Clinical aspects of scalp cooling in chemotherapy induced alopecia

Komen, M.M.C.

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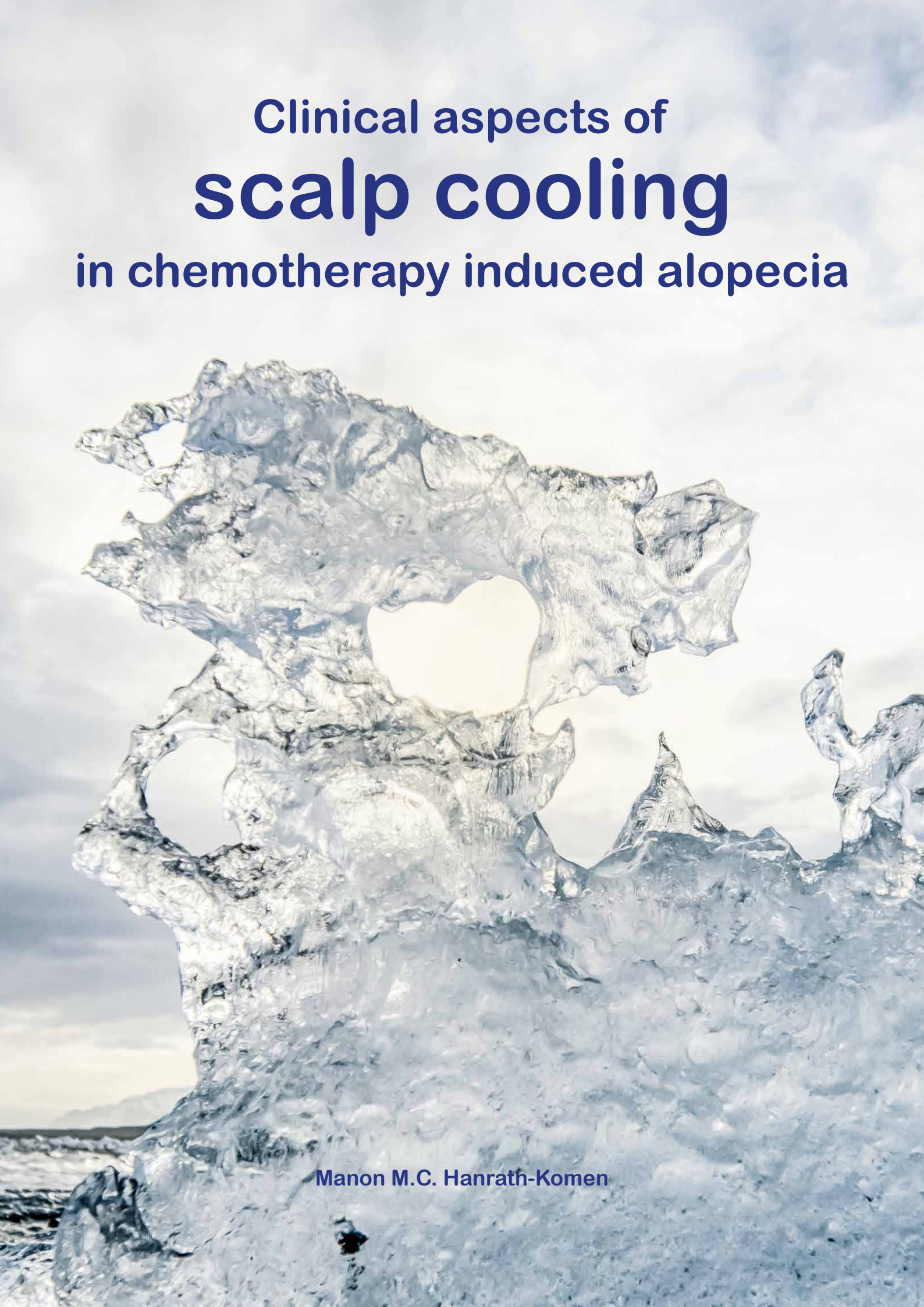

Manon M.C. Hanrath-Komen

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Colophon

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door

Manon Maria Catharina Komen

geboren te Alkmaar
in 1981

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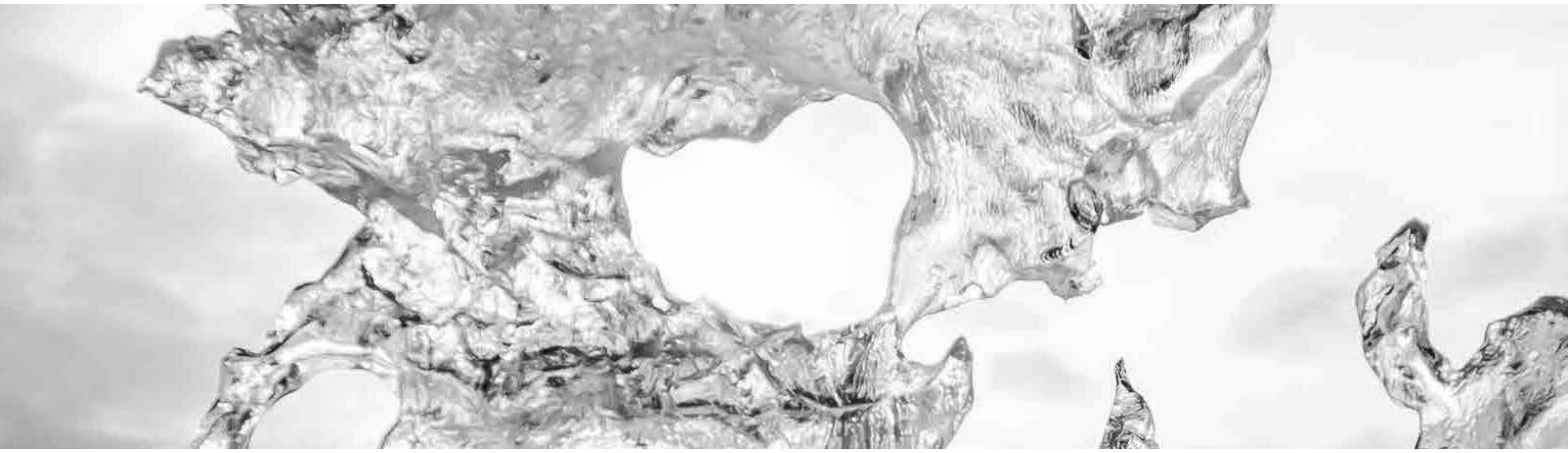
CONTENTS

Chapter 1



Introduction

INTRODUCTION

Hair loss (alopecia) is one of the most feared side effects of chemotherapy.(1-4) About one quarter of patients with cancer is at risk for chemotherapy-induced alopecia (CIA).(5) This also applies to men, although this is often not discussed.(6) Whereas about 80% of patients considers CIA as an important side effect, (7) clinical research has shown that the impact of CIA is underestimated by both oncologists and nurses.(8-11)

Up to now, scalp cooling is the only method to prevent CIA. When using scalp cooling, a cold liquid is pumped through a cap which is placed on the head of the patient before, during and after intravenous treatment with chemotherapy. It is hypothesized that this causes subcutaneous vasoconstriction resulting in reduced perfusion of cytostatic drugs to the hair follicles and a reduction of biochemical activity. During the San Antonio Breast Cancer symposium in December 2016, the results of the randomized SCALP trial were presented. This randomized clinical trial assessed whether a scalp cooling device was effective in reducing CIA. The results showed that scalp cooling was safe and effective in 50.5% of patients.(12) Following this and another publication in JAMA(13), scalp cooling has been approved for cancer patients with solid tumors by the FDA in the United States.(12,13) A poll at the St. Gallen International Breast Cancer Conference in March 2017 showed that 83% of the participants felt that scalp cooling was a good option to prevent hair loss during (neo) adjuvant chemotherapy.

At present, scalp cooling is available in almost all hospitals in the Netherlands. It is frequently used in women with breast cancer receiving chemotherapy, but also in women with other tumor types and in men with prostate cancer receiving docetaxel. This introduction provides an overview of the main points of interest when scalp cooling is applied in clinical practice.

The hair cycle and p53 mechanism in CIA

Under normal circumstances, the growth cycle of a hair follicle consists of three main phases: the anagen, catagen and telogen phase.(14) The active growth (anagen) phase lasts for three to seven years and involves the growth of a hair from a hair follicle. During the transition (catagen) phase (2-3 weeks), the hair stops growing and releases itself from the blood supply. During the resting (telogen) phase (3-4 months), the hair stays attached to the hair follicle and does not grow. When the old hair is shed, the telogen phase ends (Figure 1).

