Diploma Thesis

PODOPHYLLOTOXIN VERSUS PULSED DYE LASER

In the Treatment of HPV-related genital warts

Submitted by

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Statutory declaration

I hereby declare that I wrote this diploma thesis on my own accord and without any help from outside, that I only used the listed sources and that I indicated whenever I utilized a source as citation or in content.

Graz, 21.11.2016 *Jana Studnicka eh.*

Abstract

Background

Anogenital warts are one of the most common sexually transmitted diseases, caused by the human papillomavirus (HPV). The therapy with topical 0.5 % podophyllotoxin solution (Condylox®) is one of the most effective treatment options, and is applied by the patient over several weeks. The pulsed dye laser (PDL) represents a new treatment alternative, which eradicates the warts in a fast and potent manner. This study compares both treatment options, laser and podophyllotoxin, in terms of efficacy (reduced number of warts and infected area) as rated by the physician, and also regarding side effects (perceived pain) and quality of life. (How satisfied was the patient with the treatment) rated by the patient. The study was designed as an equivalence trial.

Methods

From the year 2012 to 2014, 48 patients (46 male, 2 female) with anogenital warts took part in a randomized and controlled, observer -blinded, monocentric trial at the outpatient venereology department in Graz. The patients were randomly assigned to either therapy with self-applied podophyllotoxin over a duration of several weeks or a PDL treatment, carried out by a trained physician. A 595 nm PDL *Candela Vbeam Perfecta* was used with the following specifications: energy density 12 J/cm², length of impulse 1.5 milliseconds, spot size 7 mm. Two laser impulses per wart were applied. The first treatment took place on day one, with a second treatment (if necessary) three weeks later. At a follow-up appointment about six weeks later, the efficacy of the therapy was evaluated by a physician, and the patients were asked to fill out a questionnaire regarding the experienced side effects (pain), and how comfortable the procedure was perceived (quality of life).

Results

After a strict selection through the inclusion/exclusion criteria, 36 male patients were selected for the statistical analysis. Statistics did not find any significant difference in efficacy (measured by the reduced number of warts and reduced infected area in mm^2), the significance level being 0.403 and 0.855 with α-level of <0.05. The experienced pain was also not significantly different (0.120), the rated level of QOL also showed no significant difference (0.078). However, a slight trend favouring the PDL treatment regarding QOL could be observed

Conclusion

The current study, comparing topical podophyllotoxin solution and PDL as therapy options for anogenital HPV induced warts, found that both treatments can be regarded as equal in terms of efficacy, side effects and QQL. Moreover, the laser treatment tended to be rated as slightly more comfortable for the patient than the established podophyllotoxin treatment. It can be concluded that the treatment of HPV related anogenital warts with PDL represents a safe and effective treatment option.

Zusammenfassung

Hintergrund

Anogenitale Warzen zählen zu den häufigsten sexuell übertragenen Krankheiten und werden von Humanen Papillomaviren (HPV) verursacht. Die Behandlung mit 0,5% Podophyllotoxin (Condylox®) Lösung ist eine der effektivsten Therapieoptionen. Die Substanz wird hierbei über einen Zeitraum von mehreren Wochen aufgetragen. Der gepulste Farbstofflaser (PDL- Pulsed dye laser) stellt eine Methode dar, welche die Warzen schnell und wirkungsvoll zerstört. Die vorliegende Studie vergleicht die Effektivität (reduzierte Anzahl an Warzen und infiziertem Hautbereich) sowie die Nebenwirkungen (gemessen am empfundenem Schmerz) und Lebensqualität (wie angenehm wurde die Therapie wahrgenommen).

Methoden

Vom Jahr 2012 bis 2014 nahmen 48 Probanden (46 Männer, 2 Frauen) mit genitalen HPV-induzierten Warzen an einer randomisierten, monozentrischen Studie an der dermatologischen Ambulanz in Graz, Österreich, teil. Sie wurden entweder der Podophyllotoxin-Therapiegruppe, bei der eine Podophyllotoxin-Lösung über einen Zeitraum von mehreren Wochen aufgetragen wurde, oder der PDL-Gruppe zugeteilt. Der verwendete 595 nm PDL *Candela Vbeam Perfecta* wurde mit folgenden Einstellungen benutzt: Energiedichte 12 J/cm², Impulsdauer 1,5 Millisekunden, Impulsgröße 7 mm. Pro Warze wurden 2 Laserimpulse abgegeben. Die erste Behandlung fand am Tag 1 statt, eine zweite Behandlung wurde – falls notwendig - 3 Wochen später durchgeführt. Im Rahmen der Nachfolgeuntersuchung, welche etwa sechs Wochen nach der initialen Therapie erfolgte, wurde die Effektivität der Behandlung vom zuständigen Arzt oder der zuständigen Ärztin evaluiert, zusätzlich wurde von Seiten der Probanden Fragen bezüglich der Nebenwirkungen (Schmerz) beantwortet Die Frage nach der Lebensqualität zielte darauf, herauszufinden, wie angenehm die Therapie erlebt wurde.

Ergebnisse

Unter Anwendung der Inklusions- /Exklusions-Kriterien wurden letztlich 36 männliche Patienten in die Studie eingeschlossen um im Rahmen eines Äquivalenztests Effektivität, Nebenwirkungen und Lebensqualität zu bewerten. Es fand sich statistisch gesehen kein signifikanter Unterschied hinsichtlich Effektivität (reduzierte Anzahl an Warzen und infiziertem Hautbereich gemessen in mm^2), die Signifikanz Level betrugen hierbei 0,403 und 0, 855 bei einem α-Wert von <0, 05. Auch bezüglich der Nebenwirkungen ließ sich kein signifikanter Unterschied feststellen (0,120), ebenso wie beim Parameter Lebensqualität (0,078). Dennoch konnte ein gewisser Trend beobachtet werden, dass Patienten die Lasertherapie als insgesamt angenehmer bewerteten.

Zusammenfassung

Die vorliegende Untersuchung zeigt, dass Podophyllotoxin und PDL bezüglich der Faktoren Effektivität, Nebenwirkung und Lebensqualität gleich gute Behandlungsoptionen für genitale Warzen darstellen. Zudem wurde die Behandlung per PDL von den Patienten als „angenehmer“ bewertet als die etablierte Medikation mit Podophyllotoxin. Die Anwendung von PDL bei HPV-induzierten, genitalen Warzen kann bezüglich Effektivität, Sicherheit und Lebensqualität als empfehlenswert eingestuft werden.

Acknowledgments

First and foremost, I want to thank Dr. Peter Komericki for making this thesis possible, and the Medical University of Graz for giving me the opportunity of studying human medicine, and my family and friends for all their love and support.

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List of Abbreviations

DNA… Deoxyribonnucleic acid
FDA… United States Food and Drug Administration
GCP… Good clinical practice
HIV… Human Immunodeficiency virus
HPV… Human papillomavirus
HSV… Herpes simplex virus
ICH… International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
Laser... Light Amplified Stimulated Emission Radiation
LGB... Lesbian/gay/bisexual individuals
MSM... Men who have sex with men
PAP... George Papanicolau, inventor of the PAP smear, a screening test for cell dysplasia of the cervix
PCR... Polymerase Chain Reaction)
PDL... Pulsed dye laser
QOL... Quality of life
STD… Sexually transmitted disease
US... United States of America

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CHAPTER I: BACKGROUND

Introduction

The human papillomavirus (HPV) is a pathogen that causes different sorts of warts, but some subtypes of HPV can also cause cancer. In this thesis we will focus on the non-oncogenic subtypes of HPV in the anogenital area and their manifestations. (1) (2)

The formation of anogenital warts is predominantly a cosmetic problem; though in immunocompromised patients more severe complications like tumorous growth are possible. However, the psychological aspect of warts in the anogenital area cannot be underestimated, as there still exists a big stigma concerning STDs in general. (1) (2)

