Non-invasive treatment of epidermal keratinocyte neoplasms

Chapter 5

General discussion and Valorization

"It is more important to know what sort of person has a disease than to know what sort of disease a person has"
- Hippocrates

This thesis describes the outcomes of several studies conducted on non-invasive interventions for actinic keratosis (AK) and superficial basal cell carcinoma (sBCC). For AK, it focuses on the comparison of the most frequently used field directed treatments. For superficial BCC, it focuses on one specific non-invasive therapy – photodynamic therapy (PDT) – and compares the efficacy of different PDT modalities.

In this chapter, I will outline the major conclusions of this research, discuss and interpret the results and reflect on the relevance for scientists, clinicians and patients.

Relevance of the research

Over the past decades, there has been a rise in the incidence of keratinocyte skin cancer and its precursor lesions such as Bowen's disease and actinic keratosis. The life-time risk of developing BCC in the Dutch population is estimated 1:5-6.[1] A recent Dutch study showed that the European age-standardized incidence rate (ESR) for basal cell carcinoma (BCC) in the Netherlands quadrupled between 1973 and 2009.[2] It is important to realize that there is a lack of BCC registries in most countries.[3,4] Because of this, the incidence can only be estimated. For premalignant conditions such as AK, the number of studies investigating its incidence is even fewer. Similar to BCC, there is no registry system for AK either and there is a need for decent cohort studies. Flohil et al. were the first to describe the prevalence of AK in the Netherlands in a population-based cohort study. In their study population, 37.5% of the participants had at least one AK.[5] Based on extrapolation of these data, they suggested that a total of 1.4 million Dutch citizens aged 50 years or older, have AK.[5] It is estimated that 12% of the consultations in Dutch dermatology practice are because of AK.[6,7] Moreover, people continue to attain higher ages; estimates indicate that in 2040 there will be 4.6 million people aged 65 years and older in the Netherlands.[8] On the other hand, there is a trend towards the development of innovative treatments, new technologies and more empowered healthcare consumers. This has resulted in awareness of increasing healthcare costs, while maintaining a high-quality standard of care. In this context, we need to remain critical regarding our treatment choices.

Treatment of actinic keratosis

Whenever we discuss treatment options for AK, the question raises: is it necessary to treat? There is still some controversy about the exact risk of developing an invasive SCC from a pre-existing AK. Some state that AK is an in situ variant of a squamous cell carcinoma (SCC) that needs to be treated to prevent further development into an invasive

SCC.[9] Rates in the literature vary from 0.025% up to 20% of AK progressing into SCC per year.[10-14] Several studies on SCC estimate that 40-80% develop from pre-existing AK.[10,12,15] Holmes et al. summarized this as follows: we do not know how often AK progresses into SCC, but the majority of SCC develops from a pre-existing AK.[16] On the other hand, there is the opinion that treatment is not required to prevent development into SCC and that the available evidence arguing this malignant potential is of insufficient strength.[17] Furthermore, Werner et al. concluded that estimates of the frequency of AKs developing into SCC are not reliable.[18] When individual lesions were assessed, it was found that treatment is not essential to prevent malignant progression.[19] A Cochrane review could not observe a reduction in SCC after treatment of AK.[20] The presence of AK was merely presented as an indicator for sun damage and therefore relevant as a predictor for the risk of developing further AK and KC.[20] Other than possible malignant transformation, the question to treat or not to treat also depends on patient complaints related to their AK. AK can lead to itching, mechanic irritation, cosmetic impairment or pain, all of which can be relieved by treatment.

When it is desirable to treat AK, there is a great number of treatment options. Relatively invasive treatments are available, such as laser resurfacing, cryotherapy and dermabrasia. In addition, less invasive treatments such as 5-fluorouracil (5-FU) cream, imiquimod cream, photodynamic therapy (PDT), ingenol mebutate (IM) gel or diclofenac sodium 3% gel can be used. In the Netherlands, the most frequently used lesion-directed therapy is cryotherapy. For field-directed therapies, 5% 5-FU cream, 5% imiquimod cream, PDT and IM gel are registered.

