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HPV type in plantar warts influences natural course and treatment response: Secondary analysis of a randomised controlled trial☆

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ABSTRACT

Background: Cryotherapy is effective for common warts, but for plantar warts available treatments often fail.
Objectives: Within a pragmatic randomised controlled trial, we examined whether subgroups of common and plantar warts have a favourable natural course or response to treatment based on wart-associated HPV type.
Study design: Consecutive patients with new common or plantar warts were recruited in 30 Dutch family practices. Patients ($n = 250$) were randomly allocated to liquid-nitrogen cryotherapy, 40% salicylic acid self-application, or wait-and-see policy. Before treatment, swabs were taken from all separate warts and analysed by a broad spectrum HPV genotyping assay. At 13 weeks, cure rates with 95% confidence intervals of common and plantar warts on intention to treat basis were compared between treatment arms for the different wart-associated HPV types.
Results: In total, 7% of swabs tested negative for HPV DNA and 16% contained multiple types, leaving 278 of 371 common swabs (75%) and 299 of 373 plantar swabs (80%) with a single type for analysis. After wait-and-see policy, cure rates were 2/70 (3%, 95% confidence interval 1–10) for HPV 2/27/57-associated common warts, 4/58 (7%, 3–16) for HPV 2/27/57-associated plantar warts, and 21/36 (58%, 42–73) for HPV 1-associated plantar warts. After cryotherapy, cure rates were 30/44 (68%, 53–80), 6/56 (11%, 5–21), and 15/23 (65%, 45–81); after salicylic acid 16/87 (18%, 12–28), 15/60 (25%, 16–37), and 24/26 (92%, 76–98), respectively.
Conclusions: HPV type influenced the natural course and response to treatment for plantar warts. HPV testing potentially optimises wart treatment in primary care.

1. Background

Cutaneous warts are benign papillomas of the skin of which common warts (verrucae vulgaris) and plantar warts (verrucae plantaris) are most common.[1,2] Up to one-third of all primary schoolchildren have warts, of which two-thirds resolve spontaneously within 2 years.[3,4] Since warts frequently result in discomfort,[5] 2% of the general population and 6% of schoolchildren present warts to their general practitioner (GP) for treatment,[6,7] at a reported cost of £40 million per year in the UK.[8] A range of treatment options are available, [9,10] the most common being liquid-nitrogen cryotherapy or topical salicylic acid application.[11] For common warts cryotherapy showed to be most effective, but for plantar warts available treatments often fail.[12,13] Because of the benign natural course and limited effectiveness, side-effects and costs of treatments, some physicians promote a wait-and-see policy.[8,14–16] Definition of subgroups that will better respond to specific treatment could improve treatment results, reduce costs, and limit the burden of side-effects.[17]

Warts are caused by infection with human papillomavirus (HPV). More than 120 HPV types, distributed over 5 genera and 16

Abbreviations: GP, general practitioner; OTC, over-the-counter; HPV, human papillomavirus; HSL, hyperkeratotic skin lesion; PCR/MPG, polymerase chain reaction/multiplex genotyping; CI, confidence interval.

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species, have been described based on their DNA sequences.[18,19] Development of the HSL-PCR/MPG (hyperkeratotic skin lesion – polymerase chain reaction/multiplex genotyping) assay has recently paved the way for large-scale cutaneous wart-associated HPV typing.[20] HPV 2, 27, and 57 from the alpha genus, and HPV 1 from the mu genus are the most prevalent types detected in cutaneous warts.[21–27] Since specific HPV types are related to clinical characteristics such as type of wart (common or plantar) and age of the patient, we questioned whether these HPV types could influence the natural course or response to treatment.[27]

2. Objectives

Within a randomised controlled trial comparing liquid nitrogen cryotherapy, topical salicylic acid application, and a wait-and-see policy, we examined whether subgroups of common and plantar warts have a favourable natural course or response to treatment based on wart-associated HPV type.