An HPV infection of the anogenital region has been found to occur in >50% of sexually active women, other studies found a similar prevalence in men. (3) (4) Also, regarding first-time treatment seekers for HPV related anogenital warts a rise of >500% in the past decade has been detected. The Center of Disease Control estimated in 2005 that 20 million people in the US (total population of US: 320 million people) had HPV. (5) (6) (7) (8)

Fortunately, there are many medical treatment options for patients with anogenital warts. The choice of therapy is made by evaluating the size and quantity of the warts; for more extensive manifestations, surgical methods as excision by scalpel or spoon are feasible. Smaller lesions can be treated by laser, cryotherapy, or by application of a local remedy, like imiquimod or podophyllotoxin. Those different options have varying pros and cons- some take time, others are rather painful; some are rather expensive, or have a better success rate than others. The different aspects of each therapeutic option will be explained in greater detail in the following chapters. (9) (10) (11) (12)

Another protective option is the immunization against HPV. Several vaccines are currently on the market, and protect against different types of HPV. It has been demonstrated that the immunization poses a good protection against HPV-related genital warts, but long term data regarding the protection against HPV-induced cell dysplasia and cancer is still a work in progress. Also it remains a highly discussed topic if both sexes should be vaccinated, and at what age a vaccination is purposeful. (13) (14)

HPV and anogenital warts

The HPV (human papillomavirus) infects humans, but other forms of the papillomavirus can also infect different vertebrate. (3) Over 100 HPV straits have been found in the human species so far. They are most likely to infect the squamous stratified epithelium (skin and mucous membrane). Of those, about 35 types of HPV have a tropism for the anogenital region. Some HPV subtypes cause cancer and are classified as oncogenic, others as non-oncogenic or low-oncogenic. (15)

The oncogenic HPV types (16, 18, 31, 33, 45, 52 und 58) cause intraepithelial neoplasia and cancer by integrating the HPV-DNA into the human cellular genome of keratinocytes of the cervix, vagina, vulva, anus, penis or, in rare cases, the larynx. (16) The risk to develop a HPV induced cancer is higher in women, but men can also be affected by oncogenic HPV types. (17) (18) (19)

The low-risk HPV subtypes (predominantly 6, 11) can cause anogenital warts and have never been proven to integrate DNA into other cells. These warts tend to grow in an exophytic pattern and can be treated with several remedies, including topical lotions or surgical options (For greater detail, see Chapter “Treatment options”). However, HPV induced warts tend to return after initial clearance. The recurrence rate is reported with approximately 50%, although this percentage may vary. In about 10-20% of genital warts, also oncogenic HPV types can be detected. In immuncompromised patients, Buschke-Löwenstein tumors may develop, caused by HPV 6 and 11. This manifestations can produce an abundant mass of tissue and may even be invasive. (15) (16)

Other non-oncogenic HPV types (1, 2, 4, 7) can induce plantar or common warts, most predominantly on hands and feet. HPV 3 and 10 can cause flat warts, mostly found in the face and hands. In any of these manifestations, different kinds of HPV types may be detected. (16)

Iit is quite difficult to obtain reliable numbers about HPV infections in Austria, since only a few sexually transmitted diseases (STDs) like HIV, syphilis, ulcus molle, gonorrhea, hepatitis, lymphogranuloma inguinale have to be reported to the public health department. (20) Therefore, reliable statistics of non-notifiable infections like HPV, chlamydia, herpes or trichomoniasis are not available in Austria. Additionally, many patients without symptoms or only light symptoms stay undetected, but may still spread the disease. The complications of undetected STDs- sterility in young women from chlamydia, the oncologic consequences of HPV, late symptoms including severe neurologic changes in syphilis and the consequences for the immune system of an HIV infection, could be easily prevented by improved education and prevention programs. (17) (20)

Diagnosis

The diagnosis of HPV induced genital warts is usually made by visual inspection through a skilled physician. In the vinegar (acetic acid) solution test, vinegar solution is applied to the infected area. If an HPV infection is present, the area turns white. This may be especially helpful in identifying flat lesions. A suspect finding may be furthermore confirmed by biopsy, particularly in atypical lesions (e.g. pigmented, bleeding or ulcerated lesions). However, the results of a biopsy in patients with typical lesions have little clinical value. (12) (15)

Pathogenesis and Infection

It is estimated that about 80% of the population gets infected with genital HPV at least once in their life. (21) A transmission of the virus usually happens via direct skin to skin contact. It is important to note that also invisible infections without noticeable symptoms like dysplasia or warts may cause a transmission of the virus. As the virus is able to infest the external and internal genital area, including the mons pubis and adjacent regions, a condom only reduces the risk of an infection, yet cannot fully protect against an infection with HPV during sexual intercourse. (15) (22)

In several studies, the risk for HPV infection has been examined, including factors like oral contraception or smoking. However, those factors only showed a minimal impact on the risk of infection; the most important determining factor remained an increased number of lifetime sexual partners. Also, a sexual relationship with a person who has an increased number of lifetime sexual partners showed to increase the risk of HPV infection. (8) It is estimated that the rate of infectivity between sexual partners is about 60%. (23)

Also, vertical transmission to newborns by passage through an infected birth canal is possible. In some cases, a caesarian section can be a viable option to protect the newborn from an HPV infection. (8) (15)

The clinical approach

A patient presenting genital warts should be handled with respect and sincerity, since there still exists a big stigma regarding all genital diseases, especially sexually transmitted diseases (STDs). It should be emphasized that a relationship built on trust and mutual respect between doctor and patient will allow the patient to speak openly about his or her symptoms. This is especially important in cases of STD, to ensure acceptance of the therapy and compliance, and to prevent a further spreading of the pathogen. Furthermore, the patient should feel comfortable to return in case of enduring or recurring disease. Also, a screening for other STDs is highly advised, as the co-infection rate is given with about 6 %. (17) Common co-infections include chlamydia, gonorrhea, trichomonas, HIV (human immunodeficiency virus), syphilis, hepatitis B and C. Those diseases can be diagnosed by a urethral, cervical and vaginal swab (chlamydia, gonorrhea, and trichomonas) or a blood test (HIV, syphilis, hepatitis B and C). In the case of suspicious lesions, it is also advised to screen for herpes simplex virus (HSV). (1) (2) (11) (15) (17)

Especially in young patients, HPV and other STDs can have a high impact on the individual psyche, and may result in a lowered self-esteem, withdrawal from social activities or depression. Also, a total withdrawal from all sexual activities can sometimes be observed, with dramatic consequences for the life of young, otherwise sexually active persons. (1) (2) (11)

Treatment options for HPV-induced anogenital warts

Genital warts are not a modern invention, but a sexually transmitted disease that has been travelling and evolving with mankind for a long time. (16) Considering this, it is not surprising that the range of possible therapies is rather large, from simple excision modalities by knife, cauterization, cryotherapy to high tech laser applications; and an array of topical agents: podophyllotoxin, imiquimod and sinecatechin compromise the most important remedies, treatment with 5-fluorouracil or trichloroacetic acid represent some less common options. (9) (24) (25)

This vast array of therapies may seem overwhelming when choosing the best therapy for a patient. Still, according to the size, number and recurrence rate of the genital warts, and also in terms of comorbidity and general health, the optimal treatment for every patient should be carefully chosen and administered to allow the best possible outcome. (12)

Podophyllotoxin (Condylox®)

Podophyllotoxin is a non-alkaloid toxic lignan extracted from the roots of the Podophyllum, an African plant. (26) It represents the established first-line therapy for anogenital warts and has been first described for this purpose in 1942. Podophyllotoxin induces the healing of lesions by binding to microtubules and causing mitotic arrest in the metaphase of cell division. This leads to an inhibition of the proliferation of skin keratinocytes, and leads to a diminishing of the lesions. (27) Placebo-controlled studies found a clinical cure rate ranging from 37% to 83% in cases of HPV induced anogenital warts. (9)

The substance is usually administered as solution, the typical dosage is 0.5%. It is recommended for the treatment of external genital warts caused by HPV and other viruses. (9) (27) In Austria, the drug is marketed as *Condylox®* 0.5% solution by Takeda Austria GmbH. (28) Typically, the topical agent is recommended to be administered for 4 weeks by the patient, with pausing days in between were the drug is not administered on the infected site. (9)