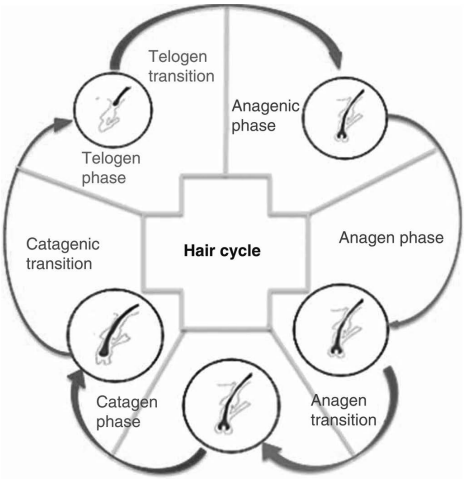


Figure 1: Hair cycle. Adapted from: Drug discovery for alopecia: gone today, hair tomorrow. Santos et al. Expert Opin Drug Discov. 2015;10(3):269-292.(15)

Since 90% of all hair is in anagen phase and rapidly proliferates, hair follicles are highly at risk to be affected by chemotherapy, resulting in CIA.(16,17) Hair shaft shedding begins days to weeks after the initiation of chemotherapy. The exact mechanism is not completely understood, but it is known that regression of the hair follicle activates a variety of signaling pathways, which induce apoptosis.(18) Although the role of many molecular factors in the DNA-damage response remain to be explained, P53, a key mediator of cellular mechanism of stress response, has a crucial role in the occurrence of apoptosis.(16) (Figure 2) In a mouse model for CIA, Botchkarev demonstrated that p53 is essential in this process. Administration of cyclophosphamide was associated with rapid increase of p53 concentrations in hair-matrix keratinocytes, followed by apoptosis. By contrast, genetic p53 ablation in mice rendered hair follicles completely resistant to cyclophosphamide.(16)

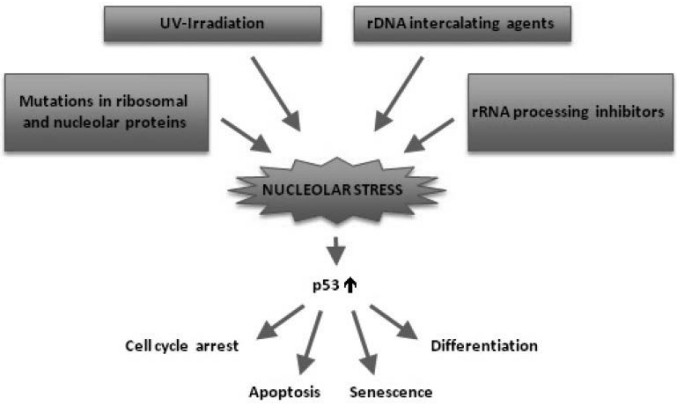


Figure 2: Apoptosis depends on p53. Adapted from p53 -Dependent and -Independent Nucleolar Stress Responses. Olausson et al. Cells. 2012 Dec; 1(4): 774–798.(19)

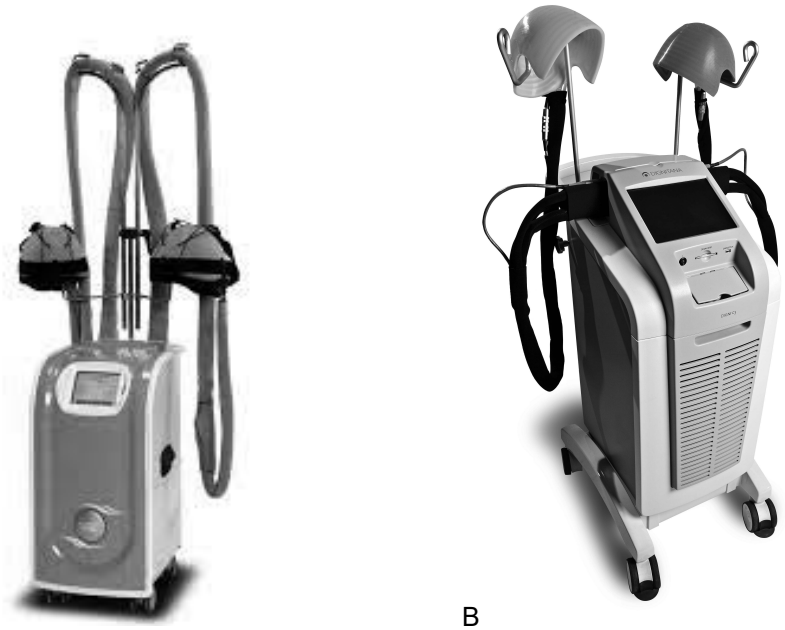
Prevention of chemotherapy induced alopecia

Several researchers have investigated pharmacological as well as nonpharmacological measures to prevent CIA. Little information is available in terms of medications. At present, there are no approved drug treatments for preventing CIA. Minoxidil is well recognized to promote hair growth, but topical administration of minoxidil, as well as other hair growth cycle modifiers, does not prevent CIA.(14,20) Other agents with different action mechanisms like cytokines, growth factors, antioxidants, proliferation modifiers and inhibitors of apoptosis showed some effect, but only in animal CIA models.(14) Reliable preventive pharmacological therapy to prevent CIA in human is still sought. Nonpharmacological measures such as scalp tourniquets were designed to reduce the blood flow to scalp hair follicles during chemotherapy infusion.(2,20) However, most studies investigating the effect of tourniquets used inconsistent techniques and involved small numbers of patients. Therefore, it was difficult to determine the exact effect.(20) Tourniquets are no longer recommended due to patient discomfort. Currently, most research on preventing CIA focuses on scalp cooling.

Scalp cooling

Methods

In the Netherlands cooling machines both from Paxman (England) and from Dignitana (Sweden) are used. These are the two largest suppliers on the market and although both types of coolers differ slightly from each other, no differences in results are seen in practice.



A B

Figure 3: Cooling machines from Paxman (A) and Dignitana (B)

Liquid circulates through the devices at a constant temperature of -10°C. For the best possible result, the cooling cap, which is connected to the device, must be optimally connected to the head. Scalp cooling is applied during the infusion of chemotherapy, with a pre-cooling time of 30 minutes to ensure the scalp is at the required temperature, and a follow-up time of usually 90 minutes.

Results

Within the literature, efficacy results for scalp cooling vary because of variability in study design, such as population, chemotherapy regimen, outcome measure, type of cooling system/device, and cooling duration.(21) In most trials, scalp cooling has shown a positive effect in reducing CIA following a number of different chemotherapy regimens.(2,12,13,22) A meta-analysis of Shin et al. in 2015 showed that the risk of hair loss became three times smaller due to the use of scalp cooling.(23) These results were confirmed in 2017 with a meta-analysis of randomized scalp cooling studies,(24,25) and a review of controlled clinical trials(25), which added additional support to the finding of statistically significant higher rates of hair preservation with scalp cooling compared to chemotherapy administered without cooling.

In the Netherlands, scalp cooling has been used since 1970. A Dutch open patient registration study was started in 2006, in which more than 7000 patients have now been included. Scalp cooling results in this registry were positive for most chemotherapy regimens.(22) Successful scalp cooling was the patient's self-determined need to wear a wig or other head covering. It was concluded that results were best for monotherapy with low dose taxanes (95% effect) and poor in the TAC regimen, a combination chemotherapy with docetaxel, adriamycin and cyclophosphamide (8% effect). These findings are confirmed in other studies.(12,13)

Factors that influence the result of scalp cooling

The type of chemotherapy, the number of cycles, the dose, administration route and the speed of administration can strongly influence the result of scalp cooling.(22,26) The influence of all other patient-related factors (age, gender and hair type) is less convincing or lacking.(2,22,23,27) Factors such as liver function and menopausal status might influence the efficacy, but there is no convincing evidence yet.(2,22,23,27) The influence of the scalp skin temperature is probably the most important factor influencing the result of scalp cooling.(28) However, the scalp skin temperature in patients using scalp cooling does not always decrease to optimal temperature levels.(28,29) Both a better connection between the cap and the head and individual temperature adjustment could overcome this problem. Temperature has also been proven to be critical in in vitro research. Laboratory studies provided biological evidence for a positive correlation between the

degree of cooling and the survival of hair follicles. Al Tameemi et al. reported a study in which they found that lowering the temperature from 22 °C to 18 °C and even further to 14 °C, resulted incrementally in a better degree of rescue from drug cytotoxicity. Cytoprotection was detectable even for the maximal drug doses tested, which had previously resulted in complete loss of cells.(30) Despite the increased knowledge about the effect of temperature on cell survival, there still is a lack of knowledge about the optimal temperature of scalp cooling and how to reach this. This lack of knowledge also applies for the post-infusion cooling time, another factor which could influence the effect of scalp cooling. Shortening the post-infusion cooling time from the usual 90 minutes to 20 minutes is justified for docetaxel, but for other regimens this time is unknown and should be studied.(6)

Tolerance

Scalp cooling is usually well tolerated.(23,31,32) Several publications report a visual analogue scale (VAS) score of 6.9-8.3 (0 'not tolerable' to 10 'very well tolerated').(2,6,33,34) Side effects include chills, dizziness, headache, nausea and a sensation of cold.(12,21) However, these side effects are mild (mostly grade 1) and are for less than 5% of patients a reason to stop scalp cooling.(2,21-23)

Safety

The lack of safety research has limited the use of scalp cooling for years. In particular, it was feared that scalp cooling would protect possible occult metastases in the scalp from chemotherapy, with the danger of scalp metastasis. Therefore, scalp cooling is not recommended in patients with hematological malignancies. In one patient with leukemia and another patient with mycosis fungoides, who both chose to use scalp cooling, cutaneous disease recurred on the scalp after several years, with no other evidence of disease.(35,36) However, in solid tumors, there has never been evidence for the occurrence of scalp metastases due to scalp cooling.(2,5,23,37) Scalp metastases rarely appear in patients with breast cancer (0.003-3%).(5,24) This risk is not increased in patients who use scalp cooling (0.04-1%).(5,24) A retrospective study with 6-8 years follow up found no difference in the occurrence of scalp metastases between patients with and without scalp cooling.(38) A large study by Van den Hurk et al. with a follow-up of 5 years also showed no increased risk of developing scalp metastases.(22) These results were confirmed by a meta-analysis, performed by Rugo(24) and Shah(25) in 2017. When scalp metastases occurred during scalp cooling in the metastasized setting, they always appeared simultaneously or after occurrence of metastases elsewhere.(37) During many years of application of scalp cooling, there has never been an adverse effect of scalp cooling on the disease.

The risk of possible metastases in the skull or even in the brain is very unlikely. Research has shown that there is no or only a minimal decrease in temperature both in the skull and in the brain.(39)
Scalp cooling is contraindicated in patients who suffer from cold sensitivity, cold agglutinin disease, cryoglobulinaemia, cryofibrinogenaemia or cold posttraumatic dystrophy.

