known cutaneous wart-associated virus were assessed, that is, wart-associated HPV types from the alpha (HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma (HPV4, 48, 50, 60, 65, 88 and 95), mu (HPV1 and 63) and nu genera (HPV41). This sensitive and specific assay has been previously described and evaluated.[18]

Morphological differences between wart types – plantar or common warts – were calculated using two-sided χ^2-test for dichotomous variables and Student's *t*-test for continuous variables. The correlations of morphology and patient characteristics with HPV genotype and treatment response were calculated using a generalized estimating equation (GEE) model with robust standard errors, thereby correcting the standard error for possibly incorrect assumptions regarding the covariance structure in the data, induced by the multiple measurements in patients, to estimate univariable and multivariable odds ratios with all nine morphological characteristics, age, sex and duration and location of the warts in the model. Subsequently, stepwise logistic regression with backward elimination was manually performed to identify the factors that significantly contributed to the model.

The linearity assumption for age and duration of warts was tested by first centring the variables age and duration of warts (centring in general decreases the collinearity between, for example, age and its square) and then entering centred age and age square or centred duration of warts and duration of warts square into the GEE model. The quadratic age and quadratic duration of warts were not statistically significant in any of the GEE models. The linearity assumption was thus not rejected for age and duration of warts. Data were analysed with SPSS Version 23·0 (IBM, Armonk, NY, U.S.A.).

Results

In the original trial 415 patients were stratified into a common wart group and a plantar wart group.[10] In total, the original study contained 611 common warts and 790 plantar warts. During the trial 356 warts from 164 patients were photographed and genotyped for HPV. Photographs of 15 warts (4·2%), were excluded because of poor photo quality or because the patients were lost to follow-up (nine warts in five patients). In total 341 warts could be scored for morphological characteristics. For 30 warts no (complete) material was available for HPV testing, resulting in 311 warts (159 patients) with a complete set of data.

The baseline characteristics of the 159 patients with a complete set of data are shown in Table 1. There were more female patients included, and this was most prominent in the plantar wart group. The patients in the common wart group were somewhat older and the duration of warts somewhat longer than in the plantar wart group. The number and size of the warts were evenly distributed among common and plantar warts and treatment arms.

Table 2 illustrates the morphological wart characteristics stratified according to common and plantar warts. Most common and plantar warts were solitary, elevated, rough, sharply circumscribed with white skin flakes, black dots (capillary thrombosis) and callus. In a minority of the warts a red colour or border erythema was present. Compared with common warts, plantar warts were statistically significantly more often characterized by a rough surface with white flakes, black dots and callus. An erythematous border was significantly more often seen in common warts. The size of the warts, the arrangement (solitary vs. multiple/confluent) and the sharpness of the border did not significantly differ between common and plantar warts.

The distribution of the different HPV types among the 159 patients is shown in Table 3.

There were 34 warts in 18 (11·3%) patients with no detectable HPV DNA in their warts; this occurred more frequently in longer existing common warts in patients who were aged ≥ 12 years. Altogether 43 warts of 37 (23·3%) patients contained two or more HPV types. These multiple types could be present in a single wart or in different warts in the same patient. HPV2 was detected in 47 warts of 29 (18·2%) patients and was most frequently observed in common warts of men who were aged ≥ 12 years. HPV27 was detected in 96 warts of 47 (29·6%) patients and occurred more frequently in longer existing plantar warts of patients who were aged ≥ 12 years. HPV57 was detected in 52 warts of 29 (18·2%) patients and occurred more frequently in longer existing warts of patients who were aged ≥ 12 years. HPV1 was detected in 62 warts of 45 (28·3%) patients and occurred more frequently in shorter existing plantar warts of patients who were younger than 12 years old.

The morphological characteristics of the 311 warts in relation to the HPV genotype are presented in Table 4. There were some statistically significant differences between the morphological characteristics of wart-like lesions containing no HPV and warts containing HPV2, HPV27, HPV57 and HPV1, which are shown in Table 5, Tables S1–5 and Figure S1 (see Supporting Information).