Side effects of podophyllotoxin include burning, redness, pain, swelling and itching. Also small sores, itching and peeling of the skin may appear hours to days after application. It should not be used during pregnancy as the drug may be harmful for the fetus. Podophyllotoxin is listed in the WHO Model List of Essential Medicine as one of the most important medications in basic health care. Its efficacy and safety, and the advantage of self-application by the patient makes podophyllotoxin an appealing option for the treatment of external anogenital warts (9) (12) (24) (27) (29)

Imiquimod (Aldara®)

Imiquimod (Aldara®) is a locally applied immune response modifier. It is used to treat various skin conditions: anogenital warts, small superficial skin cancers and actinic keratosis. It is available at different dosages- the standard being 5%, but new studies have been made with a 3,75% cream, which induced less side effects and equal clearance rates. (24) (27)

The drug functions by binding to immune cells in the skins, which leads to a release of cytokines including interferon- α, interleukin-1, interleukin-6, tumor necrosis factor-α and granulocyte colony-stimulating factor. (27) These cytokines stimulate the local immune system; this seems to have a positive effect in the treatment of viral infections like HPV. (9) (12)

The side effects of a therapy with topical imiquimod are similar to those of a podophyllotoxin treatment: swelling, itching, redness, burning, pain, hardening or peeling of the skin, or leaking of a clear fluid. In a few cases the therapy can lead to erosions, pruritus, bacterial infection, fever and scarring. Also, changes in skin color may occur, which may prove persistent. (9) (12) (27)

A study comparing the efficacy and safety of imiquimod and podophyllotoxin, conducted 2010 with 45 patients, found both substance of equal safety and efficacy. (9)

Sinecatechin/Polyphenon E 10%/15% (Veregen®)

Sinecatechin is a green tea *(Camellia sinensis)* extract, compromised of >85% catechins, and green tea polyphenols. (25) Mostly a dosage of 10% or 15 % is recommended to be applied thrice daily over 12-16 weeks or until complete clearance of the warts is achieved. It has been approved by the FDA (United States Food and Drug Administration) in 2008. (12) (24). (25)

Common side effects of the ointment include erythema, pruritus, pain, ulceration, edema, induration, and vesicular rash. The treatment is not recommended in patients with HIV infection or other immune compromised diseases. (12)

A meta-analysis by Tzellos et al. (2011) examined three placebo controlled studies regarding efficacy and safety of Polyphenon E 15% and 10% in the therapy of external anogenital warts with a total of 1247 patients (660 men, 584 women). Overall, the polyphenon treatment showed higher likelihood of clearance compared to controls. Observed recurrence rate was low, the most common side effects included erythema and itching. (30)

Destructive therapy

Destructive therapy targets to damage or remove lesions, rather than aiming to eradicate the pathogen. The options range from surgical excision, cautery, curettage, cryotherapy to laser application. The exophytic growth pattern of most HPV induced warts promotes these techniques. Especially in larger wart formations, destructive therapy is often the treatment option of choice. (15) (27)

Surgical excision

The surgical treatment of genital warts has the benefit of eliminating most warts in a single visit, although recurrence of the disease is often observed. It takes a skilled physician, additional equipment and often an elongated procedure to surgically remove anogenital warts. (12) (15) (27)

Prior to the excision, local anesthesia can be applied to the chosen area. As next step, the area will be carefully cleaned and disinfected. For the destruction of the warts, several surgical options are available: The warts can be removed by tangential excision with a pair of scissors or a scalpel. Also, electrocautery can be used to destroy warts, but this technique entails the risk of scarring. In case of larger lesions, an operation under general anesthesia may be advised. In female patients presenting extensive HPV lesions on the cervix, curettage is often the intervention of choice. (12) (15) (27)

Cryotherapy

Cryotherapy uses thermal-induced cytolysis to destroy warts by applying liquid nitrogen. The treatment must be applied by a trained health practitioner to ensure efficacy of the treatment and to prevent complications. It is common for the patient to experience pain during the application, followed by necrosis of the wart cells and sometimes blister formation. The use of local anesthesia can facilitate the treatment for both the health care practitioner and the patient. (12) (15)

Pulsed dye laser (PDL)

Unlike a standard light beam, a laser (Light Amplified Stimulated Emission Radiation), is a source of monochromatic, coherent and unidirectional light. It allows higher precision than other techniques, promotes faster healing and is associated with a lower risk of infection. It is currently used in the fields of dermatology, surgery and ophthalmology, but other new possibilities in various medical areas are also being investigated. (27) (31) (32) (33)

The application of 595 nm pulsed dye laser (PDL) is a fast and effective way of eradicating verrucae vulgares. In this study, a 595 nm PDL *Candela Vbeam Perfecta* was used with the following specifications: energy density 12 J/cm², length of impulse 1.5 milliseconds, spot size 7mm. (34) Two laser impulses per wart were applied. The first treatment took place on day one, with a second treatment (if necessary) three weeks later. (10)

The 595 nm pulsed dye laser utilizes a special technique, called selective photothermolysis, to specifically target a certain chromophore (hemoglobin). The laser impulse passes through the epidermis and dermis, where it is being absorbed by the hemoglobin in the blood vessels, but not by the surrounding tissue. The absorbed laser energy is being transformed into heat; this induces a coagulation in the blood vessels of the wart, which are unable to regenerate. The duration of the laser impulse is long enough to induce the coagulation of the vessels, but too short to cause thermal injury to the tissue, so that in the ideal case the laser energy is only absorbed by the hemoglobin and induces vessel damage, but is only marginally absorbed by different chromophores in the skin. (27) (34)

Using the pulsed dye laser system, the vessels in the warts are being destructed, which leads to a necrosis and eradication of HPV-infected cells. (10) Removal of blood supply, a cell mediated immune response and thermal damage are all believed to contribute to the healing of the lesions. (27)

The application of pulsed dye laser causes a sensation compared to being snapped by a rubber band, though some patients report even more severe intraoperative pain. The postoperative pain has been described as minimal, the healing takes 2 to 4 weeks. (27)

The process takes seconds to few minutes, depending on the number of warts. A complete remission of lesions after PDL application in 48% to 95% of cases has been observed. PDL poses a successful alternative for the treatment of HPV-induced genital warts, but is not yet a commonly applied technique. (10) (27) (35) (36)

Immunization

The first vaccine against HPV (Cervarix®) became available in 2006, and protects against the high-oncogenic subtypes of HPV, 16 and 18. Later, a quadrivalent vaccine, Gardasil®, came on the market, targeting the HPV subtypes of 6, 11, 16 and 18. (16) (24)

HPV 6 and 11 cause about 90% of cases of genital warts, HPV 16 and 18 have been specified as high risk/oncogenic subtypes and cause approximately 70% of cervical, vulvar and vaginal cancers in women. (16) (24)

A new immunization vaccine, Gardasil 9®, offers immunity against the types 6, 11, 16, 18, 31, 33, 45, 52 und 58. It is recommended for girls from the age of 9 to 26 and for boys from 9 to 15, and should prevent cancer formation caused by HPV in approximately 90% of cases, as the additional five HPV types 31, 33, 45, 52 und 58 make up for about 20 % of HPV-induced cancer formations. (13)

A recent review by Mariani et al. (2015) evaluated a total of 56 publications regarding the impact of the quadrivalent vaccine (Gardasil®) on the formation of genital warts. The author concluded that for a vaccine uptake rate of at least 70% of 3 doses, a 92.6 % reduction of genital wart formation was observed. (14)

Genital warts usually form weeks or months after infection. Cell dysplasia and cancer on the other hand, takes years to decades to evolve. For this reason, it is impossible to state if the vaccination actually protects against HPV-induced cancer, since the vaccine has been only available for only ten years so far. Adding to the controversy, the HPV vaccination is one of the most expensive ones of the market. Many countries, including Austria, target this obstacle by subsidizing the vaccine. (21)