Developments and recommendations

The use of scalp cooling in oncology patients treated with chemotherapy in the Netherlands is still low (29%).(7) There are large differences in the supply of scalp cooling between hospitals and sometimes scalp cooling is not used in (neo)adjuvant chemotherapy regimens. This limited application lies both in the limited supply and the limited demand. Only 30% of potential candidates for scalp cooling is aware of the existence of scalp cooling. (Multiscope, personal communication, 2016-2017)

Offering the possibility of scalp cooling largely depends on the opinion of doctors and nurses on the efficacy of scalp cooling to prevent CIA.(40) As a result, there are large differences in the availability of scalp cooling for men and women, for different age groups and for different types of chemotherapy. In addition, the administration of scalp cooling is often limited because of staff shortages or logistical problems.

Given their role in the use of scalp cooling, nurses are perhaps best equipped to inform patients about scalp cooling. Together with oncologists and managers, preconditions such as training and extra time could be created, so that every patient with a desire to try scalp cooling can be informed and given the opportunity to use it.
It appears that the knowledge on CIA and scalp cooling is evolving slowly, but it is expected that this will change due to the breakthrough of scalp cooling in the USA. Further research should focus on improving the results of scalp cooling and on personalizing scalp cooling. In addition, implementation of scalp cooling should be included in (international) oncology guidelines.

OBJECTIVES AND OUTLINE OF THIS THESIS

The aim of this thesis was to study the mechanism of scalp cooling in patients treated with chemotherapy and to refine and personalize the technique.

In **chapter 2** we examine various factors influencing the effectiveness of scalp cooling in the prevention of chemotherapy-induced alopecia and provide a critical appraisal of clinical studies.

In **chapter 3** we discuss the relation between scalp skin temperature and the efficacy of scalp cooling. Apart from the type or dose of chemotherapy, as described in chapter 2, the scalp skin temperature during scalp cooling is a very important factor to prevent hair loss. To obtain optimal results, we analysed which threshold should be reached below which hair loss can be prevented.

In **chapters 4 and 5** we compared different post-infusion scalp cooling times to investigate its effect on the outcome of scalp cooling. The duration of post-infusion cooling implies a prolonged stay on the chemotherapy ward, which is potentially a disadvantage both for patients and for the logistics of the clinic. **Chapter 4** describes a shorter post-infusion time in the low dose docetaxel chemotherapy regimen, in which cooling is very effective. On the other hand, scalp cooling is also offered in regimens with a more limited effect. Therefore, a prolonging of the post-infusion cooling time was investigated in an anthracycline containing chemotherapy regimen. **Chapter 5** presents the results of a randomized study, investigating a longer post-infusion scalp cooling time.

In **chapter 6** the various methods used to evaluate the outcome of scalp cooling are assessed. In this study the common subjective methods to evaluate hair loss were compared with a new objective method in order to standardize the measurement of hair loss in clinical trials. Standardization of measurement would simplify the evaluation and comparison of potential therapies to prevent CIA.

In **chapter 7** we explored molecular damage-response pathways in hair follicles from patients treated with chemotherapy, to provide a better understanding of the scalp cooling working mechanism. Investigating hair follicles of patients treated with chemotherapy, is the only way to demonstrate the working mechanism of scalp cooling and to explain why scalp cooling works in one patient, but not in the other.

This thesis ends with concluding remarks and future prospects in **chapter 8**.

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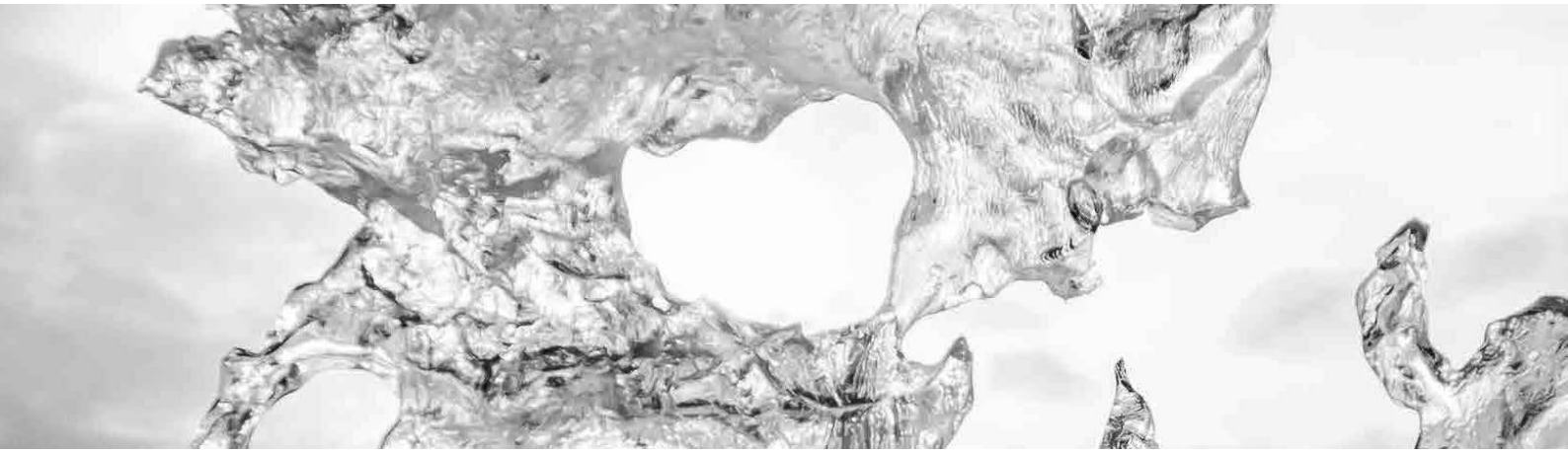
Chapter 2



Factors influencing the effectiveness of scalp cooling in the prevention of chemotherapy-induced alopecia

The Oncologist. 2013, 18:885-891.

Komen M.M.C., Smorenburg C.H., van den Hurk C.J.G., Nortier J.W.R.

ABSTRACT

Introduction: The success of scalp cooling in preventing or reducing chemotherapy induced alopecia (CIA) is highly variable between patients and chemotherapy regimens. The outcome of hair preservation is often unpredictable and depends on various factors.

Methods: We performed a structured search of literature published from 1970 till February 2012 for articles which reported on factors influencing the effectiveness of scalp cooling to prevent CIA in cancer patients.

Results: Literature search identified 192 reports of which 32 studies were considered relevant. Randomised studies on scalp cooling are scarce and there is hardly information on the determinants of the result. Hair preserving results of scalp cooling depend at least on dose and type of chemotherapy, with less favourable results at higher doses. Temperature seems to be an important determinant. Various studies suggest that a subcutaneous scalp temperature below 22°C is required for hair preservation.

Conclusions: The hair preserving results of scalp cooling are variable and mainly depend on type and dose of chemotherapy and probably on degree and duration of cooling.

INTRODUCTION

Chemotherapy induced alopecia (CIA), although being reversible, is for patients one of the most distressing side-effects. It has psycho-social implications and may affect body image and acceptance of treatment.(1-5) For some patients CIA is a reason to refuse chemotherapy, and up to 8% of patients may choose less effective chemotherapy regimens if these regimens do not cause hair loss.(6;7)

In healthy persons, scalp hair follicles show a pattern of cyclic activity. The hair growth (anagen) phase involves the growth of a hair from a hair follicle and lasts for three to seven years. During the transitional (catagen) phase, the hair follicle atrophies and migrates upwards to a resting level in the skin. During the resting (telogen) phase, the hair does not grow but stays attached to the hair follicle. The telogen phase ends when the old hair is shed and a new hair is regenerated in the anagen phase. In the adult scalp, approximately 90% of the follicles is in the growth phase.(8;9)

Chemotherapy acts on rapidly growing cells including hair follicles and chemotherapy induced shedding of hairs usually occurs seven to fourteen days after infusion.(8;10) The incidence and severity of alopecia depends both on the type (table 1) as well as the dose of chemotherapy.(6;9;11-13) Apart from hair loss from the scalp, patients may also loose their eyebrows, eyelashes and pubic hair after several cycles of chemotherapy. Although alopecia is a reversible side effect, permanent alopecia has been reported incidentally after high dose chemotherapy.(10)

Table 1. Cytotoxic drugs that cause chemotherapy induced alopecia

Mild alopecia (0,1-1%)	**Moderate alopecia (1-10%)**	**Severe alopecia (>10%)**
Bortezomib	Bleomycin	Busulfan
Cabazitaxel	Cyclophosphamide	Docetaxel
Carboplatine	Epirubicin	Doxorubicin
Catumaxumab	Trastuzumab	Etoposide
Cisplatin	Panitumumab	Gemcitabine
Cytarabin		Idarubicin
Dactinomycin		Ifosfamide
Ixabepilone		Irinotecan
Lomustine		Mitomycine
Methotrexaat		Oxaliplatin
Pemetrexed		Paclitaxel
		Topotecan
		Vinorelbine
		Procarbazine

Source: Investigator brochures

Since the 1970s, scalp cooling is being used to reduce and prevent CIA.(14) It reduces skin temperature, thereby affecting the exposure and metabolism of cytotoxic agents in the hair follicles. However, the effectiveness of scalp cooling in preventing alopecia is highly variable and unpredictable. We therefore wanted to explore possible reasons why scalp cooling works in one patient but fails in another.

MATERIALS AND METHODS

We designed a search strategy to identify relevant literature that described the use of scalp cooling in preventing chemotherapy induced hair loss among patients treated for cancer. We performed our search on February 24, 2012 in the electronic databases PubMed, Embase, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) for literature published from 1970 through February 24, 2012, linking the subject search headings with text word and MESH terms.

A combination of the following search terms was used: ((("chemotherapy"[all fields] OR "antineoplastic protocols"[Mesh] OR "antineoplastic agents"[Mesh] OR "neoplasms/drug therapy"[Mesh Terms] OR "chemotherapy-induced"[all fields]) AND ("hair loss"[all fields] OR "alopecia"[Mesh] OR "alopecia"[all fields])) OR "alopecia/chemically induced"[Mesh Terms]) AND ("scalp cooling"[all fields] OR "scalp hypothermia" OR "cold cap"[all fields] OR ("hypothermia, induced"[Mesh] AND ("scalp"[Mesh] OR "scalp"[all fields])))

We did not restrict the search strategy to a particular type of study design.