Black dots (capillary thromboses) were highly predictive for the presence of any HPV. HPV-containing warts also more often had a sharply defined border. Longer existing warts less frequently contained HPV DNA (Table 5 and Table S1; see Supporting Information). HPV2-positive warts were more common among male patients who had common warts showing erythema at the border of the wart (Table 5 and

Table 1 Baseline characteristics per wart group and treatment arm restricted to 159 patients with a complete dataset

	Common wart group[a]			Plantar wart group[a]			All patients (n = 159)
	Treatment arms			Treatment arms			
	MCA (n = 34)	Cryotherapy (n = 39)	All together (n = 73)	MCA (n = 40)	Cryo + SA (n = 46)	All together (n = 86)	
Sex, n (%)							
Female	20 (59)	23 (59)	43 (59)	25 (62)	31 (67)	56 (65)	99 (62·3)
Male	14 (41)	16 (41)	30 (41)	15 (38)	15 (33)	30 (35)	60 (37·7)
Age (years), mean ± SD							
4–11	10·0 ± 29·4	13 ± 33	23 ± 31·5	23 ± 57·5	20 ± 43·5	43 ± 50·0	66 ± 41·5
≥ 12	24·0 ± 70·6	26 ± 67	50 ± 68·5	17 ± 42·5	26 ± 56·5	43 ± 50·0	93 ± 58·5
Number of warts							
Mean ± SD	2·9 ± 2·5	2·7 ± 2·7	2·8 ± 2·6	2·6 ± 2·5	3·5 ± 3·1	3·1 ± 2·8	3·0 ± 2·7
Median	2·0	2·0	2·0	1·5	2·0	2·0	2·0
Size (mm)							
Mean ± SD	5·4 ± 1·8	5·5 ± 1·9	5·4 ± 1·9	5·1 ± 2·1	5·4 ± 2·0	5·3 ± 2·1	5·4 ± 2·0
Median	5·0	5·0	5·0	5·0	5·0	5·0	5·0
Duration (months), n (%)							
0–12	17 (50)	25 (64)	42 (58)	25 (62)	32 (70)	57 (66)	99 (62·3)
13 or more	17 (50)	14 (36)	31 (42)	15 (38)	14 (30)	29 (34)	60 (37·7)

MCA, monochloroacetic acid; Cryo, cryotherapy; SA, salicylic acid. [a]As some patients had both common and plantar warts, six plantar warts of six patients with common warts were treated with cryotherapy and eight common warts of seven patients with plantar warts were treated with Cryo plus SA.

Table 2 Morphological characteristics of all common and plantar warts for which photographs were available

Characteristic	Common wart group (n = 143)	Plantar wart group (n = 168)	All warts (n = 311)
Size at baseline in mm: mean ± SD	4·9 ± 2·0	4·9 ± 2·0	4·9 ± 2·0
Arrangement, solitary, n (%) (Other lesions: confluent)	116 (81·1)	126 (75·0)	242 (77·8)
Level, elevated, n (%) (Other lesions: skin level)	116 (81·1)	123 (73·2)	239 (76·8)
Aspect, rough/lobed, n (%) (Other lesions: smooth/not lobed)	96 (67·1)	159 (94·6)[a]	255 (82·0)
Border, sharply defined, n (%) (Other lesions: not sharply defined)	112 (78·3)	121 (72·0)	233 (74·9)
White skin flakes present, n (%) (Other lesions: no white skin flakes)	97 (67·8)	137 (81·5)[a]	234 (75·2)
Black dots present, n (%) (Other lesions: no black dots)	48 (33·6)	120 (71·4)[a]	168 (54·0)
Red colour, n (%) (Other lesions: skin or yellow colour)	45 (31·5)	41 (24·4)	86 (27·7)
Border erythema present, n (%) (Other lesions: no border erythema)	68 (47·6)	24 (14·3)[a]	92 (29·6)
Callus present, n (%) (Other lesions: no callus)	68 (47·6)	149 (88·7)[a]	217 (69·8)

[a]Difference between common and plantar wart group is statistically significant ($P < 0·05$).