3. Study design

This study is a secondary analysis within the WArts Randomized Treatment Study (WARTS, trial registration ISRCTN 42730629). For detailed information on study design and treatment protocols we refer to the publication of the original trial.[12]

3.1. Patients and samples

All patients from 4 years of age and older who attended one of the 30 participating general practices between May 1st 2006 and January 26th 2007 with one or more new cutaneous warts were eligible. We defined new cutaneous warts as common or plantar warts on the skin that were diagnosed in general practice and were presented for the first time without treatment from a physician or dermatologist in the previous year, regardless of previous self-treatment with over-the-counter (OTC) medication, and excluded immunocompromised patients. Trained research nurses visited the patients at home to confirm eligibility and collect baseline characteristics, including number, size, location and duration (<6 versus ≥6 months) of warts.

3.2. Randomisation

We stratified patients by number of warts (<6 versus ≥6 warts) and type of warts (plantar [warts on the soles of the feet] versus common [all other locations, mainly on the hands]). Patients who had both plantar and common warts were stratified according to where the majority was located. All warts of patients with multiple warts received the same treatment.

3.3. Treatments

We trained all GPs and assistants in the three 13-week protocols, which were designed to reflect best practice.[10] In the cryotherapy protocol, we used a high intensity regimen of one session every 2 weeks until all warts were completely gone. In the salicylic acid protocol, salicylic acid 40% in a vaseline album solution was self-administered every day. In the wait-and-see protocol, participants were informed about the benign natural course of warts and were advised not to undergo treatment for at least 13 weeks.

3.4. Outcome assessment

The trained research nurses assessed wart cure during home visits at 13 weeks of follow-up, independently of the treating general practice. A wart was considered cured if the wart had visually disappeared (skin colour and skin lines re-established) and could no longer be palpated by hand.

3.5. HPV identification

At baseline, the nurses took swabs from each single wart by firmly rubbing a wetted cotton-tipped stick over the surface of the wart five times. This swab technique adequately detects HPV types compared to wart scab or biopsy.[28] We considered multiple warts as a cluster when the distance between warts was less than 1 cm. Only when warts were too close to take separate swabs, a single swab was taken from the cluster. All swabs were stored in 1 ml of saline solution.

To determine HPV type, a broad spectrum PCR–MPG assay was used for genotyping all known wart-associated HPV types from the alpha (HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma (HPV4, 65, 95, 48, 50, 60 and 88), mu (HPV1 and 63) and nu genus (HPV41). This sensitive and specific assay (HSL-PCR/MPG assay; Labo Biomedical Products BV, Rijswijk, The Netherlands) has been well described and evaluated.[20] In short, 10 μl of the saline solution was used in the single-step HSL-PCR, generating a biotinylated amplimer of 76–84 bp from the L1 region. Subsequently, simultaneous identification of the 23 HPV genotypes was performed with bead-based xMAP suspension array technology.

3.6. Statistical analysis

Baseline characteristics of the patients and warts, as well as all outcomes, were stratified for common and plantar warts. Because HPV type is associated with separate warts, we used warts instead of patients as unit of analysis. The primary outcome measure was the crude cure rate of separate warts associated with a single HPV type per treatment arm per specific wart-associated HPV type at 13 weeks on an intention-to-treat basis. The software package SPSS, PASW Statistics, release 17.02 was used.

We only compared cure rates for HPV types which had at least 10 warts per treatment arm. To identify subgroups of common and plantar warts that have a favourable natural course, cure rates of wait-and-see arms were compared between specific HPV types using 95% confidence intervals (CIs). To examine subgroups that have favourable response to treatment, cure rates of treatment arms were compared within specific HPV types using 95% CIs, relative risks and risk differences.

In addition, per-protocol analysis was performed based on reported treatment adherence. To explore whether we had created a specific subgroup of warts by including only warts with a single HPV type, we compared cure rates of warts negative for HPV DNA and cure rates of warts with multiple HPV types with cure rates of warts with single HPV type within treatment arms.