The current HPV immunization schedule in Austria advises for children of both genders to be vaccinated between the ages of 9 to 12. For a full immunization from the age of 9 to 15, a double vaccination is recommended. For this age group, the immunization is fully subsidized by public health insurance. For the age of 12 to 15, the vaccine is only partially subsidized. After the age of 15, three vaccinations are necessary for full protection. The vaccination program supports the immunization with all three vaccines currently on the market (November 2016): Cervarix, Gardasil and Gardasil 9. It is recommended that the immunization should take place before the first sexual contact, however for sexually active teens and adults the immunization is also currently advised. (21)

It is important to note, that even when immunization is completed, a regular PAP smear screening for HPV dysplasia in women is still recommended for the early detection of cervical dysplasia which may result in cancer. (21)

There is still an ongoing discussion whether men have a greater benefit from HPV immunization, with regard to protection from HPV related cancer. The current data seems to suggest that vaccinating men would prevent the spread of HPV, minimizing the risk of women to develop HPV-related cancer, and also to protect men from HPV-induced penile and anal cancer. It is also especially advised for men who have sex with men (MSM) to get vaccinated, because this population group tends to have an increased STD infection risk. (17) (18) (19)

CHAPTER II: MATERIALS AND METHODS

Aim and Motivation

The treatment of HPV related warts with PDL or podophyllotoxin is routinely applied in the STD walk-in clinic of the dermatology department in Graz, Austria. The primary aim of this study was to prove that the two treatment options can be regarded as, at least, equally efficient, in the sense of an equivalent study. As a secondary outcome, the patient's acceptance of the different methods was studied (designated as Quality of life), and the side effects regarding pain of PDL and podophyllotoxin were compared. Clinical experience would suggest that the laser therapy has greater acceptance among patients, even though the PDL application is sometimes considered painful. (27)

Study design

The study was designed as a moncentric, prospective, randomized-controlled, observer-blinded study. It represents an equivalence trial: the number of participants required according to the sampling plan was based on a power of 0.8, with an α- level of 0.05 and a standard deviation of the difference of 10%. A difference between the two treatments of ≤ 10% was considered an equivalent result. This power calculation resulted in a minimum of 36 participants. The patients were randomly assigned to either the PDL or podophyllotoxin group, using the Research Randomizer system prior to the inclusion of the first patient. The Research Randomizer is an online available software which allows to design various studies by generating random assignments and sampling. (37)

The protocol was approved by the local Ethics Committee of the Medical University of Graz and conducted according to the principles of good clinical practice (GCP) and to the ICH-guidelines. The ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) has been established in 1990 and its mission is to prevent the harmful use of pharmaceutics. Its guidelines focus on the topics efficacy, quality, safety and multidisciplinary. (38)

Patients

In this prospective study, patients of the Outpatient STD clinic of the Department of Dermatology and Venereology of the Medical University of Graz, Austria from the years 2012 to 2014 were included. They were randomly assigned to either the PDL or podophyllotoxin group, using the Research Randomizer system. (37)

Inclusion criteria

Patients with uncomplicated HPV-induced genital warts which were amenable for the treatment with podophyllotoxin or PDL and were older than 18 years of age were included. Also, only patients with no history of former treatment for genital warts, or a treatment ≥ 6 months in the past were enclosed. Written informed consent had to be given by all patients prior to study entry.

Exclusion criteria

The exclusion criteria were orientated on the guidelines for the usage of podophyllotoxin (Condylox®): high-grade infected or bleeding warts, or pregnancy. (28) Also patients in need of extensive surgical removal of warts were also excluded from the study.

Therapy options: Podophyllotoxin versus Pulsed Dye Laser (PDL)

In the current study, a 595 nm PDL *Candela Vbeam Perfecta* was used with the following specifications: energy density 12 J/cm², length of impulse 1.5 milliseconds, spot size 7 mm. Two laser impulses per wart were applied. The first treatment took place on day one, with a second treatment (if necessary) three weeks later.

The treatment plan for the podophyllotoxin group included two self-applied topical administrations per day for three days, followed by a four day pause. The pause could be elongated if strong adverse reactions like inflammation would occur. This pattern was repeated for up to four weeks. After six weeks, the therapy progress was controlled by a physician at a follow-up appointment.

Efficacy

The efficacy of the treatment was measured by comparing the evaluation of the infection site regarding number of warts and the site of infection in mm² by the physician prior to and after the respective treatment.

This factor should shed some light on the reaction of HPV-induced warts to the PDL system, as compared to podophyllotoxin response to the given therapy in terms of results.

Side effects (Pain)

The patients were further asked to rate the experienced side effects of the treatment. The experienced pain was rated on a scale from 0-10, a rating of ‘0’ representing none to very weak pain and whereas ‘10’ representing quite severe pain.

This parameter did not only enclose the perceived pain of the application of either podophyllotoxin or PDL itself, but also the subsequent reactions of the body to the treatment, like infection, irritation or ulcera. This may pose a less serious factor regarding the PDL application, since the healing after the treatment takes only 1 to 2 weeks, but may be more of a burden for a patient during a 4 week treatment with self-applied podophyllotoxin.

Quality of life (QOL)

Moreover, the quality of life for the experienced treatment option was evaluated by the patient. The question was “On a range of 0 to 10, with 10 being most patient friendly, how would you rate the given treatment?” This question was asked at the follow up appointment or via phone in case of full remission which would have made a second visit at the clinic redundant.

Of course, every treatment option entails its own advantages and disadvantages. While the podophyllotoxin solution often takes weeks of application to show results, the PDL treatment only takes a few seconds to minutes to achieve a destruction of warts. On the other hand, the application of PDL to the skin is often conceived as painful by the patient, even if the pain level is quite moderate and can be compared with the snapping of a rubber band for most patients. (27)

Another factor, influencing quality of life is also the longer duration of side effects, as the healing after PDL application takes 1-2 weeks for any more symptoms to subside, the podophyllotoxin solution on the other hand however can create ulcera and erosion for the whole length of treatment (4 weeks) and often even for days to weeks afterwards. (9) (10)

Still, one cannot neglect that for some patients it is comfortable to perceive more control in their therapy regimen, and may prefer the self-application of podophyllotoxin over the PDL, which is applied by a trained physician. However, in terms of compliance the application of PDL is far easier to monitor than with podophyllotoxin, as in self-applicable medication the physician can never be entirely sure how cautiously the patient follows his or her treatment regimen.

Supplementary factors

Along the main parameters (efficacy, side effects, QOL), also other parameters were investigated.

Routinely, the patients were asked about their age and gender. Moreover, the physician in charge noted down the site of infection, which was for male patients: glandiopraeputial, at the shaft/scrotum/groin area or perianal. For women, the options were: introital/interlabial, labia majora/mons pubis/groin area or perianal. In some patients, the lesions were also documented by photography. Also, the skin type of every patient was noted down. Further the patient was asked since when he or she had first noticed the symptoms, i.e. the formation of warts in the genital area.

Additionally, the patients were asked about their sexual history. Options were composed of homosexual/heterosexual lifestyle, how many partners they have had in the past, if they lived in a monogamous relationship at the moment, and if so, how long the current relationship has already lasted. Furthermore, in patients living in sexually exclusive partnerships it was questioned whether the partner also presented HPV related genital warts at the moment. Since a lot of data about the relationships of patients were missing, and also quite varied, the data regarding partnership was not analyzed in greater detail in this thesis.

Another question at the follow up appointment was targeting the quality of side effects. Patients undergoing the podophyllotoxin treatment were asked if they experienced redness of the skin and erosions/ulcerations. The laser study group was asked if any bleeding or erosions/ulcerations had occurred on site.

CHAPTER III: RESULTS

Patient Data

In this study, originally 48 patients who presented with an uncomplicated case of HPV-induced genital warts and gave their formal written consent to participate in this study were examined. They compromised of 46 men and 2 women. Later the data analysis was restricted to 36 patients (accidentally exactly according to the needed number of participants), further details on exclusion/inclusion criteria are listed in the chapter 'Materials and Methods'.