Table S2). HPV27 was more often found in plantar warts showing white skin flakes (Table 5 and Table S3). HPV57 was more prevalent in multiple confluent warts without black dots (Table 5 and Table S4) and HPV1 was more frequently detected in solitary warts with a smooth aspect, a sharp, nonerythematous border and black dots (capillary thrombosis) among younger people (Table 5 and Table S5).

Table 6 and Tables S6–9 (see Supporting Information) show the most relevant predictive factors for a favourable treatment response for the three different treatment regimens.

Table 3 Distribution of human papillomavirus (HPV) types in 311 warts of 159 patients[a]

	No HPV detected (n = 18)	Alpha species 4			Mu species 1			
		HPV2 (n = 29)	HPV27 (n = 47)	HPV57 (n = 29)	HPV1 (n = 45)	Other HPV[b] (n = 26)	Multiple HPV[c] (n = 37)	All patients (n = 159)
Sex, n (%)								
Female	11 (61)	13 (45)	28 (60)	19 (66)	30 (67)	18 (69)	21 (57)	99 (62·3)
Male	7 (39)	**16 (55)**	19 (40)	10 (35)	15 (33)	8 (31)	16 (43)	60 (37·7)
Age (years), n (%)								
4–11	2 (11)	7 (24)	11 (23)	6 (21)	36 (80)	15 (58)	14 (38)	66 (41·5)
12 or older	**16 (89)**	**22 (76)**	**36 (77)**	**23 (79)**	**9 (20)**	11 (42)	23 (62)	93 (58·5)
Location, n (%)								
Common	13 (72)	19 (66)	16 (34)	14 (48)	16 (36)	11 (42)	17 (46)	76 (47·8)
Plantar	**5 (28)**	**10 (35)**	31 (66)	15 (52)	29 (64)	15 (58)	20 (54)	83 (52·2)
Duration (months), n (%)								
0–12	6 (33)	19 (66)	21 (45)	12 (41)	37 (82)	19 (73)	18 (49)	99 (62·3)
13 or more	**12 (67)**	10 (35)	**26 (55)**	**17 (59)**	**8 (18)**	7 (27)	**19 (51)**	60 (37·7)

[a]Statistically significant differences are indicated in bold. The patients in each column are compared with the rest of the patients that can be calculated by subtracting the specific column from the last column, which is representing all patients. [b]Altogether 26 patients had other HPV types in 46 different warts. Two patients had two and one patient three of these different types in the same wart or in different warts on the same person, which is reflected here by the fact that the sum of the HPV types (31) exceeds the number of patients (26). The alpha genus HPV10 was found in five warts of three patients; the gamma genus HPV4 was found in 20 warts of 11 patients, HPV28 in one wart, HPV65 in 15 warts of nine patients and HPV88 was only found in combination with HPV27 in two warts of one patient; the mu genus HPV63 was found in four warts of three patients; the nu-genus HPV41 was found in three warts of three patients. The following HPV types were not found: alpha genus HPV3, HPV7, HPV29, HPV40, HPV43, HPV77, HPV91 and HPV94, and the gamma genus HPV48, HPV50, HPV60 and HPV95. [c]Thirty-seven patients had more than one different HPV type in the same wart or in different warts on the same person. Out of 29 patients with HPV2 in one or more warts, seven patients also had HPV27; three also had HPV57; two also had HPV1 and none had another HPV type in their warts. Out of 47 patients with HPV27 in one or more warts, six also had HPV57; four also had HPV1 and three also had another HPV type in their warts. Out of 29 patients with HPV57 in one or more warts, one also had HPV1 and two also had another HPV type in their warts. Out of 45 patients with HPV1 in one or more warts, five also had another HPV type in their warts. There were three patients with three different HPV types in their warts (HPV1, HPV27 and HPV57; HPV1, HPV27 and HPV65; and HPV1, HPV41 and HPV57) and one patients with four different HPV types (HPV1, HPV4, HPV41, HPV65).