4. Results

4.1. Patients and samples

In the original trial, 250 patients with 391 common and 379 plantar warts were included.[12] No swabs were available from 20 common and 6 plantar warts (13 warts belonged to four patients without consent for swabs, and 13 swabs were lost in transport to the laboratory). A total of 45 common and 4 plantar warts (7%) swabs tested negative for HPV DNA, and 48 common and 70 plantar warts (16%) contained multiple HPV types per swab, leaving 278 common warts (75%) and 299 plantar warts (80%) with single HPV type for analysis. A further 6 patients with 7 common and 8 plantar warts (3%) were lost to follow-up (Fig. 1). Patients were evenly distributed over treatment arms, but by chance the cryotherapy arm

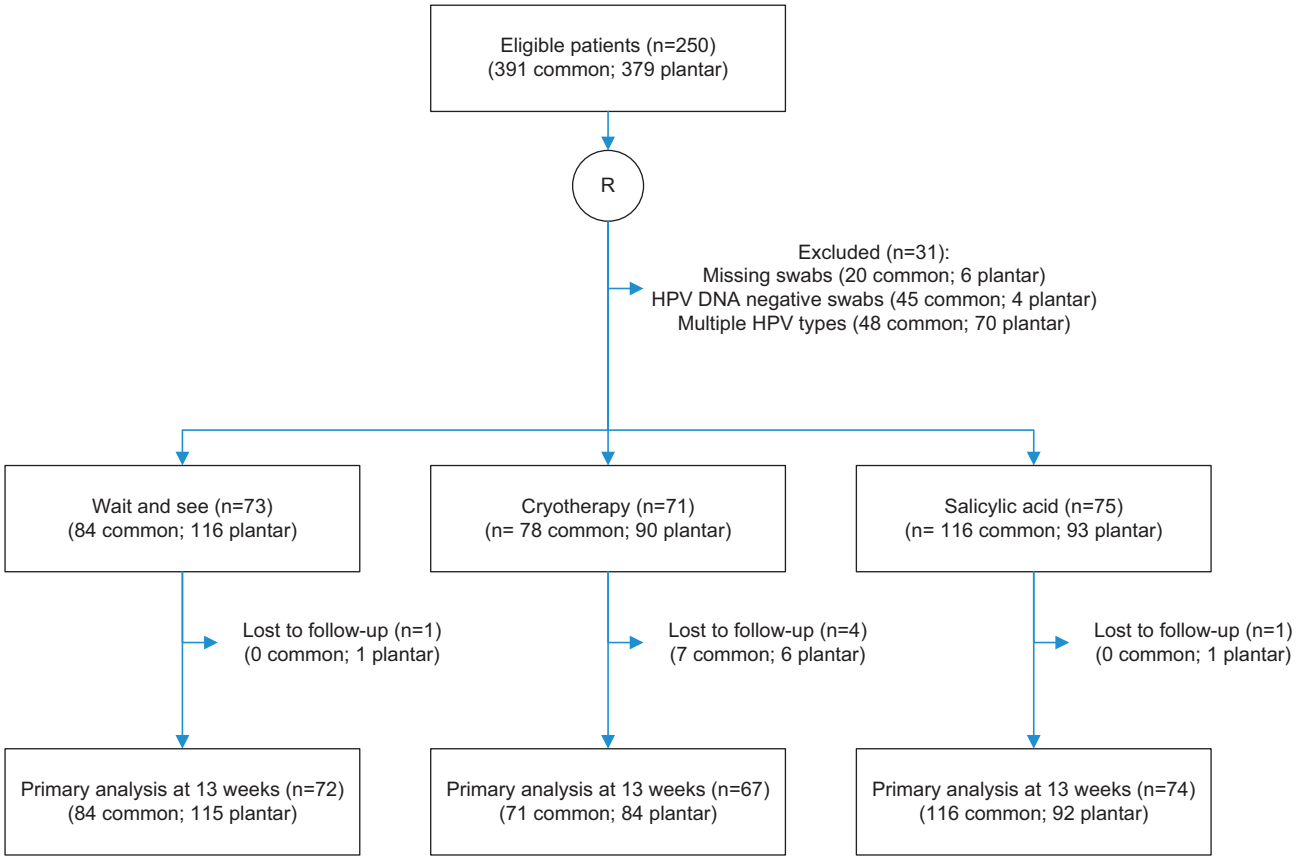

Fig. 1. Flowchart of common and plantar warts.

contained less warts than the salicylic acid and wait-and-see arm for common warts ($p = 0.003$) as well as plantar warts ($p = 0.069$).