Articles were selected if they assessed any possible factors affecting the effectiveness of scalp cooling in preventing alopecia after chemotherapy. Only full text articles in English and Dutch were considered. We also did a manual search for any relevant references used in the articles found. Papers that described scalp cooling as a safety issue or focussed solely on impact or tolerance were excluded.

RESULTS

The initial search resulted in a total of 192 citations (76 Hits in PubMed, 92 in Embase, and 24 in CINAHL). After removing duplicates, 102 citations remained; 70 were discarded based on title or abstract because they did not meet the inclusion criteria and the 32 citations that were considered relevant, were included in this review (table 2). A manual search of references in the relevant articles did not yield any additional study. The majority of the articles (20 out of 32) was published between 1980 and 2000. Since 2010, only 3 articles have been published on possible factors influencing the effectiveness of scalp cooling in the prevention of CIA. Excluding one large multicenter observational study (ref 14), the median number of reported patients was 35 (range 9-180).

Table 2. Factors influencing the result of chemotherapy induced alopecia

	Author	Year	N of patients	Patient charateristics	Chemotherapy characteristics	Scalp cooling characteristics	Scalp cooling techniques
1	Anderson	1981	31			X	
2	Hunt	1982	22		X	X	X
3	Guy	1982	12				X
4	Gregory	1982	24			X	
5	Dean	1983	33			X	X
6	Wheelock	1984	11		X	X	
7	Satterwhite	1984	26		X	X	
8	Vendelbo	1985	61			X	
9	Middleton	1985	60			X	X
10	Bulow	1985	10			X	
11	Symonds	1986	26			X	X
12	Robinson	1987	32			X	
13	David	1987	180			X	
14	Giaccone	1988	39		X		
15	Hillen	1990	48			X	X
16	Adams	1992	34		X	X	
17	Tollenaar	1994	35		X	X	
18	Lemenager	1997	98		X	X	X
19	Peck	2000	10				X
20	Katsimbri	2000	70			X	X
21	Christodoulou	2002	83			X	X
22	Ridderheim	2003	74			X	X
23	Macduff	2003	40			X	X
24	Massey	2004	94			X	
25	Grevelman	2005	Review			X	
26	Janssen	2005	Model	X		X	
27	Janssen	2007	9			X	
28	Auvinen	2010	64		X	X	
29	Komen	2011	27			X	
30	Kargar	2011	63			X	X
31	Van den Hurk	2012	76			X	
32	Van den Hurk	2012	1411	X	X		X

The effectiveness of scalp cooling in cancer patients depends on many factors, which can be related to patient characteristics, chemotherapy characteristics and the procedure of scalp cooling (table 2). Only one study reported on a relationship between patient characteristics and scalp cooling effectiveness, while in 9 articles various chemotherapy schedules were tested and in 31 articles technical aspects of scalp cooling were described.

Patient characteristics

In a large multicenter observational study in the Netherlands Van den Hurk et al.(15) concluded that scalp cooling was more effective at younger age, in male patients and in patients with a Caucasian type of hair. In a computer model study, Janssen et al.(16) found that the thickness of the hair layer correlated with the scalp skin temperature during scalp cooling. This may explain the lower effectiveness of scalp cooling in patients with Afro hair, who have a thick layer of hair which acts as an insulating layer between the cooling cap and the scalp.

Chemotherapy characteristics

Type and dose

The incidence and severity of CIA using scalp cooling depends on the type and dose of chemotherapy.(1;1;2;12;15;17-22) Only three of these reported studies randomised patients to chemotherapy either with or without scalp cooling. Therefore, results of effectiveness of scalp cooling have to be compared with historical series with identical chemotherapy regimens. An ongoing Dutch observational study collects data on the effectiveness of scalp cooling with various types and doses of chemotherapy regimens (table 3)(15) For anthracycline containing regimens, a higher dose of anthracycline was correlated with a worse outcome of scalp cooling. (15;17) With 5-Fluorouracil-Epirubicin-Cyclophosphamide (FEC) chemotherapy, 33% of the patients treated with epirubicin at a dose of 100 mg/m^2 did not require a head cover versus 52% of the patients treated with epirubicin at a dose of 90 mg/m^2. Likewise, 59% of the patients treated with docetaxel at a dose of 100 mg/m^2 did not require a head cover versus 79% of the patients treated at a dose of 75 mg/m^2 (table 3).(15) Of notice, scalp cooling failed to prevent alopecia in most patients who were treated with the combination of docetaxel, adriamycin, cyclophosphamide (TAC) chemotherapy for early breast cancer.

Table 3. Overview of scalp cooling results in The Netherlands 2006-2009

Indication and chemotherapy type[1]	Total number of patients using scalp cooling	% patients not wearing a wig or head cover
Breast cancer		
FEC-T (500/100/500-100)[2]	45	47
FEC (500/100/500)	123	33
FEC (500/90/500)	552	52
FAC (500/50/500)	38	55
P (70-90) (mono/combinations)[3]	39	82
T (100) (mono/combinations)[4]	42	59
T (75) (mono/combinations)[4]	58	79
TAC (75/50/500)[5]	66	8
AC-TH (60/600-100)[2]	16	63
AC-PH (60/600-175)	21	29
AC-PH (60/600-80)	29	48
AC (60/600)	74	39
Ovarian cancer		
P-Carbo(175-5/6)	49	37
Colon cancer		
Irinotecan (350)	41	29

[1]Dosage in mg/m^2

[2]Sequentially: FEC followed by T/ AC followed by T or P and H

[3]Weekly schedule

[4]Docetaxel combinatons with exception of docetaxel/adriamycine/cyclofosfamide (TAC)

[5]T,A en C simultaneously

C: Cyclofosfamide; Carbo: Carboplatin; A: Adriamycine; T: Docetaxel; E: Epirubicine; F: 5-fluorouracil; H: Herceptin (trastuzumab); P: Paclitaxel

Source: Breed W, van den Hurk CJ, Peerbooms M. Presentation, impact and prevention of chemotherapy induced hair loss: scalp cooling potentials and limitations: *dermatology* 2011; 6: 109-125

Liver function

According to several studies, scalp cooling does not prevent CIA in most patients with biochemical evidence of abnormal liver function.(2;23-27) This may be predicted on the basis of pharmacokinetics of chemotherapy that is metabolized by the liver. For these drugs, an impaired liver function is associated with higher and more prolonged plasma concentrations.(23) In contrast, Grevelman et al.(11) concluded that in only six out of 13 studies, impaired liver function seemed to be related to less benefit from scalp cooling.(11) In these studies patients were treated with doxorubicin or epirubicin.

Scalp cooling characteristics

Temperature

It is evident that optimal fitting of the cold cap is an important factor for success. Bald areas are seen where the cap did not fit properly (figure 1). Contact between the cold cap and the scalp skin is decisive for lowering the skin temperature.(16) In 1982, Gregory et al.(28) found a relation between the degree of decrease in scalp skin temperature and the protective effect of scalp cooling against hair Ioss in patients treated with doxorubicin. They concluded that to prevent CIA the subcutaneous scalp skin temperature should be reduced below 22ºC corresponding to an epicutaneous scalp temperature below 19ºC to prevent CIA .(29) Hillen et al.(30) attributed the success of their air-cooling methods in part to achieving epicutaneous scalp temperatures below 15ºC and Bülow reported that in two out of ten healthy volunteers it was impossible to obtain a subcutaneous scalp temperature below 28ºC, which is in agreement with the findings of Gregory and Janssen and implies that some persons consistently respond to scalp cooling with only a minor reduction in subcutaneous temperature.(28;29;31) As these studies used different and obsolete scalp cooling techniques and report different cut-off levels of scalp skin temperature, a study on scalp temperature using the modern Paxman® system is presently being done at our centre. Janssen demonstrated that wetting the hair increased the conductivity of the hair layer, resulting in a further decrease in scalp skin temperature.(16) However, there are no randomized studies regarding the influence of wetting on scalp skin temperature and scalp cooling success rates, while wetting the hair increases the burden for the patient.

Figure 1. Bald areas are seen where the cold cap did not fit properly

Perfusion

To gain more insight into the effect of cooling, Janssen et al. studied the relationship between skin temperature and skin perfusion during a cooling experiment in 9 healthy subjects.(16) During scalp cooling, relative perfusion of the scalp skin gradually dropped

down to 28%. A plateau in perfusion was reached after prolonged cooling to lower skin temperatures. This reduction in perfusion was in line with the findings of Bülow(29) and Hillen(30), who found that blood flow during scalp cooling was reduced to 25% of the basal value. Bülow also stated that blood flow was not reduced any further when the subcutaneous scalp temperature was below 30ºC.(29)

Scalp cooling time

The duration of scalp cooling might influence the hair protective effect of scalp cooling. In most studies the pre-cooling time (time between start of scalp cooling and start of intravenous infusion of chemotherapy) ranged from 5 to 30 minutes.(12;17-23;25-27;30;32-37) At the Medical Centre Alkmaar we have measured serial scalp skin temperatures during scalp cooling in healthy subjects and patients to determine the optimal pre-infusion cooling time. In 27 persons treated with scalp cooling using the Paxman® PSC1 system, scalp temperature reached a constant level of approximately 18ºC after 45 minutes. These preliminary data suggest that as no further reduction in temperature occurred, a pre-infusion cooling time of 45 minutes seems optimal when a non pre-cooled cap is used.(38)