Table 4 Morphological characteristics of 311 warts in relation to the human papillomavirus (HPV) genotype[a]

	No HPV detected (n = 34)	Alpha species 4			Mu species 1			
		HPV2 (n = 47)	HPV27 (n = 96)	HPV57 (n = 52)	HPV1 (n = 62)	Other HPV[b] (n = 46)	Multiple HPV[c] (n = 43)	All warts (n = 311)
Arrangement, solitary	29 (85)	39 (83)	**68 (71)**	**31 (60)**	**56 (90)**	36 (78)	32 (74)	242 (77·8)
Level, elevated	24 (71)	35 (75)	73 (76)	40 (77)	51 (82)	35 (76)	31 (72)	239 (76·8)
Aspect, rough/lobed	**18 (53)**	39 (83)	**89 (93)**	43 (83)	51 (82)	38 (83)	**37 (86)**	255 (82·0)
Border, sharply defined	22 (65)	37 (79)	67 (70)	**32 (62)**	**56 (90)**	38 (83)	35 (81)	233 (74·9)
White skin flakes present	**17 (50)**	38 (81)	**84 (88)**	43 (83)	44 (71)	**27 (59)**	33 (77)	234 (75·2)
Black dots present	**1 (3)**	28 (60)	**66 (69)**	24 (46)	**41 (66)**	28 (61)	**28 (65)**	168 (54·0)
Red colour	9 (27)	18 (38)	26 (27)	11 (21)	22 (36)	10 (22)	16 (37)	86 (27·7)
Border erythema present	11 (32)	**23 (49)**	25 (26)	18 (35)	14 (23)	9 (20)	17 (40)	92 (29·6)
Callus present	**14 (41)**	28 (60)	**77 (80)**	38 (73)	**51 (82)**	29 (63)	28 (65)	217 (69·8)

Data are n (%). [a]Statistically significant differences as calculated by $\chi 2$-test compared with the rest of the warts and are indicated in bold; [b]The other HPV types are described in the legend of Table 3; [c]43 warts had more than one different HPV type in the same wart, which is reflected here by the fact that the sum of the HPV types exceeds the number of warts.

Elevated common warts without an erythematous border and without callus had the best therapeutic results when using cryotherapy, which therapy was most effective did not depend on the HPV type that was present in common warts (Table 6). Combination treatment was more effective in sharply defined smooth plantar warts, although this was not statistically

Table 5 Potential predictive factors for the presence of different human papillomavirus (HPV) types calculated for 311 warts using a generalized estimating equation (GEE) model with manual backward elimination[a]

	Any HPV	HPV2	HPV27	HPV57	HPV1
Age (per year)	–	–	–	–	**0·95 (0·91–0·99)**
Male vs. female patients	–	2·20 (0·96–5·30)	–	–	–
Duration of warts (months)	**0·99 (0·98–1·00)**	–	–	–	–
Plantar vs. common warts	–	0·49 (0·21–1·10)	**2·40 (1·20–4·70)**	–	–
Confluent multiple vs. solitary	–	–	–	**3·70 (1·80–7·70)**	**0·35 (0·13–0·90)**
Elevated level vs. skin level	–	–	–	–	–
Rough/lobed vs. smooth	–	–	–	–	**0·25 (0·08–0·80)**
Border sharply defined	**3·40 (1·30–9·00)**	–	–	–	**2·70 (1·00–6·90)**
White skin flakes present	–	–	**2·70 (1·30–5·70)**	–	–
Black dots present	**37·40 (4·50–309·00)**	–	–	**0·49 (0·24–1·00)**	2·00 (0·96–4·10)
Red colour of the wart	–	–	–	–	–
Border erythema present	–	**1·90 (1·00–3·60)**	–	–	**0·48 (0·22–1·00)**
Callus present	**3·10 (1·30–7·10)**	–	–	–	**3·90 (1·6–9·60)**

Data are odds ratios with 95% confidence intervals. –, not included in the final model. [a]The GEE model with manual backward elimination was performed with all the factors in this Table. Statistically significant differences are indicated in bold.