Baseline characteristics of patients with complete follow-up showed that, in the common wart group ($n = 103$), 54 patients (56%) were female, median age was 16 (range 4–73) years, and median number of warts was 2 (interquartile range [IQR] 1–4). In the plantar wart group ($n = 110$), 68 patients (62%) were female, median age was 11 (range 4–69) years, and median number of warts was also 2 (IQR 1–4). In total, 91 patients had common warts only, 90 had plantar warts only, and 32 had both common and plantar warts.

The patients had a total of 271 common warts and 291 plantar warts (Table 1). The common warts were mainly located on hands (78%). The combined contribution of the four most prevalent HPV types (HPV 1, HPV 2, HPV 27 and HPV 57) was 82% in common and 88% in plantar warts. For detailed information on the HPV type prevalence and their relation with patient characteristics we refer to a recent publication.[27]

Table 1
Characteristics of warts associated with a single HPV type ($n = 562$).

	Common warts ($n = 271$)	Plantar warts ($n = 291$)
Associated HPV type[a]		
HPV 1	20 (7)	85 (29)
HPV 2	80 (30)	35 (12)
HPV 27	65 (24)	71 (24)
HPV 57	56 (21)	68 (23)
Other HPV types	50 (18)	32 (11)[b]
Location		
Sole of the foot	–	291 (100)
Dorsum of the foot	26 (10)	–
Hand	211 (78)	–
Rest of the body	34 (13)	–
Wart duration < 6 months	56 (21)	125 (43)
Size of wart in millimetre (median, IQR)	3 (4–5)	3 (4–5)

Values are numbers (percentage of warts) unless stated otherwise.

[a] HPV3, 10, 28, 2, 27, 57 and 7 from the alpha genus, HPV4, 65, and 95 from the gamma genus, HPV1 and 63 from the mu genus, and HPV41 from the nu genus.

[b] Sum of percentages is $\neq$ 100 due to rounding off.

4.2. HPV types and wart cure

Only the three most prevalent types (HPV 2, 27, and 57) for common warts, and the four most prevalent types (HPV 2, 27, 57, and 1) for plantar warts had sufficient numbers (>10) to compare treatment arms (supplemental table). Since the CIs of cure rates of the three highly prevalent HPV types 2, 27, and 57 from the alpha genus species 4 overlapped for common as well as for plantar warts, we combined cure rates for these HPV types. Thus, we identified three subgroups of warts for which we could make reliable comparisons: common warts with HPV 2/27/57 ($n = 201$), plantar warts with HPV 2/27/57 ($n = 174$), and plantar warts with HPV 1 ($n = 85$).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.02.021.

For common warts with HPV 2/27/57, the cure rate after a wait-and-see policy was 2/70 (3%, 95% CI 1–10). Cryotherapy was the most effective treatment for common warts with HPV 2/27/57: 30/44 (68%, 53–80) cured compared to 16/87 (18%, 12–28) cured after salicylic acid (Fig. 2 and Table 2).

For plantar warts, the subgroup with HPV 1 had a favourable natural course compared to those with HPV 2/27/57: 21/36 (58%, 95% CI 42–73) cured versus 4/58 (7%, 3–16) cured after a wait-and-see policy (Table 2). For plantar warts with HPV 2/27/57, salicylic acid [15/60, 25% (16–37) cured] was more effective compared to wait-and-see, whereas cryotherapy [6/56, 11% (5–21) cured] was not more effective than wait-and-see. For plantar warts with HPV

Table 2
Natural course and treatment response of the three largest groups[a] of warts based on type of wart (common or plantar) and wart-associated HPV type ($n = 460$).