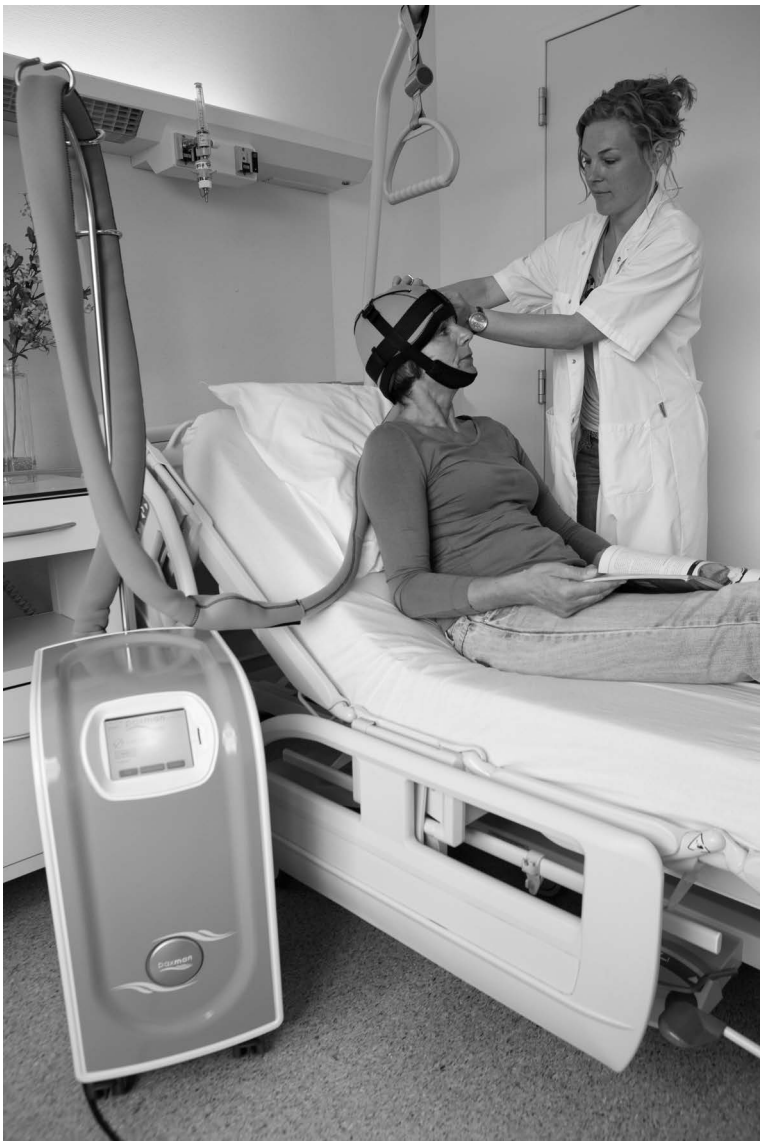
While the pre-infusion cooling time is well known, there is much uncertainty about the post-infusion cooling time. Theoretically, the cooling period after infusion of chemotherapy should be related to pharmacokinetics of exposure to the cytostatic agent and its active metabolites.(11;22) However, research on post infusion cooling time is very scarce. In daily practice, post-infusion cooling times range from 15 minutes to 4 hours.(12;17-23;25-28;30;32;34-37) A study comparing the effect of a shorter post-infusion time in patients treated with docetaxel (90 versus 45 minutes) showed no difference in results on hair preservation (95% versus 79% did not need head covering).(39) Therefore, a new docetaxel study has started in which patients are randomized between 45 versus 20 minutes of post-infusion cooling time. In contrast, in breast cancer patients treated with adjuvant FEC chemotherapy for which scalp cooling is less effective (about 50% no head covering), it is investigated whether prolonging the post-infusion cooling time to 150 minutes is favorable over 90 minutes.

Scalp cooling techniques

Several techniques have been used to induce hypothermia: chilled air, bags with crushed ice, frozen cryogel packs or packs with an endothermic cooling reaction, special caps with cryogel and an insulation layer, and caps connected to a cooling device using air or fluid as a medium and equipped with a thermostat.(2;12;18;20;26;30;32-37;40;41) Few studies compared the effectiveness of different methods of scalp cooling.(30;32;34) Dean et al compared a Kold Kap® device with ice packs in the treatment of 62 patients

with doxorubicin.(34) Sixty-three percent of Kold Kap® patients and 56% of ice pack patients did not require wigs. Although cooling devices using air or fluid as a medium and equipped with a thermostat (figure 2) provide more constant cooling that can be maintained for longer, there is no conclusive evidence that permanently cooled caps give better hair preservation. Studies comparing skin temperatures and skin perfusion as obtained with various methods of scalp cooling are lacking. Although bags with ice as well as special caps are both well tolerated, caps are lighter in weight and easier to apply, which might offer a comfort advantage.

Figure 2. Cooling device equipped with a thermostat



Side effects and contraindications of scalp cooling

Scalp cooling is generally well tolerated.(2;3;11;42) Results obtained from patients appear to indicate high levels of comfort and acceptability with evidence of only minor and reversible side-effects.(1) The most often reported side-effects of scalp cooling are:

headaches, complaints of coldness and/or uncomfortable sensations and among others claustrophobia.(11;20;42) Scalp cooling is contra-indicated in cases of cold sensitivity, cold agglutin disease, cryoglobulinemia, cryofibrinogenemia and post-traumatic cold dystrophy.(11) Scalp metastases have rarely been reported in the literature but caution regarding its development has been a limitation for the broad-scale application of scalp cooling during chemotherapy.(6;43) Theoretically, tumour cells that have seeded in the scalp might not receive adequate chemotherapy during hypothermia allowing them to grow at a later date.(6) Since various studies have reported recently on the safety of scalp cooling (44;45), a feasibility studies on scalp cooling in oncology patients have recently started in the United states.

DISCUSSION

While the incidence and severity of alopecia as a side effect of chemotherapy depends on the type and dose of chemotherapy(6;9;11-13), the outcome of hair preservation by scalp cooling is often unpredictable and varies between patients. The effectiveness of scalp cooling to prevent CIA depends on various factors such as patient characteristics, chemotherapy characteristics and scalp cooling characteristics. Unfortunately, there are hardly any randomized studies on the effectiveness and safety of scalp cooling in CIA. Only few studies have investigated which patient characteristics could be of influence and which method of scalp cooling is the most effective. In this review we found that scalp cooling results are better with certain chemotherapy types (taxanes). Results are less favourable at higher doses of chemotherapy. Skin temperature seems to play an important role, but until now, there is no evidence for a cut-off point under which alopecia can be prevented by scalp cooling. There are suggestions in the literature that a subcutaneous scalp temperature below 22°C is required for hair preservation, but as these studies on temperature used different and obsolete scalp cooling techniques, there is no conclusive evidence so far. Ideally scalp cooling should be applied more patient tailored. If a threshold level of scalp temperature is to be a critical issue, timing and technique of scalp cooling should be adapted to individual measurements of skin temperature. To advise patients on an individual basis on scalp cooling in preventing CIA, factors like optimal temperature and post-infusion cooling time should be investigated further. At present, various hospitals in Europe, Canada and Japan are already using scalp cooling routinely.

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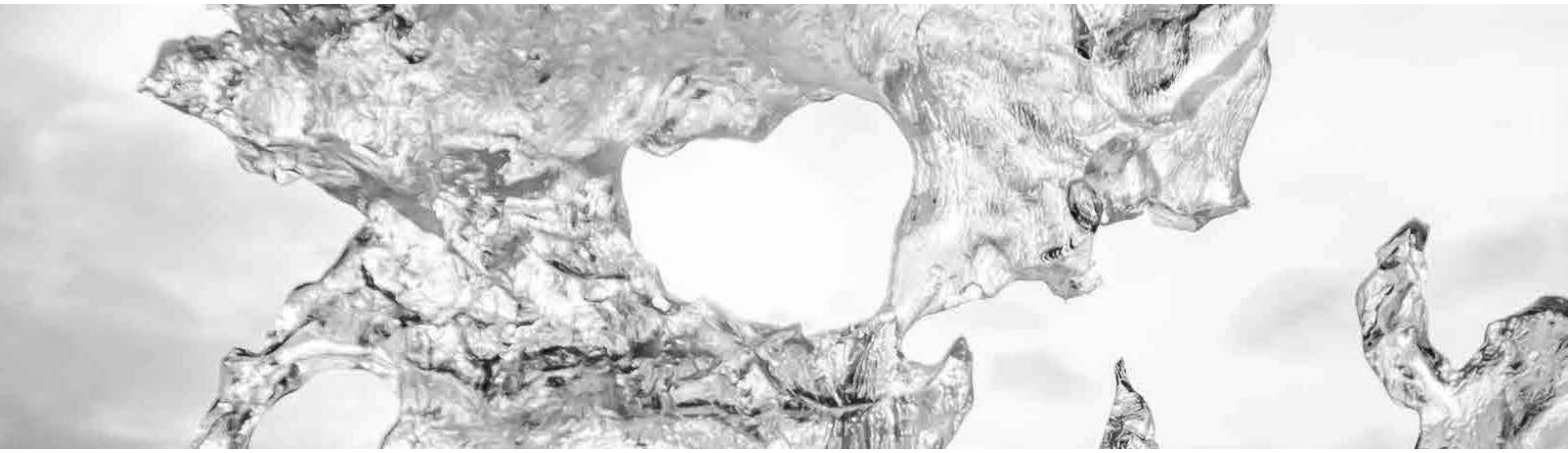
Chapter 3



Results of scalp cooling during anthracycline containing chemotherapy depend on scalp skin temperature

Breast. 2016, 30: 105-110.

Komen MM, Smorenburg CH, Nortier JW, van der Ploeg T, van den Hurk CJ, van der Hoeven JJ.

ABSTRACT

Objectives: The success of scalp cooling in preventing or reducing chemotherapy induced alopecia (CIA) is highly variable between patients undergoing similar chemotherapy regimens. A decrease of the scalp skin temperature seems to be an important factor, but data on the optimum temperature reached by scalp cooling to prevent CIA are lacking. This study investigated the relation between scalp skin temperature and its efficacy to prevent CIA.