Table 6 Relevant predictive factors for a favourable treatment response calculated for 311 warts using a generalized estimating equation (GEE) model with manual backward elimination[a]

	Common warts		Plantar warts	
	MCA	Cryotherapy	MCA	Cryo + SA
Age (per year)	–	–	–	–
Male vs. female	–	–	–	–
Duration of the warts (months)	**0·99 (0·98–1·00)**	–	–	–
Confluent multiple vs. solitary	2·30 (0·90–5·80)	–	–	–
Elevated level vs. skin level	–	**4·80 (1·10–20·40)**	–	–
Rough/lobed vs. smooth	0·31 (0·08–1·20)	–	–	0·16 (0·03–1·10)
Border sharply defined	–	–	–	3·40 (0·85–13·60)
White skin flakes present	–	–	–	–
Black dots present	–	–	–	–
Red colour of the wart	–	–	**4·20 (1·30–13·40)**	–
Border erythema present	–	**0·22 (0·05–0·90)**	–	–
Callus present	–	**0·13 (0·03–0·51)**	–	–
HPV2	–	–	**0·10 (0·02–0·48)**	–
HPV27	–	–	**0·17 (0·04–0·74)**	**0·03 (0·00–0·15)**
HPV57	–	–	–	–
HPV1	–	–	–	4·90 (0·99–24·10)

Data are odds ratios with 95% confidence intervals. MCA, monochloroacetic acid; Cryo, cryotherapy; SA, salicylic acid; –, not included in the final model; HPV, human papillomavirus. [a]The GEE model with manual backward elimination was performed with all the factors in this table. Statistically significant differences are indicated in bold.

significant. HPV2- or HPV27-positive plantar warts compared with warts that contained HPV1 or other HPV types were much more resistant to treatment with MCA or a combination of cryotherapy and salicylic acid whereas HPV1-positive plantar warts had a favourable nonsignificant outcome when they were treated with combination therapy (Table 6).

Discussion

Although the distinction between common and plantar warts is widely used in clinical practice and research,[19] this is the first study that provides a systematic and standardized approach to acquire data with a clinical tool based on morphological characteristics to discern the two main wart types. Our observations demonstrate that plantar warts are more often characterized by a rough surface with white skin flakes, black dots and callus compared with common warts.

Also, this is the first study providing systematic data on clinical appearance in relation to HPV genotype or treatment response. For some lesions it was possible to predict the HPV type in a specific wart using the age and sex of the patient, the location and duration of the wart and nine morphological

wart characteristics. Black dots (capillary thrombosis) strongly predicted the presence of HPV in a wart and therefore are an indicator that HPV typing should be performed. However, the absence of black dots did not guarantee the absence of HPV.

The predictive value for HPV presence of the other characteristics was too small, and for accurate determination of the HPV type in the wart we relied on HPV DNA genotyping. Although Jablonska *et al.* attempted to associate clinical (and histological) features to HPV type, systematically acquired data or statistical analyses were not reported.[14]

For treatment purposes it may be important to know the HPV type in the wart, because of all the characteristics tested, the HPV type most strongly predicted the treatment response. HPV2 and HPV27 of the alpha genus were significantly associated with a decreased treatment response in the case of the treatment of plantar warts with MCA or the combination of cryotherapy and salicylic acid. By contrast, HPV1-positive plantar warts favourably responded to treatment with a combination of cryotherapy and salicylic acid. These data confirm our earlier finding in a separate group of patients showing that HPV1-positive warts had a much better response to treatment with salicylic acid than HPV2- or HPV27-positive warts.[15]

When cryotherapy was used for common warts, HPV type did not appear to play a role. It seems the response to this destructive therapy with liquid nitrogen does not depend on HPV type. This is in line with a trial by Tomson *et al.* that showed no correlation between HPV type and treatment response to cryotherapy.[16] Cryotherapy for common warts was less effective in the presence of callus and when the warts were located deeper in the skin (nonelevated warts), which confirms our clinical experience.