Of the initial study group containing 48 patients, seven male patients were excluded because important data regarding the treatment and outcome was missing and could not be retrieved. One patient presented with a history of genital warts < 6 months in the past, which was another exclusion factor, as this was rated as a case of recurrence rather than a fresh infection. Also it was decided to exclude the two female patients in order to produce a more homogenous data set of only men. Most women with a STD tend to visit a gynecologist or the gynecology department for diagnosis and treatment, not a venereal outpatient clinic; for that reason mostly men compromised the original study group.

Of the 36 male patients, seven patients had not returned for a follow-up appointment after therapy because the initial therapy had been successful; those patients have been contacted by phone to ask about their outcome and their ratings of given therapy.

Six patients of the selected 36 males did not want to share information regarding their sex life, but since this data was only a subsidiary factor in our study, those individuals were still enclosed.

The 36 patients were randomly chosen for either Podophyllotoxin or PDL treatment, which resulted in the end that 19 subjects formed the podophyllotoxin group, the PDL group was composed of 17 patients.

Patient Age

The 36 patients of the study are all male and range from age 19 to 68. On average, the patients were 36 years old, with a median of 30.5 years.

The gathered data accorded with the expectations that the patients would mostly be comprised of sexually active adults.

In other studies concerning the therapy of HPV, the patient's group average age was similar with 30.3 years (9) or 37.2 years (10).

Patients (n)	36
Range (years)	19-68
Mean average (years)	36
Median (years)	30.5

Table 1- Patient Data Analysis

Sexual habits

To evaluate how the sexual lifestyle would influence the HPV prevalence, also data on the sexual habits of the patients with anogenital warts was collected. Of the 36 patients, six patients did not provide any information regarding their sexual habits.

25 of 30 patients (83.3%) reported to live a heterosexual lifestyle, the remaining five patients (16.6%) reported of a homosexual lifestyle i.e. to have sex with men (MSM-men having sex with men). MSM patient groups usually tend to correlate with a higher prevalence of STDs and should be checked thoroughly for co-infections like HIV, Syphilis, Gonorrhea or Herpes. (39)

Sexual orientation	Heterosexual	Homosexual
30 patients	25	5

Table 2 - Sexual data analysis- Sexual orientation

The number of previous sexual partners ranged from 2-200, the median was found to be 15. The mean number of sexual partners was calculated as 29.8. The high average of sexual partners, diverting from the low median can be explained as followed: the number of sexual partners in most patients ranged mostly from 2-30, only three patients (10%) reported to have had up to 100- 200 previous sex partners.

Average sexual partners	29.8
Median sexual partners	15
Range of sexual partners	2-200

Table 3 - Sexual habits data analysis, depiction and analysis of the number of sexual partners

According to “A global survey of sexual behavior” by Wylie (2009) including 542 participants in Austria, Austrian males reported to have had 17 sexual partners at that point of their life on average. (40) Compared with the median of 15 sexual partners in this research, a more promiscuous lifestyle in HPV-infected patients can hereby not be concluded.

As predicted, the risk group MSM for STDs showed a higher HPV-prevalence in this study as compared to the general population. Current studies suggest that 1.8% to 11% of the population report to have experienced at least same-sex attraction and/or had engaged in same-sex sexual behavior at least once in their life, and about 1.2% to 3.7% report a LGB (lesbian-gay-bisexual) identity (compared to the 16.6 % MSM in our research). (41) Though the data set is too small to make any real statement, it can be interpreted as confirming of an often observed and described trend. (21) (19) (39)

Skin Type

In this study, the Fitzpatrick skin type scale was utilized to measure the skin type of the study patients. This scale is one of the most commonly used tools for describing skin types. (42)

36 patients were categorized accordingly by a trained physician with following results: 1-I, 23-II, 12-III, 2-IV, 0-V and 0-VI (number of patients- skin type). As expected, most of the patients of the outpatient venereology department in Graz, Austria had a light Caucasian skin type.

Regarding the laser or podophyllotoxin treatment, no correlations between skin type and side effects were found. As found in previous studies, the colour of skin does not seem to influence the efficacy, safety or life quality of either therapy in any way. (10)

Number of patients	36
Average skin type	2.39
Standard deviation	0.73
Median	2

Table 4 - Data of Skin Type analysis

Efficacy

At the first visit, the physician in charge rated the HPV induced genital warts by the number of visible warts and estimated the size of the infected area (mm^2) and recorded his observations in the given study data sheet. After their given consent to participate in the study, the patients were randomly designed to the PDL or Podophyllotoxin group and the treatment was explained in great detail. Afterwards the patients either received a PDL treatment or a recipe for the podophyllotoxin solution.

At the follow-up appointment, about six weeks later, the number of warts and size of infected area was reevaluated, to find the efficacy of the treatment. Some patients did not return for a follow-up treatment and were called by phone to be questioned about the success of their therapy. Those patients, both of the PDL and podophyllotoxin group reported that the first therapy had successfully erased all lesions and all signs of genital infections. The remaining number of warts and the size of infected area were interpreted as zero warts and zero mm^2.

The difference of number of warts and reduction of infected area before and after the treatment was calculated for both treatment options, and statistically analyzed.

Reduction of number of warts

The results show that under treatment with podophyllotoxin (Condylox®), a reduction of the number of warts of 7.16 as average could be achieved, with a standard deviation of 8.859.

In the laser group, a reduction of 5.18 warts was achieved, with a standard deviation of 4.019.

Total reduction was 6, 22 warts for both treatments and a standard deviation of 6.982.

Condylox (C) /Laser (L)		Mean Value	Standard Deviation	N
Reduced number of warts	C	-7.16	8.859	19
	L	-5.18	4.019	17
	Total	-6.22	6.982	36

Table 5- Descriptive Analysis: Efficacy: reduced number of warts

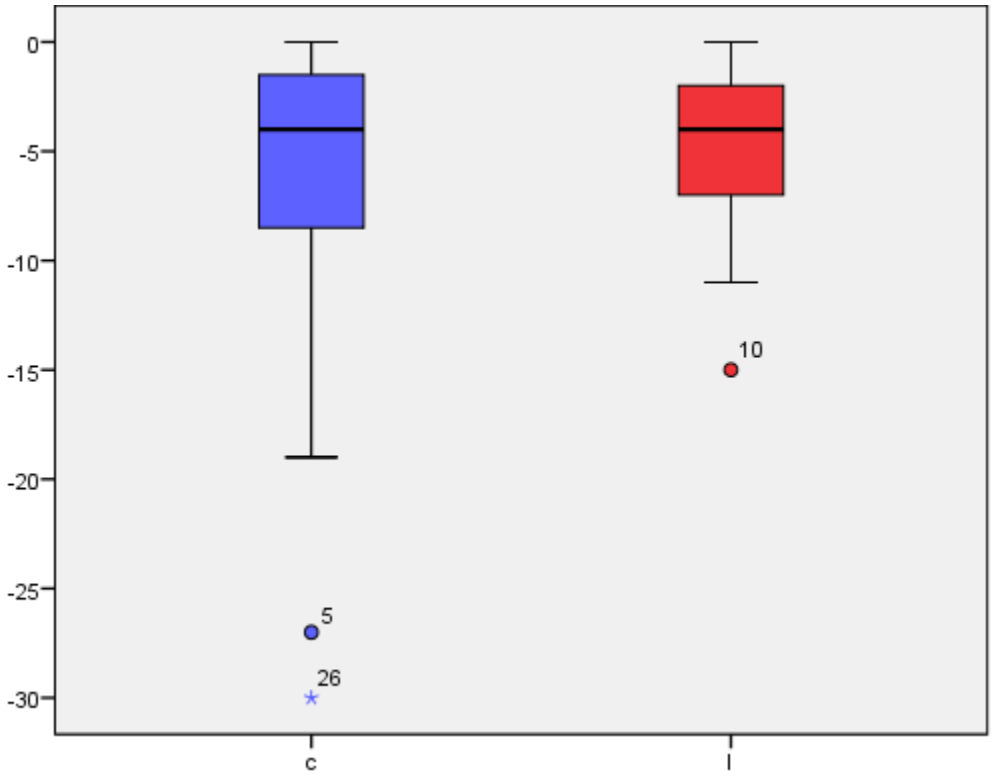

Figure 1- Reduction of the number of warts, blue (c): Condylox treatment, (l): laser treatment; Range of reduced number of warts: 0-30 warts

Following table shows the results from the statistical analysis, which were calculated using a test of between-subjects effects whether a significant statistical difference between the two treatment options could be found. A value of significance <0.05 would be interpreted as significant difference, a value near 1 on the other hand would suggest that both options can be seen as equal.