Materials and methods: In this explorative study, scalp skin temperature was measured during scalp cooling in 62 breast cancer patients undergoing up to six cycles of anthracycline containing chemotherapy. Scalp skin temperature was measured by using two thermocouples at both temporal sides of the head. The primary end-point was the need for a wig or other head covering.

Results: Maximal cooling was reached after 45 minutes and was continued for 90 minutes after chemotherapy infusion. The scalp skin temperature after 45 minutes cooling varied from 10°C to 31°C, resulting in a mean scalp skin temperature of 19ºC (SEM: 0,4). Intrapersonal scalp skin temperatures during cooling were consistent for each chemotherapy cycle (ANOVA: p=0,855). Thirteen out of 62 patients (21%) did not require a wig or other head covering. They appeared to have a significantly lower mean scalp skin temperature (18ºC; SEM: 0,7) compared to patients with alopecia (20ºC; SEM: 0,5) (P=0,01).

Conclusion: The efficacy of scalp cooling during chemotherapy is temperature dependent. A precise cut-off point could not be detected, but the best results seem to be obtained when the scalp temperature decreases below 18ºC.

INTRODUCTION

Alopecia is a much feared side effect of chemotherapy and may have an impact on treatment decisions(1-6). Scalp cooling still remains the only current intervention to prevent chemotherapy induced alopecia (CIA). It is assumed that scalp cooling works by inducing local vasoconstriction and reduction of metabolism of the administered cytostatic agents(7,8). Vasoconstriction reduces the blood flow to the hair follicles in the period of peak plasma concentration of the relevant chemotherapeutic agent. Reduced metabolic activity makes hair follicles less vulnerable to the damage caused by chemotherapy. Although a decrease of the scalp skin temperature seems to be relevant for the results of cooling, data on the optimal temperature required for hair protection are scarce. There are suggestions in the literature that a subcutaneous scalp skin temperature below 22 degree Celsius (ºC)(9) (corresponding to an epicutaneous scalp temperature below 19ºC(7)) is required for hair preservation, but considerable variations have been reported on the desirable scalp temperature reached during scalp cooling.(7,9-13) Hillen et al.(11) attributed the success of their air-cooling method in part to achieving epicutaneous temperatures below 15ºC, whereas the average epicutaneous scalp temperature of three volunteers recorded in a study of Massey(13) was 16ºC. Al-Tameemi et al.(14) used in vitro models to provide evidence that temperature conditions may be critical in the efficacy of cooling by rescuing cells from drug mediated toxicity. Although previous in vitro reports concluded that further cooling below 22ºC would not provide any further protection against doxorubicin-mediated keratinocyte cytotoxicity(15), it was shown that lowering the temperature from 22ºC to 18º and even further to 14ºC in human keratinocyte models resulted in a better degree of rescue from drug cytotoxicity. Based on the current available knowledge, it is not possible to draw conclusions on the optimal scalp temperature for effective cooling.

To investigate the relation between the obtained scalp skin temperature during scalp cooling and its outcome in preventing CIA, we measured scalp skin temperatures during the procedure of scalp cooling in breast cancer patients treated with anthracycline containing chemotherapy.

MATERIALS AND METHODS

We conducted an explorative single-centre study between August 2010 and January 2014 at the department of Internal Medicine of the Medical Centre Alkmaar, the Netherlands. The study enrolled patients with primary breast cancer who were planned for adjuvant chemotherapy with up to six cycles of 5-Fluorouracil-Epirubicin-Cyclophosphamide (FEC) or Adriamycin-Cyclophosphamide (AC) and who were willing to use scalp cooling to prevent CIA. The study was approved by an independent ethics committee and institution review board. All procedures were conducted in accordance with the 1964 Helsinki

Declaration and its subsequent amendments. Written informed consent was obtained from all patients included in the study.

Inclusion criteria were primary invasive breast cancer without distant metastases. Patients had to be planned for treatment with three to six cycles FEC combination chemotherapy with an epirubicine dose of 90-100 mg/m^2 at 3-weekly intervals or with AC combination chemotherapy with doxorubicin at a dose of 60 mg/m^2. Subsequent chemotherapy cycles consisting of docetaxel monotherapy (100 mg/m^2) were allowed after 3 FEC cycles. Patients were excluded if they lacked basic proficiency in Dutch, if they were unable to understand the patient information brochure or if they suffered from cold sensitivity, cold agglutinin disease, cryoglobulinaemia, cryofibrinogenaemia or cold posttraumatic dystrophy.

The one-person Paxman cooling machine (PSC-1) was used in this study. The temperature of the coolant in the refrigeration tank was -10°C. This temperature is a standard set-up installed by the manufacturer. The cool cap was applied before cooling, with a pre-infusion cooling time of 45 minutes before the start of intravenous infusion of chemotherapy. Scalp cooling was continued during the administration of the chemotherapy with a post-infusion cooling time of 90 minutes after the end of chemotherapy infusion. Scalp cooling was applied in all planned cycles of chemotherapy, unless the patient decided to stop the cooling procedure because of hair loss, side effects or for patients' preference.

At baseline, patient characteristics and objective hair quantity were collected. Objective hair quantity was measured with a Hair Check. The mechanical device compresses a bundle of hair in a disposable cartridge from a delineated area of the scalp and measures its cross-sectional area (Hair Mass Index, HMI). HMI incorporates both density and diameter and was measured at both temporal sides. Tolerance of scalp cooling was measured during all visits by a Visual Analogue Scale (VAS) of 0-10, in which 0 represented 'not tolerable at all' and 10 meant 'very tolerable'. Patients were also asked whether they experienced other side effects such as headaches. The success of scalp cooling was defined in terms of the patient's self-determined need to wear a wig or other head covering. Patients were considered evaluable for hair preservation if they were treated with at least three cycles of chemotherapy or if they discontinued scalp cooling due to severe hair loss. The epidermal temperature at the surface of the scalp was measured using two calibrated J type thermocouples that were fixed with medical glue at the left and right temporal side. To ensure that the registered temperature was a good measure for the skin temperature, each thermocouple end was modified with a specially developed aluminium disc with a diameter of 4 mm and a thickness of 0.5 mm. This facilitated the attachment to the scalp skin and, in combination with the medical glue, ensured that the thermal resistance between the thermocouple and the scalp skin was

lower than the thermal resistance between the thermocouple and the cold cap. The temperature was measured continuously from the start until the end of the scalp cooling process.

Statistical analysis

Data were collected using standard forms, which were compiled into a SPSS database (SPSS version 20.0).

A paired t-test was used to check differences between the two measuring positions on the left and right temporal side. Differences in temperature between patients with and without head covering were analysed by the Mann-Whitney test. Repeated analysis of variance (ANOVA) was used for intergroup differences. All tests of significance were two-sided, and differences were considered statistically significant when $p<0,05$. All tests were performed using SPSS software (version 20.0) for Windows XP.

RESULTS

Patient characteristics

In this study a total of 62 female patients with breast cancer were included. Patient characteristics and the efficacy of scalp cooling are listed in Table 1. The median age of the patients was 60 years. The mean baseline HMI was 64 (range 24-101).

All patients were treated conform the protocol, with a median of 3 cycles of chemotherapy and scalp cooling. The median duration of scalp cooling was 195 minutes per cycle. All patients were evaluable for hair preservation and side effects. Four patients were not evaluable for temperature measurements because of probe dislocation or because probes came loose. At the time of data cut-off (January 1, 2014), the median follow-up of patients was 29 months.

Table 1. Patient characteristics

	N (%)	No head covering *N* (%)	Head covering *N* (%)
Patients included	62	13 (21%)	49 (79%)
Median age, years (range)	60 (32-74)		
Epirubicin	50 (81%)	8 (16%)	42 (84%)
6x F500/E100/C500	28 (45%)	5 (18%)	23 (82%)
3x F500/E100/C500 followed by 3xT100	22 (36%)		
Overall 3xFEC, 3xT		3 (14%)	19 (86%)
Result after 3xFEC		8 (36%)	14 (64%)
Adriamycin	12 (19%)	5 (42%)	7 (58%)
4x A60/C600	10 (16%)	4 (40%)	6 (60%)
6x A60/C600dd	2 (3%)	1 (50%)	1 (50%)
Mean HMI (range)	64 (24-101)		
Median number of cycles with scalp cooling (range)	3 (1-6)		

F: 5-fluorouracil; E: Epirubicine; C: Cyclofosfamide; A: Adriamycine; T: Docetaxel
dd: every two weeks

Scalp temperature

Temperature measurements at the left and right temporal side of the head did not show significant differences. Scalp skin temperatures were therefore reported as the mean of the two measuring points. Maximal cooling was reached after 45 minutes and was continued for 90 minutes after chemotherapy infusion. The scalp skin temperature following 45 minutes cooling varied between patients from 10°C to 31°C, resulting in a mean scalp skin temperature of 19ºC (SEM: 0,4). However, in each individual patient, a consistent temperature was obtained on repeated measurement (ANOVA: *P*=0,855) (figure 1).