Current AK guidelines do not provide clear advice on the treatment of primary choice. The 2010 Dutch guidelines advised treating all patients with AK, primarily because of the uncertain malignant potential.[21] The 2017 updated version states there is no direct oncological need to treat AK, but factors such as complaints or cosmetic outcomes can be reasons to treat.

One AK treatment for all?
Different patient categories might have different needs and require distinctive therapeutic approaches. The younger (working) population, for example, might prefer treatments with a shorter down-time such as PDT or IM gel. The elderly population, on the other hand, might have trouble with a self-applied treatment. As AK tends to have a high recurrence rate and repetitive treatments are often needed, compliance is an important factor in treatment choice.

Because of its in-clinic setting, PDT is an example of a treatment that might be preferred by patients who are not able to complete a self-applied treatment or prefer a one-day

treatment. Unfortunately, pain is an important limiting factor during PDT treatment, especially in AK patients.[22] In ***chapter 2.2***, we compared conventional methyl-aminolevulinate (MAL)-PDT with laser mediated MAL-PDT using a pulsed dye laser (PDL). Previous literature indicated that PDL illumination leads to lower pain scores.[23,24] We aimed to assess whether the efficacy is comparable to conventional MAL-PDT and whether pain scores are lower after PDL illumination. In a split-face study, we found no statistically significant differences between the two illumination sources when assessing the mean change in the number of lesions from baseline, one year post-treatment. However, pain scores were significantly lower after laser-mediated PDT. Furthermore, patients indicated a preference for PDL illumination (78.8%) above conventional PDT (32.8%). Unfortunately, in our study, newly developed lesions were not differentiated from pre-existing lesions when counting the AK numbers post-treatment, a frequently observed problem in AK studies. Because of this, it was not possible to conclude on the exact effect of these two treatments on the lesions at baseline. An important drawback of the treatment itself is the limited availability of laser devices in clinical practice and the costs. Besides the high expenses for the laser device, the treatment is still a complete in-clinic treatment which involves additional costs, e.g. for healthcare workers.

Another solution for the pain during PDT is daylight PDT, a treatment presented for grade I-II AK a few years ago.[25-28] Exposure to daylight after application of a photosensitizer leads to a continuously lower amount of protoporphyrin IX (PpIX) formation, instead of a rapid peak with conventional illumination devices. In daylight PDT, there should be continuous exposure to daylight for 2 consecutive hours instead of a couple of minutes in conventional PDT. The use of daylight leads to lower pain scores, fewer side effects and comparable cure rates to those after conventional PDT.[27,28,29] However, the use of daylight comes with weather and season uncertainties. Perhaps due to these practical concerns, currently there is only a small number of hospitals that offer daylight PDT in the Netherlands.

What field-directed treatment should be preferred?

The majority of patients will choose the most effective treatment. Ideally this coincides with a short treatment duration, a minimum of side effects, a short down-time and of course low costs. With the background of increasing costs in medical care, however, this is challenging. In ***chapter 2.1*** we described a literature review in which we concluded that most AK studies are very heterogeneous in terms of the study population and outcome measures. It is desirable to support treatment choices with more evidence, preferably with head-to-head trials comparing the most frequently used therapies for AK. The gap in the available literature led to the initiation of a large multi-center randomized controlled effectiveness trial at our center, described in ***chapter 2.3*** We included 624 patients with the aim of determining the

most effective field-directed treatment for AK. Data after three months of follow-up indicated that 5-FU appears to be the most effective field-directed treatment. The proportion of treatment success at three months of follow-up was significantly higher for 5% 5-FU cream (90.6%), compared to 5% imiquimod cream (76.4%), MAL-PDT (76.0%) and 0.015% IM gel (67.3%). In the 5-FU group, 4 patients who had initial treatment failure refused re-treatment. For imiquimod, PDT and IM this occurred for 12, 13 and 15 patients. When assessing the side effects, our data show that patients treated with MAL-PDT reported significantly more pain and burning sensations compared to patients treated with the other topical ointments. Patients treated with 5% 5-FU, on the other hand, experienced erosions more frequently in the two-week post-treatment period, compared to the other treatments.

What are the implications of these results for physicians?
The primary outcome of this trial was the proportion of patients with ≥75% lesion clearance at 12 months follow-up. Hence, it is too early to draw definite conclusions; the lesion reduction 12-months post-treatment will provide insight into whether the superior efficacy of 5-FU is sustained.