This study was a substudy of a large, randomized, controlled trial (WARTS-2 trial).[10] With the pragmatic design of the trial, the single photographer and the closely monitored follow-up, we were able to create a relatively large dataset of standardized photographs of common and plantar warts and related information about treatment response. There was a high follow-up rate of patients, because we were able to make use of highly skilled research nurses who did a lot of home visits during the trial.

A new clinical diagnostic tool for warts, the CWARTS tool, was developed and validated in an earlier study.[17] In the current study this tool was implemented. The most predictive characteristics for the presence of an HPV type, 'presence of black dots', was also the characteristic with the strongest agreement among the observers. The intraclass coefficients for inter- and intraobserver agreement were 0·68 and 0·69, respectively, which implies substantial agreement. The use of the CWARTS tool makes a head-to-head comparison for future studies possible.

Besides the discussed advantages, the retrospective nature of the study had some disadvantages. The original trial randomized patients, but we determined morphological characteristics and HPV genotype in individual warts. This may have introduced some bias, as a single patient could have multiple (up to six) separate warts with a different morphology and HPV genotype. In addition, as the original trial did not have a wait-and-see control arm, we could not analyse the natural course of the common and plantar warts. For practical reasons not all patients were photographed, which decreased the power of our analysis compared with the original trial.

We could only analyse HPV2, HPV27, HPV57 and HPV1, the most frequently occurring HPV types in common and plantar warts. The influence of the remaining HPV types on the treatment response is unknown. However, in a study on prevalence of HPV genotype in cutaneous warts, HPV1, HPV2, HPV27 and HPV57 was found in 86% of all HPV-positive warts. Hence, influence of other wart types is estimated to be small.[12] Additionally, HPV typing was performed from swabs and not from wart biopsies, which may have increased the chance of detecting multiple infections.[20]

In terms of clinical practice, the results from our study might guide physicians in treatment decisions, for example warts with black dots may benefit more from MCA treatment or combinational treatment (salicylic acid and cryotherapy) compared with monotherapy with cryotherapy. In addition, based on our findings it might be interesting to develop a fast HPV test kit to determine HPV type in warts to optimize treatment further and enable personalized medicine (often referred to as 'precision medicine').

In summary, this study shows that reliable morphological characteristics of warts and HPV genotype influence treatment response and thus potentially influence future treatment decisions for different common and plantar warts. For the development of new wart therapies it may be important or even essential to take HPV DNA testing into account in order to determine the most optimal treatment option.

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References

1 Lynch MD, Cliffe J, Morris-Jones R. Management of cutaneous viral warts. *BMJ* 2014; **348**:g3339.
2 Bzhalava D, Muhr LS, Lagheden C *et al.* Deep sequencing extends the diversity of human papillomaviruses in human skin. *Sci Rep* 2014; **4**:5807.
3 Beliaeva TL. [The population incidence of warts.] *Vestn Dermatol Venerol* 1990; **2**:55–8 (in Russian).
4 Kilkenny M, Marks R. The descriptive epidemiology of warts in the community. *Australas J Dermatol* 1996; **37**:80–6.
5 Kyriakis K, Pagana G, Michailides C *et al.* Lifetime prevalence fluctuations of common and plane viral warts. *J Eur Acad Dermatol Venereol* 2007; **21**:260–2.
6 van Haalen FM, Bruggink SC, Gussekloo J *et al.* Warts in primary schoolchildren: prevalence and relation with environmental factors. *Br J Dermatol* 2009; **161**:148–52.