For the parameter efficacy, regarding to the number of destroyed warts, a significance level of 0.403 was found. No significant difference was found between PDL and podophyllotoxin therapy in this regard.

		Type III Sum of Squares	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Number _diff	35.225a	35.225	0.717	**0.403**	0.020

Table 6- Lack of statistical significance regarding efficacy in terms of warts reduction

Reduction of infected area

Efficiency was not only measured in the numbers of reduced warts, but also in terms of reduction of the infected area. The size of the infected area was evaluated by a trained physician.

The average reduced area under podophyllotoxin (Condylox®) therapy was 27.26 mm², with a standard deviation of 32.237.

In the PDL treatment, a reduction of infected area of 29.06 mm² was accomplished, with a standard deviation of 25.418.

A total reduction of area of both treatments was calculated as 28.11 mm² in average, with a standard deviation of 28.820.

Condylox (C) /Laser (L)		Mean Value	Standard Deviation	N
Reduced Area [mm²]	C	-27.26	32.237	19
	L	-29.06	25.418	17
	Total	-28.11	28.820	36

Table 7- Descriptive Analysis: Efficacy: Reduced infected area (mm²)

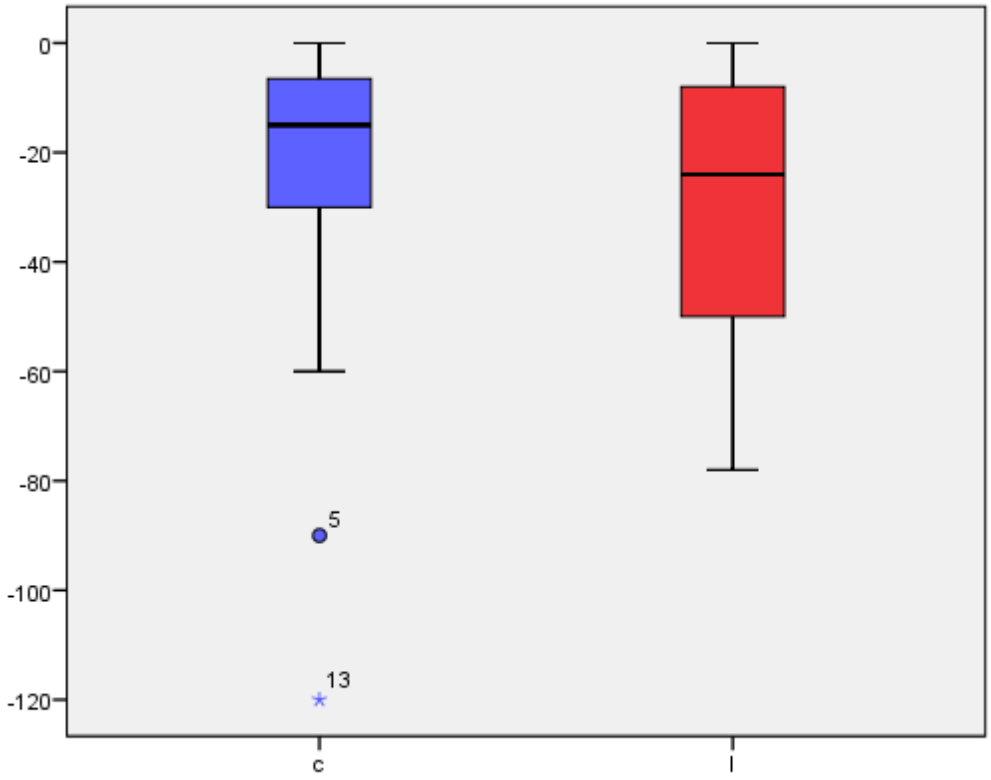

Figure 2- Reduction of the HPV-infected area, (c): Condylox treatment versus (l): laser treatment. Scale is rated in mm², range is 0-120 mm²

Efficacy, measured in mm² of reduced infected area, was calculated in the same manner as the number of warts. A test of between-subjects effect revealed a significance level of 8.55. In regards to decreased area, the two treatment options proved as almost identical in efficacy.

		Type III Sum of Squares	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Area_diff	28.930b	28.930	0.034	**0.855**	0.001

Table 8- Lack of statistical significance regarding efficacy of infected area reduction

Side effects (Pain)

Another examined factor was the side effects of treatment, be it with PDL or the podophyllotoxin (Condylox®) solution. After the therapy, on their second visit at the outpatient clinic, or, in case of remission after the first visit, via phone call, the patients were asked to rate the experienced side effects, in regards to pain, from a scale of 0 to 10, with 0 experiencing mild pain to 10 experiencing severe pain under treatment.

The podophyllotoxin (Condylox®) treatment was rated 3.11 of 10 points as average value, with a standard deviation of 2.787.

The PDL treatment was scored 4.47 of 10 points as average value, with a standard deviation of 2.294.

In total, for both treatments, the pain was rated as 3.75 of 10 as average with a standard deviation of 2.623.

Condylox (C) /Laser (L)		Mean Value	Standard Deviation	N
Side effects (Pain) [1-10]	C	3.11	2.787	19
	L	4.47	2.294	17
	Total	3.75	2.623	36

Table 9- Descriptive analysis, comparing side effects (Pain) [0-10]

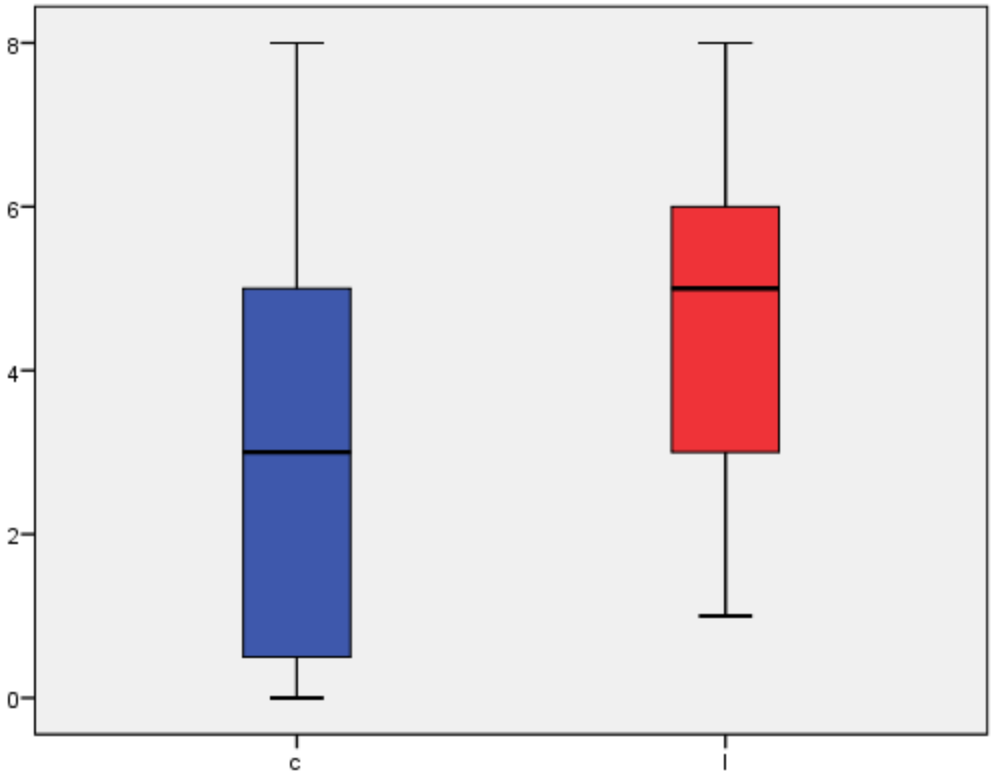

Figure 3 - Experienced pain of the treatment, (c): podophyllotoxin (Condylox®) (l): laser treatment. Scale ranging from 0-10

Regarding the quantitative measuring of side effects (pain), a statistical analysis effect was conducted. It revealed a significance level of 0.120. A value of <0.05 would have been the significance threshold. No significant difference in the aspect of rated pain experience was found between treatments.