In case of insufficient initial response, patients received a maximum of one re-treatment. It is remarkable that to achieve a high effectiveness, only 14.8% of the patients treated with 5-fluorouracil needed a second treatment cycle, compared to 35.9%, 43.6% and 47.8% after imiquimod, MAL-PDT and IM, respectively. Furthermore, a substantial larger number of patients refused re-treatment after imiquimod, PDT and IM, compared to 5-FU, which might indicate that 5-FU is better tolerated. Factors as the need for an extra treatment and the way a treatment is tolerated are important considerations to address when a treatment strategy is discussed with a patient.

Based on the current available three-month follow-up data, 5-FU should be considered the field treatment of first choice. The study we performed is the first large multi-center randomized controlled study comparing the four most common treatments head to head. This trial is also one of the few AK trials that included Olsen grade III AK and a large treatment area (up to 100 cm^2). With this, we tried to simulate daily clinical practice in a trial setting, as in daily practice AK patients usually do not present with only grade I-II AK in a limited area. The outcomes of our study are the first data to provide insight into the most effective treatment for field AK, including severe AK lesions.

Along with conclusions about the effectiveness of therapies at 12 months post-treatment, this study will also provide insight into the number of AK lesions that progress into SCC. We performed a specific lesion follow-up that allows us to register all SCC that will develop in the study area during follow-up. Because of the mapping performed in the study, we

will be able to tell if the SCC developed from a pre-existing AK. It will be of future interest to analyze the number of SCCs that develop in the study area five years post-treatment and to evaluate whether there are interesting correlations, such as the previous grade of AK at that specific location or the administered treatment.

In my opinion, until there is no consensus regarding the risk of development into SCC, patients should be informed about the uncertain malignant potential and the decision to treat or not should be based on shared decision making between the physician and the patient. AK can be left untreated, when the patient is adequately informed to return to their dermatologist in case of complaints such as pain, growth of a lesion or bleeding. When deciding to treat field change, I would primarily advise 5% 5-FU cream, based on its efficacy and tolerability at three months of follow-up in our randomized controlled trial.

Treatment of basal cell carcinoma

Can tea cure?

Besides established treatments for sBCC, it is important to gain knowledge about possible new topical treatments to strive for an optimization in efficacy. The future of cancer medicine is increasingly focusing on molecular medicine and targeted therapies by blocking tumor growth more specifically by targeting particular molecules or pathways. The exact molecular mechanism of BCC development has not been fully elucidated. It is known that the vast majority of sporadic BCC contain mutations in the patched 1 (PTCH 1) gene, which is an inhibitor of the Hedgehog (HH) pathway.[30] It remains debatable whether there is a role for the Wingless (Wnt) pathway in neoplasia in a manner that is HH-pathway driven or whether there might be cross-talk between both pathways.[31-33] Wnt plays a role in hair bud formation; HH subsequently promotes the maturation of hair follicles. Deregulation of the Wnt pathway leads to the accumulation of nuclear b-catenin, which consequently leads to tumor cell proliferation. There are some data suggesting that epigallocatechin-3-gallate (EGCG) – an active constituent of green tea – might lead to the inactivation of b-catenin signalling through the Wnt pathway.[34] Other data have demonstrated an anti-apoptotic effect of EGCG by decreasing the anti-apoptotic Bcl-2 proto-oncogene.[35-37]

Sinecatechin 10% ointment (currently available as Veregen® and indicated to treat condyloma acuminatum) contains EGCG. In ***chapter 3***, we aimed to investigate the efficacy of sinecatechin 10% ointment to treat sBCC in a randomized controlled trial. We hypothesized that this ointment would lead to histological tumor clearance; however, we found no significant differences in histopathological clearance post-treatment between the intervention and placebo groups. We also assessed whether there was decreased expression