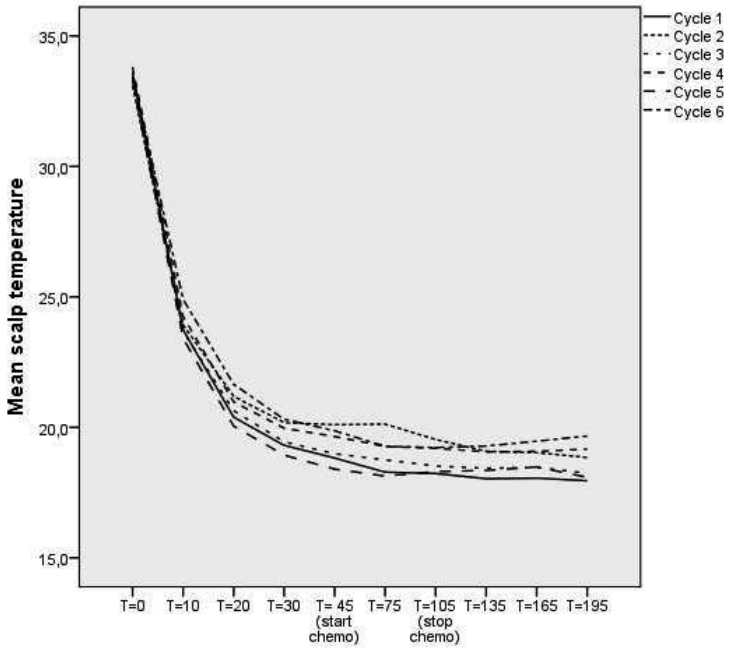


Figure 1. Mean scalp skin temperatures in degrees Celcius on repeated measurement in all patients during scalp cooling during 6 cycles of anthracycline-containing chemotherapy

Prevention of hair loss

The most pronounced hair loss was recorded after cycle 1: 40% of the patients lost their hair after the first treatment. Thirteen out of 62 patients (21%) showed satisfactory hair retention during anthracycline containing chemotherapy (Table 1). Although these patients suffered from slight hair loss, they did not feel the need to wear a wig or other head covering. The pattern of hair loss (global or patchy) was measured after every cycle of chemotherapy. 85% of the patients with global and 75% of the patients with patchy hair loss required a head covering. The baseline HMI score was not predictive for hair loss (HMI no head covering 61; HMI head covering 64; p=0.7) Figure 2 and table 2 show the mean scalp skin temperatures during scalp cooling for patients with and without head covering. Patients with good hair retention had a mean scalp skin temperature of 18ºC (SEM: 0,7) while patients with hair loss resulting in the use of a wig or other head covering had a mean scalp skin temperature of 20ºC (SEM: 0,5) (P= 0,01) (Table 3). Because of the high variation in mean scalp skin temperatures in our patients, it was not possible to detect a threshold scalp skin temperature below which hair retention was always observed.

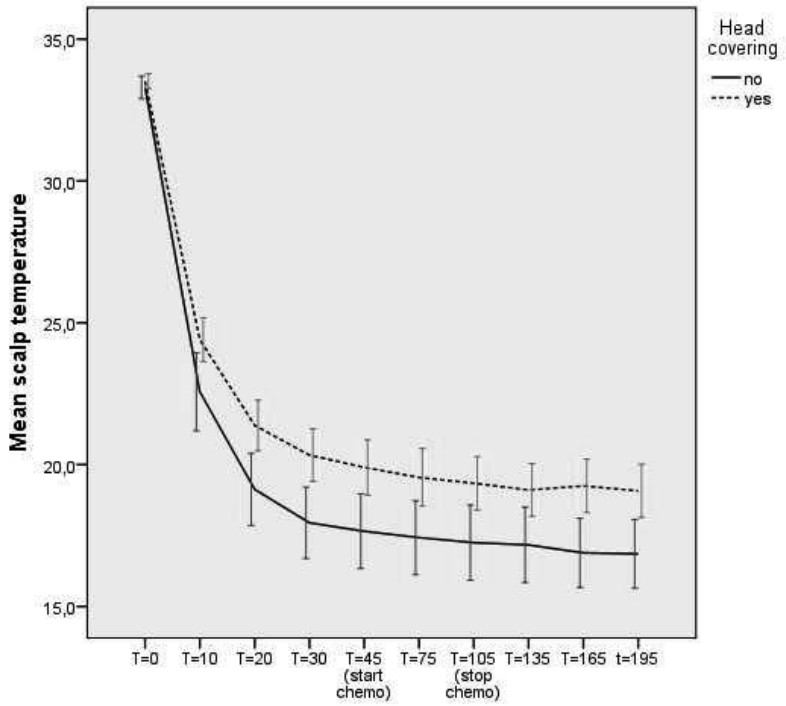


Figure 2. Mean scalp skin temperatures in degrees Celcius during scalp cooling in patients with and without head covering

Table 2. Mean scalp skin temperatures in degrees Celsius during scalp cooling in all patients

Time (minutes)	No head covering		Head covering		*P*-value
	mean	SEM	mean	SEM	
T=0 (start scalp cooling)	33	0,2	34	0,1	0,46
T=10	23	0,7	24	0,4	0,02
T=20	19	0,6	21	0,4	0,01
T=30	18	0,6	20	0,5	0,004
T=45 (start chemotherapy)	18	0,7	20	0,5	0,01
T=75	17	0,7	20	0,5	0,02
T=105 (stop chemotherapy)	17	0,7	19	0,5	0,001
T=135	17	0,7	19	0,5	0,01
T=165	17	0,6	19	0,5	0,004
T=195 (stop scalp cooling	17	0,6	19	0,5	0,01

Table 3. Mean scalp skin temperatures in degrees Celsius after 45 minutes pre-infusion cooling according to the number of chemotherapy cycles

	No head covering		Head covering		*P*-value
	mean	SEM	mean	SEM	
Cycle 1 (*N*= 52)	17	1,1	19	0,6	0,10
Cycle 2 (*N*= 29)	18	1,9	21	1,2	0,15
Cycle 3 (*N*= 22)	17	1,1	20	1,2	0,11
Cycle 4 (*N*= 9)	18	1,8	21	2,5	0,56
Cycle 5 (*N*= 7)	18	3,7	19	3,2	0,86
Cycle 6 (*N*= 7)	20	1,0	20	2,6	1,00
Mean cycle 1-6	18	0,7	20	0,5	0,01

Toxicity and tolerance

Scalp cooling was very well tolerated. A VAS score for tolerance of scalp cooling was performed after 192 cooling procedures, resulting in a mean score of 8 (SD: 1,9). Only one patient stopped scalp cooling because of intolerance after cycle 4. Information about headaches was reported after 194 cooling procedures: in 163 sessions (84%) patients reported no headache; while headache was reported as minimal, moderate or severe in 23 (12%), 4 (2%) and 4 (2%) sessions, respectively. Only fourteen percent of all patients in the study used paracetamol somewhere during their cycles to prevent headaches. No other side effects were reported. (Table 4). No scalp metastases were reported during follow up.

Table 4. Tolerance and side effects of scalp cooling

	N (%)
Tolerance (VAS 0-10[a]) ± SD	8 (±1,9)
Reasons to stop scalp cooling other than hair loss	
Intolerance	1 (2)
Chemotherapy finished or interrupted	23 (37)
Other	4 (6)

[a]0 represents 'not tolerable' and 10 means 'very well tolerable'

DISCUSSION

To our knowledge this is the first study measuring scalp skin temperature during scalp cooling with a Paxman scalp cooling machine to prevent CIA in patients treated with anthracycline containing chemotherapy. Maximal cooling was reached after 45 minutes and was continued for 90 minutes after chemotherapy infusion. Intrapersonal scalp skin temperatures during cooling were consistent for each chemotherapy cycle, but scalp skin temperatures were highly variable between patients, ranging from 10°C to 31°C, resulting in a mean scalp skin temperature of 19ºC. Thirteen out of 62 patients (21%) did not require a wig or other head covering and showed satisfactory hair preservation. These patients appeared to have a significantly lower mean scalp skin temperature (18ºC; SEM: 0,7) during cooling than patients with alopecia (20ºC; SEM: 0,5) (*P*=0,01).

The exact determinants on the efficacy of scalp cooling in the prevention of CIA are unknown(16-18). Factors like the type and dose of chemotherapy can influence the outcome of scalp cooling(16). Based on the results of this study, we can conclude that the temperature of the scalp skin is another important variable related to the efficacy of scalp cooling to prevent hair loss. Patients with good hair retention reached significant lower scalp skin temperatures than patients with hair loss. These results are in line with the study of Gregory et al.(9) who investigated scalp cooling by frozen cryogel packs in 24 patients treated with doxorubicin and vincristine. They observed a consistent temperature in each patient after repeated cooling with maximal cooling after 20 to 30 minutes. Intradermal scalp skin temperature varied from 19 to 29 degrees Celsius with significantly lower temperatures in patients with good hair retention (21 versus 24°C, $P < 0,001$). Other studies on scalp skin temperature were performed in healthy man, not treated with chemotherapy (Table 5). We could not detect a precise threshold temperature below which hair preservation was likely. However, the best results seem to be obtained when the scalp temperature decreases below 18ºC. In reports on scalp cooling, many authors refer to Gregory et al.(9) with 22°C subcutaneously (19ºC epicutaneously) as a threshold temperature which patients have to reach for effective cooling. This cut-off point is based on one study with a limited number of patients treated with chemotherapy regimens with lower dosages than used nowadays and outdated scalp cooling techniques. Our results confirm this cut-off point with modern scalp cooling techniques and adequately dosed chemotherapy regimens.

There is currently no satisfactory explanation for the wide scalp temperature variation between patients. Some persons consistently respond to scalp cooling with only a minor reduction in subcutaneous temperature. This might be due to a greater insulative power of the hairs or dermis(7). It might also be due to enhanced dissipation of heat structures below the subcutaneous tissue, or due to thermal reflex differences with regard to skin perfusion(7). Janssen et al.(19) explain the variation by anatomical differences such as

head shape and thickness of the insulating fat layer. However, Gregory et al.(9) report that the large variation in scalp temperature between patients in their study could not be explained by differences in hair thickness or density of scalp tissue. Although large variations were found between patients in our study, the reached degree of cooling in individual patients was very consistent on repeated cycles of chemotherapy. Differences between patients were therefore not due to changes in the procedure.

Initial hair mass as measured by the Hair Check was not predictive for the severity of hair loss during scalp cooling. Therefore, the efficacy of scalp cooling in preventing CIA is independent of having either thin or thick hair.