	Common warts	Plantar warts	
	HPV 2/27/57 ($n = 201$)	HPV 2/27/57 ($n = 174$)	HPV 1 ($n = 85$)
Cure rates[b]			
Wait-and-see	2/70; 3 (1 to 10)	4/58; 7 (3 to 16)	21/36; 58 (42 to 73)
Cryotherapy	30/44; 68 (53 to 80)	6/56; 11 (5 to 21)	15/23; 65 (45 to 81)
Salicylic acid	16/87; 18 (12 to 28)	15/60; 25 (16 to 37)	24/26; 92 (76 to 98)
Relative risks[c]			
Wait-and-see	1.0	1.0	1.0
Cryotherapy	23.9 (6.0 to 94.9)	1.6 (0.46 to 5.2)	1.1 (0.74 to 1.7)
Salicylic acid	6.4 (1.5 to 27.1)	3.6 (1.3 to 10.3)	1.6 (1.2 to 2.2)
Risk differences[c]			
Wait-and-see	0	0	0
Cryotherapy	65 (51 to 80)	4 (−7 to 14)	7 (−18 to 32)
Salicylic acid	16 (7 to 25)	18 (5 to 31)	34 (15 to 53)

[a] Numbers of warts > 10 per treatment arm were considered sufficiently high to compare cure rates.
[b] Cure rates are number of warts cured at 13 weeks/number of warts; percentage (95% confidence intervals [CIs]).
[c] Relative risks (95% CIs) and risk differences (95% CIs) of active treatments compared to wait-and-see policy as reference.

1, salicylic acid [24/26, 92% (76–98) cured] was also more effective compared to wait-and-see, whereas cryotherapy [15/23, 65% (45–81) cured] was not more effective than wait-and-see (Fig. 2 and Table 2).

In addition to the highly prevalent HPV types, plantar warts with HPV 4 from the gamma genus showed sufficient numbers ($n = 17$) in the wait-and-see arm to reveal a specifically favourable natural course: 16/17 (94%, 73–99) of warts cured (additional file 1). Per-protocol analysis did not reveal additional information. Cure rates per treatment arm in warts negative for HPV DNA ($n = 40$) and warts with multiple HPV types ($n = 118$) were similar to our analysis of cure rates of warts with single HPV types ($n = 562$).

5. Discussion

5.1. Main findings

HPV type influences the natural course and treatment response for plantar warts. The probability of cure after a wait-and-see policy was 8 times higher for HPV 1-associated plantar warts than for HPV 2/27/57-associated plantar warts. Using the HSL-PCR/MPG assay in our primary care study population, 80% of plantar warts provided a single HPV type of which 29% contained HPV 1. When treated, salicylic acid was more effective than cryotherapy for both HPV subgroups of plantar warts. However, for common warts, cryotherapy was most effective. Since the majority of common warts were associated with HPV 2/27/57, this study does not provide sufficient power to draw conclusions on the less prevalent HPV types.

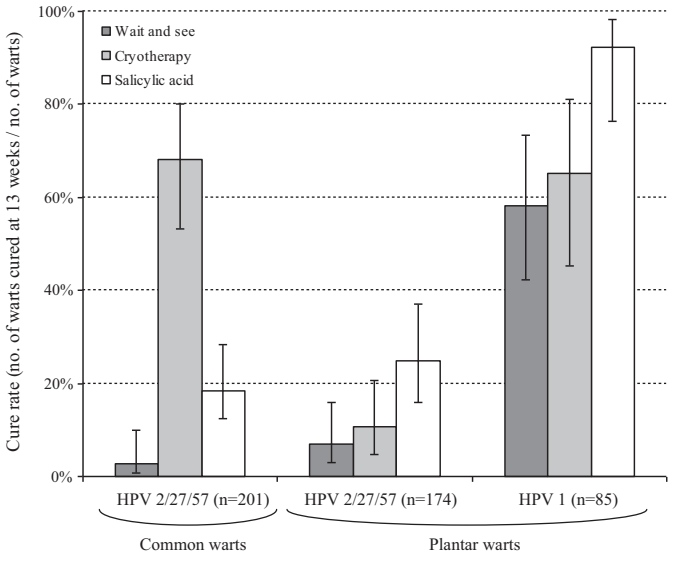

Fig. 2. Cure rates per treatment group with 95% confidence intervals stratified by type of warts (common or plantar) and warts-associated HPV type ($n = 460$).