		Type III Sum of Squares	Mean Square	F	Sig.	Partial Eta Squared
Corrected model	Side effects-Pain [1-10]	16.725c	16.725	2.538	**0.120**	0.069

Table 10- Lack of statistical significance regarding side effects- (Pain)

Side effects- Qualitative differences

Apart from rating the side effects from 1-10, also the qualitative aspects of the side effects were measured. For the PDL treatment, the options “none”, “erosion/ulcera”, “hemorrhage” were rated by the physician. For the podophyllotoxin group, the options “none”, “redness” and “erosion/ulcera” were available.

However, not all patients gave exact information regarding the appearance of side effects, hence the data remains incomplete. Especially in patients that did not show up to the follow-up appointment caused by the success of the initial treatment, and had been called up to three years after their treatment. This may also mean that those patients who benefitted of a successful therapy may be underrepresented in the data.

In the podophyllotoxin group, information regarding side effects was available in 20 patients of the initial data set of 48 patients. Of those 20 patients, 5 patients did not report any side effects like redness or erosion/ulcera. 2 patients reported only of erosion/ulcera without redness. 6 patients reported only of redness on the site of infection during the treatment. 9 patients reported to have experienced erosion/ulcera and redness under podophyllotoxin therapy.

Podophyllotoxin patients	20	%
No side effects	5	25%
Only redness	6	30%
Only erosion/ulcera	2	10%
Redness + erosion/ulcera	9	45%

Table 11- Qualitative data on side effects for podophyllotoxin (Cnondylox) group

The PDL group with available data consisted of 18 of the original 48 patients. 6 reported to had experience no side effects. 5 reported of both haemorrhage and erosion/ulcera.1 patient reported of only haemorrhage. The remaining 6 patients reported of only erosion/ulcera.

PDL patients	18	%
No side effects	6	33.33%
Only haemorrhage	1	5.56%
Only erosion/ulcera	6	33.33%
Haemorrhage + erosion/ulcera	5	27.78%

Table 12- Qualitative data on side effects for PDL group

The data shows that slightly more patients experienced no side effects like haemorrhage, erosion or ulcera in the PDL group (33.33% versus 25%). However, the Podophyllotoxin group experienced more often only redness as side effect (10% versus 5.56%). Regarding the full spectrum of side effects, haemorrhage and erosion/ulcera for PDL or redness and erosion/ulcera in the Podophyllotoxin group, the PDL treatment seems to be more lenient on the skin (27.78% versus 45%).

	podophyllotoxin patients (20)	PDL patients (18)
No side effects	25% (n=5)	33.33% (n=6)
Only haemorrhage or redness	30% (n=6)	5.56% (n=1)
Only erosion/ulcera	10% (n=2)	33.33% (n=6)
Haemorrhage or redness + erosion/ulcera	45% (n=9)	27.78% (n=5)

Table 13- Comparison of objective side effects of podophyllotoxin and PDL group

Quality of Life (QOL)

At the second clinical visit, or via phone call, the patients were asked how comfortable the treatment was perceived, with '10' being most comfortable and '0' not at all comfortable. Results are listed below.

The podophyllotoxin (Condylox®) treatment scored a value of 6.74 of 10 achievable points, with a standard deviation of 3.478.

The PDL therapy was rated with an average of 8.47 of 10 points, standard deviation was calculated with 1.940.

This results in a total count of 7.56 of 10 points, with a standard deviation of 2.951.

Condylox (C) /Laser (L)		Mean Value	Standard Deviation	N
Quality of Life [1-10]	C	6.74	3.478	19
	L	8.47	1.940	17
	Total	7.56	2.951	36

Table 14- Descriptive analysis, comparing quality of life [1-10] in the treatment with 'C'- podophyllotoxin (Condylox®) versus 'L'- Pulsed dye Laser (PDL)

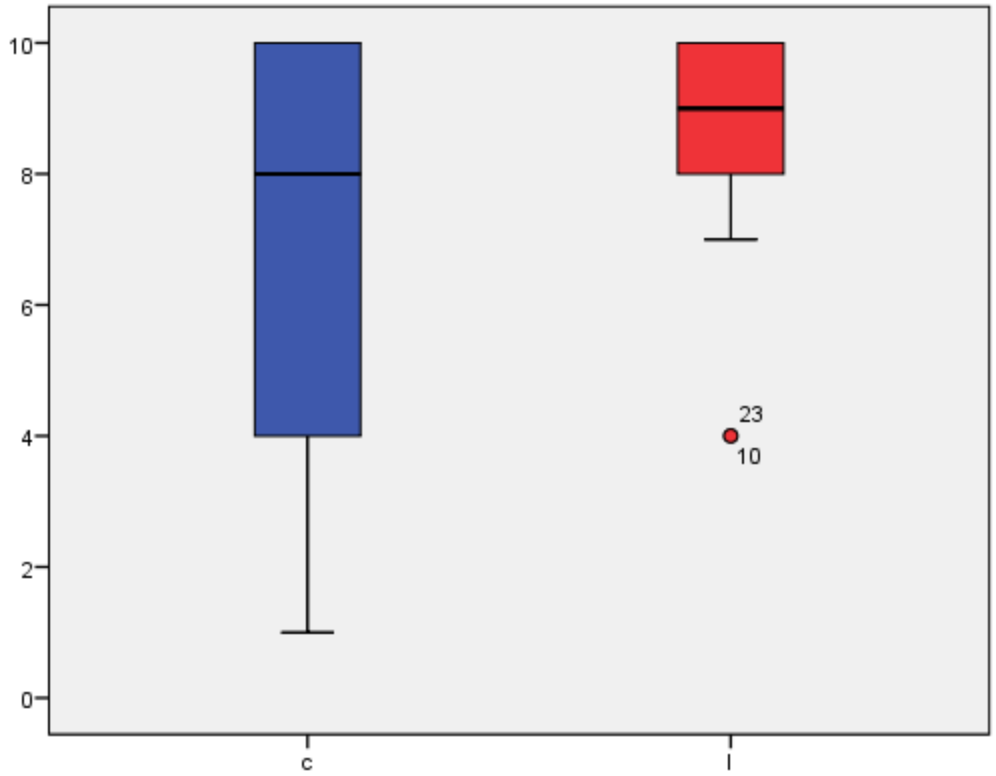

Figure 4 - Quality of life, as rated by the patients [1-10] 'C '- podophyllotoxin (Condylox®) versus 'L'- Pulsed dye Laser (PDL)

In the parameter -Quality of Life- a significance in difference of 0.078 was found, favoring the PDL treatment. As predicted, patients rated the laser system as more comfortable, however, this difference was still >0.05 and therefore not statistically significant.

		Type III Sum of Squares	Mean Square	F	Sig.	Partial Eta Squared
corrected model	Quality of Life [1-10]	26.969[d]	26.969	3.299	**0.078**	0.088

Table 15- Lack of statistical significance regarding Quality of Life (how "comfortable" is the treatment?)

Site of infection

Another additional parameter in this research was the site of the genital warts. For the study group, compromising of only men, following options were available: glandiopraeputial, at the shaft/scrotum/groin area or perianal. Multiple appointments were possible.

Of the 36 males, 11 reported their warts to be on the glans and foreskin. 26 reported their warts to be on the shaft/scrotal/groin area. Only five patients presented warts in the anogenital area.

Four patients with warts in the anal area stated to live a homosexual lifestyle, only one of them living a heterosexual lifestyle. One homosexual patient did represent warts only in the shaft/scrotal/groin area.