7 Ciconte A, Campbell J, Tabrizi S *et al.* Warts are not merely blemishes on the skin: a study on the morbidity associated with having viral cutaneous warts. *Australas J Dermatol* 2003; **44**:169–73.
8 Kwok CS, Gibbs S, Bennett C *et al.* Topical treatments for cutaneous warts. *Cochrane Database Syst Rev* 2012; **9**:CD001781.
9 Bruggink SC, Assendelft WJ. Cryotherapy for plantar warts more costly but no more effective than salicylic acid self-treatment. *Evid Based Med* 2012; **17**:156–7.
10 Bruggink SC, Gussekloo J, Egberts PF *et al.* Monochloroacetic acid application is an effective alternative to cryotherapy for common and plantar warts in primary care: a randomized controlled trial. *J Invest Dermatol* 2015; **135**:1261–7.
11 Bruggink SC, Gussekloo J, Berger MY *et al.* Cryotherapy with liquid nitrogen versus topical salicylic acid application for cutaneous warts in primary care: randomized controlled trial. *CMAJ* 2010; **182**:1624–30.
12 Bruggink SC, de Koning MN, Gussekloo J *et al.* Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. *J Clin Virol* 2012; **55**:250–5.
13 Egawa K. New types of human papillomaviruses and intracytoplasmic inclusion bodies: a classification of inclusion warts according to clinical features, histology and associated HPV types. *Br J Dermatol* 1994; **130**:158–66.
14 Jablonska S, Orth G, Obalek S *et al.* Cutaneous warts. Clinical, histologic, and virologic correlations. *Clin Dermatol* 1985; **3**:71–82.
15 Bruggink SC, Gussekloo J, de Koning MN *et al.* HPV type in plantar warts influences natural course and treatment response: secondary analysis of a randomised controlled trial. *J Clin Virol* 2013; **57**:227–32.
16 Tomson N, Sterling J, Ahmed I *et al.* Human papillomavirus typing of warts and response to cryotherapy. *J Eur Acad Dermatol Venereol* 2011; **25**:1108–11.
17 Hogendoorn GK, Bruggink SC, Hermans KE *et al.* Developing and validating the CWARTS diagnostic tool, a novel clinical assessment and classification system for cutaneous warts. *Br J Dermatol* 2018; https://doi.org/10.1111/bjd.15999
18 de Koning MN, ter Schegget J, Eekhof JA *et al.* Evaluation of a novel broad-spectrum PCR-multiplex genotyping assay for identification of cutaneous wart-associated human papillomavirus types. *J Clin Microbiol* 2010; **48**:1706–11.
19 Micali G, Dall'Oglio F, Nasca MR *et al.* Management of cutaneous warts: an evidence-based approach. *Am J Clin Dermatol* 2004; **5**:311–7.
20 De Koning MN, Khoe LV, Eekhof JA *et al.* Lesional HPV types of cutaneous warts can be reliably identified by surface swabs. *J Clin Virol* 2011; **52**:84–7.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Morphological characteristics of warts in relation to the human papillomavirus genotype.

Table S1 Potential predictive factors for the presence of any human papillomavirus type using a generalized estimating equation model with manual backward elimination.

Table S2 Potential predictive factors for the presence of human papillomavirus type 2 using a generalized estimating equation model with manual backward elimination.

Table S3 Potential predictive factors for the presence of human papillomavirus type 27 using a generalized estimating equation model with manual backward elimination.

Table S4 Potential predictive factors for the presence of human papillomavirus type 57 using a generalized estimating equation model with manual backward elimination.

Table S5 Potential predictive factors for the presence of human papillomavirus type 1 using a generalized estimating equation model with manual backward elimination.

Table S6 Potential predictive factors for a favourable treatment response in common warts treated with monochloroacetic acid using a generalized estimating equation model with manual backward elimination.

Table S7 Potential predictive factors for a favourable treatment response in common warts treated with cryotherapy using a generalized estimating equation model with manual backward elimination.

Table S8 Potential predictive factors for a favourable treatment response in plantar warts treated with monochloroacetic acid using a generalized estimating equation model with manual backward elimination.

Table S9 Potential predictive factors for a favourable treatment response in plantar warts treated with the combination of cryotherapy and salicylic acid using a generalized estimating equation model with manual backward elimination.

Video S1 Author video.