of Ki-67 (proliferation) or Bcl-2 (anti-apoptosis) by immunohistochemical staining pre- and post-treatment. We could not observe statistically significant differences, but we did observe a tendency towards a greater decrease in Bcl-2 expression in the sinecatechin 10% group compared to placebo (41.2% versus 23.5%). A decrease in Ki-67 was observed in similar proportions (29.4% versus 31.3%). This study could not confirm the theoretically hypothesized efficacy of sinecatechin 10% ointment in sBCC. It remains debatable as to why decreased Bcl-2 expression was observed in the intervention group. Is it just coincidence? Perhaps the current formula contains insufficient EGCG to be effective to treat sBCC. Another explanation could be that the ointment was unable to reach the nucleus of tumor cells and thereby failed to increase apoptosis and decrease proliferation sufficiently to lead to histological tumor clearance. We can conclude that, based on our study, there is no need to further assess the use of topical sinecatechin 10% ointment in the current formula to treat sBCC. More pre-clinical (laboratory and animal studies) might be needed to assess the effect of EGCG on BCC tumor cells and to determine the optimal EGCG dosage.

Personalized medicine: a niche for PDT?

The gold standard treatment for all BCC is surgical excision. In recent decades, research has shown that non-invasive therapies (e.g., topical 5-FU cream, imiquimod cream or PDT), result in acceptable clearance rates for sBCC. An important disadvantage of non-invasive treatments is the lack of histological control. On the contrary, they lead to a lower workload, might be more patient friendly and – in the case of topical creams – lead to fewer in-clinic treatments. In the 20th century, PDT gained popularity as an alternative to surgery. This rise was based on a few small studies reporting on efficacy. However, with rising patient numbers, the need for alternative non-invasive treatments was high. In the Netherlands, PDT used to be a popular treatment because of reported good cosmetic results, the excellent reimbursement for the dermatologist and an international consensus mentioned PDT as first-line non-invasive treatment for sBCC.[38] Interestingly, in 2013, Arits et al. demonstrated that imiquimod cream was superior and 5-flurouracil was non-inferior and more cost-effective compared to PDT after one year of follow-up in a large multi-center randomized trial studying sBCC.[39] Recently, five-year follow-up data showed that 5% imiquimod cream is superior to both MAL-PDT and 5% 5-FU cream.[40]

Several studies have been conducted to assess whether the effectiveness of PDT could be improved by different photosensitizers, illumination sources or treatment schemes. De Haas et al. were the first to describe a two-fold 5-aminolevulinic acid (ALA)-PDT scheme, in which two illuminations were performed with 20 and 80 J/cm^2, 4 and 6 hours after a single application of aminolevulinic acid (ALA) in a randomized controlled trial. The results were promising, with 12-month clearance rates (CR) of 97% compared to 89% after a single ALA application.[41] These CR, however, were not replicated by other study groups.

We tried to assess the replicability of these CR published by de Haas et al. and described the results in ***chapter 4.1 and 4.2***. We initially performed a retrospective study investigating the efficacy of this two-fold ALA-PDT scheme. We assessed a total number of 323 primary sBCC for recurrence, derived from data in electronic patient files at the outpatient department of a single non-university hospital. Our results indicate a cumulative probability of recurrence-free survival based on clinical observations of 88.8%, 81.8% and 77.1% after 12, 24 and 48 months post-treatment, respectively. Our results are comparable to a study by Star et al. showing a clearance rate of 88% at 12 months follow-up using two-fold illumination with 45 J/cm^2.[42] However, we could not confirm the high one-year clearance rate reported by de Haas et al.[41] The limitations of this study were its retrospective nature and the lack of a control group.

We tried to solve these limitations by performing a prospective multi-center randomized trial comparing the two-fold illumination ALA-PDT scheme with the conventional MAL-PDT scheme, which is mostly used by dermatologists in Europe. Our results showed a lower probability of treatment success in the conventional MAL-PDT group compared to the two-fold ALA-PDT group at 12 months follow-up (92.3% versus 83.4%). Even though not statistically significant (p=0.091), these results do show a trend towards better efficacy of the two-fold ALA PDT scheme and support the previous findings from the group of de Haas et al.[41,43] We also observed a higher pain score and more local adverse events after two-fold ALA-PDT compared to conventional MAL-PDT. Pain during PDT is a well-known drawback for patients. This is especially observed in AK patients, but it is also reported in BCC patients. Our findings might be explained by the double illumination on one day. Middelburg et al. found a significantly higher degree of PpIX in the dermal vasculature after ALA compared to MAL.[44] It is suggested that, by fractionation of the illumination, there might be an additional use of PpIX because of re-oxygenation during the dark interval.[45] The reported stronger effect of ALA on the vasculature and an increased local immune response might be an explanation for the higher degree of pain sensation experienced in our sample.