Location	glans and foreskin	shaft/ scrotal/ groin area	Anogenital area.
N=36	11	26	5

Table 16- Location of HPV warts, with multiple appointments possible

CHAPTER IV: CONCLUSION

This is the first report of a direct comparison of podophyllotoxin (Condylox®) solution and pulsed dye laser in the treatment of HPV-induced anogenital warts. Dye Laser application is not commonly used in HPV wart treatment and little data is available on the PDL system regarding the therapy of HPV-induced warts. However, laser technology is becoming a more and more common instrument in treating various diseases, especially in the fields of dermatology, surgery or ophthalmology, but also experimental treatments like laser-acupuncture are developing. Overall, application of laser is vindicating itself as an excellent tool in many new therapeutic strategies. (31) (33)

This research was designed as a non-inferiority study comparing PDL and podophyllotoxin, to test if those two therapy options for HPV induced anogenital warts can be regarded as equal, not only in terms of efficacy, but also regarding side effects and quality of life. Also, additional factors were investigated: The patient's data (gender, age, skin type, sexual habits and site of infection) was recorded and analyzed, to see if any correlations became apparent.

The PDL in the current study utilizes a 585 nm laser beam which interacts with the hemoglobin in the blood vessels that supply the warts, in a process called photothermolysis. The energy of the laser causes a coagulation of those vessels, which results in a necrosis of the warts and a diminishing of the infected area. The healing takes about 1-2 weeks, scar formation is usually not observed in the process. (10) (27)

The established first-line treatment utilizes podophyllotoxin (Condylox®). The extract of the Podophyllum sp. root, podophyllin resin, was first described as topical remedy against genital warts in 1942. Its active component is podophyllotoxin, which inhibits the proliferation of human skin keratinocytes and stops the proliferation of HPV infected cells. In placebo-controlled trials the podophyllotoxin displayed cure rates from 37 % to 83%. (9) (11) (27)

In the current study, a direct comparison in a randomized test was conducted to find if the PDL treatment and podophyllotoxin could be regarded as equal concerning efficacy, quality of life and side effects. 36 patients in total were thoroughly examined on the day of the first appointment at the outpatient venereology department in Graz, and a few weeks later at a follow-up appointment. Some patients experienced remission after the first session, and had been called by phone to assess the patients experience and level of contentment with the therapy.

The non-inferiority test showed that equality had been reached in all aims. There was no statistical significance regarding efficacy, side effects or quality of life. However, a trend favoring PDL was observed in the aspect quality of life (to which degree the treatment was perceived as “comfortable”).

The application of the laser involves a short and precise application of light energy to the warts, causing a coagulation of the blood vessels and inducing necrosis in the wart. The laser application is often perceived as painful for the patient and needs to be repeated for every wart (2 pulses per wart). The overall procedure however takes less than 5 minutes. The application of podophyllotoxin on the other hand takes weeks of self-applying the topical agent to the infected area. It was probably for that reason that podophyllotoxin therapy scored slightly lower on the side-effect/ pain scale, but the laser scored quite a bit better on the quality of life scale. However, both differences in score lacked statistical significance. A broader study including more patients or a more detailed questionnaire might shed more light on these factors.

An inevitable shortcoming of this study, which may however blur the results is the lack of blinding. Since the podophyllotoxin treatment includes an application of a topical agent over weeks, and the laser treatment constitutes only of a short application of high energy to the warts, a blinding of the study is neither possible nor purposeful, but also the comparison of an high-tech PDL application to the traditional ointment remedy podophyllotoxin may alter the expectations and attitude of the patients and physicians, resulting in an unmeasurable impact on the results.

Another factor is compliance: The laser therapy is not influenced in terms of compliance, since the laser is applied by a physician. However, the podophyllotoxin solution is administered by the patients themselves over a period of several weeks. Hence it is impossible to tell how accurately the application of podophyllotoxin was handled by the patients, a fact that may or may not have influenced the results, especially regarding efficacy in wart reduction and perceived side effects.

Further research could investigate the recurrence rate of HPV-related genital warts after podophyllotoxin versus PDL treatment. As recurrence of lesions appear in approximately 50 % of cases, this factor should not be neglected. (15) (16) Be that as it may, the current study focussed on the primary outcome of both therapy options, and the recurrence rate was not examined.

Another shortcoming of this research is the total absence of women in the study group. Since most women rather consult a gynaecologist in case of venereal disease such as HPV induced warts, only a few were eligible as subjects for the study. In order to produce a more homogenous set of patients, the two women who were part of the original data set, were excluded from the study. Cooperation with other clinics might be useful to create data concerning laser treatment with HPV- induced anogenital warts in both sexes.

Some interesting studies in the last decade(s) investigated the use of laser therapy in various fields of dermatology. But since almost every study utilized a different laser system, the papers are hardly comparable, even though they may allow some insights on the pro and cons of different laser systems targeting numerous skin problems.

One study by Komericki et al. (2006) investigated the efficacy of 585 nm flashlamp pulsed dye laser, and achieved complete remission in 22/22 patients (20 male, 2 female) after an average of 1.59 treatment sessions. The laser was used with the following conditions: spot diameter 7 mm, pulse duration 450 milliseconds, fluence 6-7 J/ cm^2. (10)

Another study, targeting laser efficacy on HPV induced warts of hands and feet and other sites by Kopera et al. (2002) achieved 63% total remission and 22% partial remission in 93 patients. 15% however did not respond at all to this kind of treatment. The laser system used was a 585 nm flashlamp pumped pulsed dye laser with the following laser parameters: energy density 8 J/cm2, spot size 7 mm, pulse duration 450 milliseconds. There were no relapses in a 2 to 6 month follow-up period. (43)

A study by Kauvar et al (1995) investigated the effect of flashlamp-pumped pulsed dye laser on uncomplicated and recalcitrant warts in an observational study. 142 patients with 703 recalcitrant and 25 previously untreated warts were treated from the years 1993 to 1995. Response rates were 99% for body, limb and anogenital warts, 95% for hand warts; 84% for plantar warts; and 83% for periungual warts. A complete remission was observed after a follow up three to nine months later. Higher energy fluences were used in this setup (7.25-9.5 J/cm^2). (36)

A prospective observational study by Badawi et al. (2006) examined the safety and efficacy in the treatment of anogenital warts with flashlamp-pumped pulsed dye laser. A total of 174 adult males with 550 uncomplicated anogenital warts were included. The pulsed dye laser by Cynosure® worked with following specifications: wavelength 585 nm, 450 milliseconds pulse duration, spot size 5-7 mm; fluence 9-10 J/cm2. A complete resolution was achieved in 96% of lesions, side effects were reported as limited, transient and infrequent, the recurrence rate was given with 5%. (35)

A recent study by Nistico et al (2016) utilized flash-lamp pulsed-dye laser in the therapy of various dermatological problems: angiokeratoma circumscriptum, genital and extra-genital virus-induced warts, striae rubrae, basal cell carcinoma, Kaposi's sarcoma, angiolymphoid hyperplasia, and Jessner-Kanof disease. A total of 149 patients (73 males and 76 females) received a laser treatment, with varying results, 59.7% achieved excellent clearance, 21.4% achieved good to moderate clearance, 12.7% only obtained slight clearance, and 9 subjects (6.1%) had non-responding lesions. (44)

All these studies suggest that PDL is a widely useful tool in tackling various skin diseases. Still, those studies all lacked a control group. Furthermore, if greater numbers of patients were examined with follow-ups longer than a year, deeper insights in efficacy regarding remission might be expected.

A lot of data regarding the treatment of HPV related anogenital warts is still missing. Taking into account that HPV induced anogenital warts are one of the most common STDs, a search for an efficient treatment should be a priority. (8) (10)

In conclusion, the application of pulsed dye laser proved itself as equal with the established topical podophyllotoxin therapy. PDL quantitatively reduced the size of the infected area and the number of warts same as podophyllotoxin, PDL did not cause significantly more or less side effects (pain), and trended to be perceived as more comfortable than podophyllotoxin by the patients.

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