5.2. Comparison with literature

This study confirms that treatment response in common warts is different from plantar warts,[10] even when associated with the same HPV type. Reasons for this difference are not fully understood at present. Conceivably, skin location specific factors such as callus are at play.

The short duration of warts with HPV 1 has been described earlier, but has never been prospectively investigated or related to treatment response.[24] Only one other trial has investigated the relation between cutaneous wart-associated HPV type and treatment effect. Tomson et al. (2010) studied the effect of cryotherapy on 54 common and plantar warts.[26] They found that the response to cryotherapy was unrelated to HPV type, but more likely the result of the individual's immune response to the virus. However, since all warts were treated with cryotherapy, they could not investigate differences between a wait-and-see policy or salicylic acid treatment. Furthermore, the low number of warts prevented drawing conclusions about HPV 1 associated warts.[26]

Compared to older HPV typing techniques, the HSL-PCR/MPG assay is able to distinguish closely related HPV types 2, 27, and 57.[20] The similar cure rates of these types are probably in line with their high DNA homology in the alpha genus species 4,[18] and correspond with their similarity in relation to patient characteristics.[27] Only 7% of all swabs were negative for HPV DNA in which unknown HPV types could be involved. Alternatively, lesions (like callus) could have been misdiagnosed as warts, or residual hyperkeratotic lesions following HPV clearance could have been sampled. Therefore we could not use the swabs negative for HPV DNA for clinical prediction. The assay was also capable of detecting multiple HPV types per wart. It is likely that only one HPV type is responsible for the persistence of the wart; however, it is difficult to establish which one without using a technology such as laser capture microdissection for which biopsies instead of wart swabs are needed.[27–29] Consequently, we did not use swabs with multiple HPV types for clinical prediction.

5.3. Strengths and limitations

This study combined the broad spectrum HSL-PCR/MPG assay and simple non-invasive swabs, which showed that HPV testing in

practice can be easy, quick, and reliable. The study was embedded in a high quality randomised trial in primary care.[12] The two most frequently used treatments in dermatology as well as primary care practice were included.[9,11] However, the power of our analyses is lower compared to the original trial, since we studied cure rates in subgroups based on HPV types. Nevertheless, for the four most prevalent HPV types, numbers of warts per HPV type per treatment arm were high enough to make reliable comparisons. Although the original trial randomised the patients, we determined HPV type in separate warts. Because more than half of all patients had multiple warts, the number of warts in the cryotherapy arm was less than the numbers in the other two arms due to chance.

This study allows us to conclude that the HPV type influences the natural course and treatment response, which is different from drawing conclusions about causal relations. One could argue that some wart characteristics are confounders, or are in fact in the causal pathway between HPV type and cure.[30] For example, HPV 1 is associated with a low number of warts per patient. HPV 1 often has endophytic growth patterns and high viral loads. It is hypothesised that this could trigger the immune system and limit the spread of warts, both of which could contribute to the favourable cure rates.[31] Increasing numbers of warts per patient may reflect poor immune responses to the HPV type inducing these warts. However, we did not study the immune response in this cohort of patients. Thus, for our research question related to prognosis only, we chose to present crude cure rates without adjustment.

5.4. Implications

This study reveals that HPV type may influence the choice of treatment for plantar warts. In daily practice, detection of HPV 1 in plantar warts implies a favourable natural course and may lead to advise the patient to wait-and-see. Detection of HPV 2/27/57 in plantar warts implies a persistent wart, which in most cases is resistant to treatment. However, when treatment is preferred, salicylic acid can be considered. For common warts, HPV typing does not yet contribute to the choice of treatment, because cryotherapy is effective in the majority of HPV2/27/57-associated warts. Future studies should reveal whether less prevalent HPV types causing common warts will be associated with lower cure rates. With our findings, we have opened a new direction to optimise wart treatment.

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Competing interest

Authors M.N.C. de Koning and W.G.V. Quint are employed by DDL Diagnostic Laboratory which performs HPV testing; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval

The protocol was approved by the medical ethical committee of the Leiden University Medical Centre.

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