The trade-off between treatment efficacy and side effects is a personal choice: a higher probability of treatment success after two-fold ALA-PDT or fewer side effects and lower efficacy after MAL-PDT. We did not perform a cost-effectiveness analysis, but in the Netherlands ALA cream is cheaper than MAL cream. Furthermore, an important advantage of two-fold ALA-PDT over conventional MAL-PDT is the one-day treatment, which leads to lower costs and workload. Our five-year follow-up data will reveal whether the lower probability of treatment failure after two-fold ALA-PDT will be maintained in the long term. In this same context of researching possible improvements for PDT, we assessed the efficacy of an ambulatory MAL-PDT device in a retrospective study, described in ***chapter 4.3***.

The results showed a clearance rate of 89.9% at 12 months follow-up, with the highest efficacy in tumors smaller than 10 mm in diameter. Ambulatory PDT emits red light at a low irradiance compared to conventional PDT and over a longer period of time. It is hypothesized that this leads to a greater cytotoxic effect and photobleaching efficiency.[46-48] Photobleaching is a phenomenon describing the decrease in the fluorescence signal in irradiated tissue due to photosensitizer destruction. In this process, protoporphyrin IX (PpIX) fluorescence is reduced. The amount of photobleaching during PDT is correlated with the amount of PDT-induced damage.[49] These factors might be an explanation for the greater effectiveness compared to conventional PDT. As our study was a retrospective data study and the literature is lacking in prospective, randomized controlled comparative trials, there is not enough evidence to implement the ambulatory PDT device widely in dermatology practice.

Finally, we considered the role of PDT in dermatology practice in the Netherlands. After the publication of the randomized trials by Arits et al.[39] and Roozeboom et al.,[50] reimbursement for PDT to treat sBCC was questioned, because of its proven lower efficacy and higher costs compared to 5% imiquimod cream and 5% 5-FU cream. This thesis focused on the use of PDT for sBCC, so the question is: should PDT return to the stage? In my opinion, PDT should not be eliminated as a treatment for sBCC. It is of great importance to look at the individual patient when determining a therapeutic approach. Even though less effective, PDT may still be preferred for a certain category of patients. One can think of the elderly, who are sometimes unable to apply a topical ointment themselves and/or in whom surgery might not be preferred. Also, younger patients might have a profession, which holds them back from applying a topical ointment for 4-6 weeks with a long down-time. At this moment, we cannot draw conclusions on the comparison of the two-fold ALA PDT scheme and topical creams such as imiquimod or 5-FU. A direct comparison of these treatments through randomized trials is necessary to validate conclusions on their comparative efficacy. It would also be of interest to determine the long-term outcomes of the two-fold ALA PDT regimen at three or five years follow-up.

Conclusion

In conclusion, in this thesis, different randomized controlled trials are reported in which we aimed to investigate several non-invasive treatment modalities for two keratinocyte neoplasms: AK and sBCC. Randomized controlled trials are essential to create valuable evidence regarding the efficacy of treatments. Our data show that 5% 5-FU should be the first-choice treatment for field AK. In case an in-clinic treatment is preferred, PDT using the pulsed dye laser could be an alternative to conventional PDT, when pain is a limiting factor.

Despite the fact that recent studies showed that MAL-PDT is inferior compared to 5% imiquimod for sBCC, we aimed to search to optimize its efficacy. The two-fold ALA-PDT regimen is promising. In our study, it led to fewer recurrences compared to conventional MAL-PDT. PDT might remain valuable for a certain subgroup of patients. We should realize that treating patients does not only involve choosing the most effective treatment. Although all treating physicians should be aware of the most (cost) effective approach, the treatment choice also relies on the needs and possibilities for our individual patients. As Hippocrates once stated: "*It is more important to know what sort of person has a disease than to know what sort of disease a person has*".

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