Accurate measurement of the scalp skin temperature during scalp cooling is difficult. Needle thermometers can be inserted into the scalp skin to investigate the intradermal temperature(7,9-11,20). These measurements record the exact temperature without bias from the temperature of the cooling cap, but results of different studies are difficult to compare and these intradermal measurements are a burden for patients. Surface temperatures are patient friendly and can be recorded by using thermocouples attached to the skin(7,10-13,20,21). These measurements are easier to compare, but a major disadvantage is the probable influence of the cooling cap. However, Bulow et al.(7) demonstrated a close relationship between the epicutaneous and the subcutaneous temperatures during cooling, indicating that the influence of the cooling cap can be neglected.

The position of the temperature probes on the scalp is poorly described in most studies. Researchers do not indicate in which region of the scalp the probes were placed. When described, the frontal and parietal region are mostly used to measure scalp skin temperature(7,10,13,21). The top of the head is found to be less responsive to cooling(10,13,17). Massey(13) observed that the temperature at the top of the head was 1°C higher than at other places of the scalp. This region is most extensively affected by alopecia as we also observed at images that were taken at different time points in the study (Figure 3). Despite this, there seemed no difference in the need for a head covering between patients with global or patchy hair loss. The probes in our study were placed on the left and right side of the head, which was found to be a temperature-stable region according to Ekwall et al.(10). A third measurement would have been an interesting addition to find out whether the temperature variability between the temporal area and the crown might have been a predicting factor for the requirement of a head covering. Unfortunately, for practical reasons, only the temporal scalp skin temperatures were measured. This is simultaneously a limitation of our study as relation to the crown temperature remains unknown as well as it's prediction for requirement of head covering.

Scalp cooling was very well tolerated (VAS= 8). Nevertheless 15 patients (24%) reported a (mostly mild) headache somewhere during at least one of their cycles.

However, only one patient stopped scalp cooling because of intolerance, which is comparable with the literature(8,13,22). The use of paracetamol as premedication is no standard care in the chemotherapy regimens used in this study. Only fourteen percent of all patients in the study used paracetamol somewhere during their cycles to prevent headaches. This rejects the argument of doctors and nurses who do not to offer scalp cooling because it would be too hard to tolerate(23).

Baseline After 4 cycles FEC

Figure 3: The top of the head is most extensively affected by alopecia

Table 5: Results of studies on skin temperature during scalp cooling

Author	No. of people	Cooling method	Temperature measuring method	Mean temperature	% No head covering	Chemo-therapy regimen
[a]Gregory, 1982(8)	24 patients	Cryogel packs	Invasive	22°C intradermal	42%	Doxo-rubicin, vin-cristin
Bulow, 1985(7)	10 healthy volunteers	Cooling helmet	Invasive and non-invasive	17°C intradermal 20°C intradermal	NA	NA
Hillen, 1990(11)	10 healthy volunteers	Cold air	Invasive and non-invasive	29°C intradermal 14°C epidermal	NA	NA
	11 healthy volunteers	Cryogel packs	Non-invasive	18°C epidermal		
Massey, 2004(13)	3 healthy volunteers	Paxman scalp cooler	Non-invasive	16°C epidermal	NA	NA
Janssen, 2007(12)	9 healthy volunteers	Paxman scalp cooler	Non-invasive	16°C epidermal	NA	NA
Ekwall, 2013(10)	5 healthy volunteers	Dignitana 3°C preset	Non-invasive	17°C epidermal	NA	NA
		Dignitana 8°C preset		23°C epidermal		

[a]Only Gregory et al. included patients in their study and draws conclusions about temperature in relation to the result of scalp cooling

CONCLUSION

When using scalp cooling to prevent anthracycline-induced alopecia, patients with satisfactory hair preservation appeared to have a significantly lower mean scalp skin temperature (18ºC; SEM: 0,7) during cooling than patients with alopecia (20ºC; SEM: 0,5) (*P*=0,01). To obtain optimal results of scalp cooling to prevent chemotherapy induced alopecia, a scalp skin temperature of at least 18°C should be reached. Apart from the type or dose of chemotherapy, the obtained scalp skin temperature during scalp cooling is a very important factor to prevent hair loss. Improvements in scalp cooling machines should focus on possibilities to measure scalp skin temperature and the possibility to adapt cooling temperature in individual patients.

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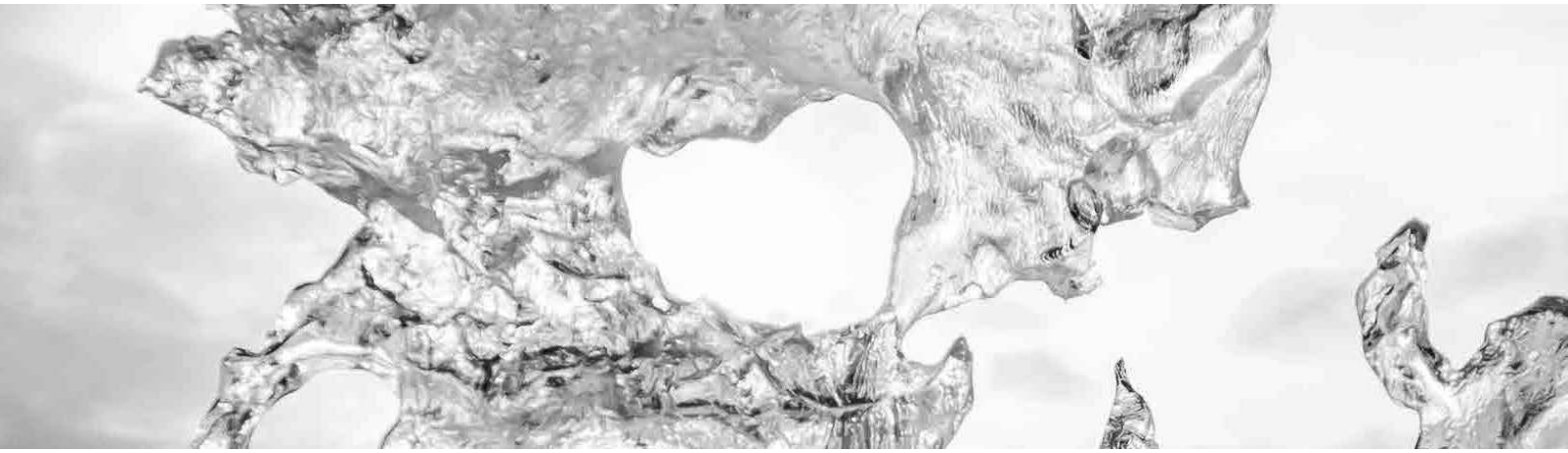
Chapter 4



Results of 20- versus 45-min post-infusion scalp cooling time in the prevention of docetaxel-induced alopecia

Support Care Cancer. 2016, 24:2735-41.

Komen MM, Breed WP, Smorenburg CH, van der Ploeg T, Goey SH, van der Hoeven JJ, Nortier JW, van den Hurk CJ.

ABSTRACT

Purpose: For patients, chemotherapy-induced alopecia (CIA) is one of the most distressing side-effects of treatment. Scalp cooling can prevent or minimise CIA; the results may depend on the duration of cooling. Since a previous study on post-infusion cooling time in patients treated with docetaxel chemotherapy found no difference between 90 and 45 minutes, we investigated whether hair-preserving results could be maintained with a shorter post-infusion cooling time.

Methods: In this prospective multi-centre randomised study, 134 patients who started treatment with docetaxel 75-100 mg/m^2 in a 3-weekly schedule were randomly assigned in a 1:1 ratio to a post-infusion cooling time of 45 minutes or 20 minutes. The primary end-point was the need for a wig or other head covering as assessed by the patient. A visual analogue scale (VAS) with a range from 0 (not tolerable) to 10 (very tolerable) was used to measure tolerance.

Results: Scalp-cooling results were similar for 45 minute and 20 minute post-infusion cooling times. Thirty-three out of 45 patients (73%) treated with 20 minutes of post-infusion cooling did not need a form of head covering, compared with 41 out of 52 patients (79%) treated with 45 minutes of post-infusion cooling (p=0.5). The procedure was well tolerated (mean Visual Analogue Score 8,3). Six patients stopped due to intolerance during the first treatment cycle.

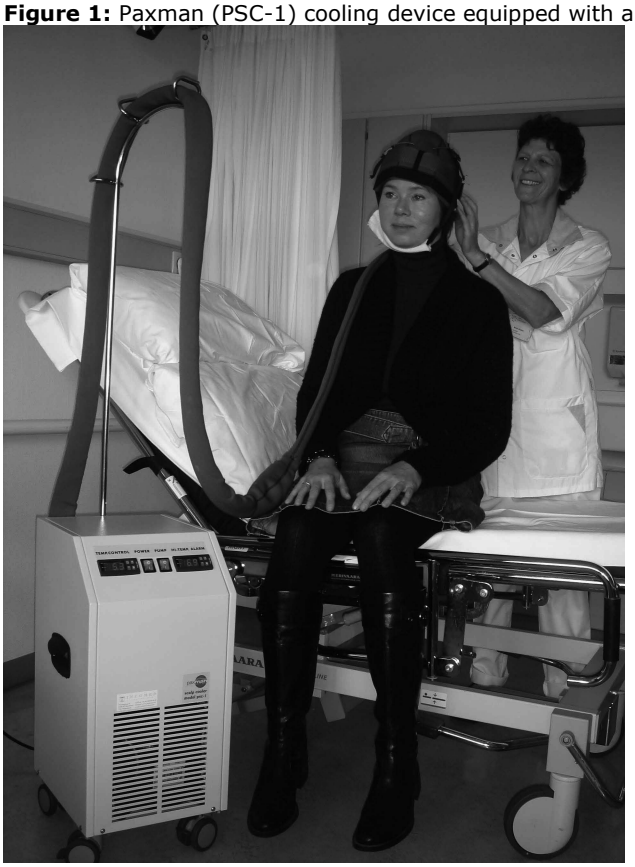
Conclusions: A 20 minute post-infusion cooling time is effective and tolerable for patients treated with scalp cooling to prevent docetaxel-induced alopecia.

INTRODUCTION

For patients chemotherapy-induced alopecia (CIA) is one of the most distressing side-effects of treatment. It has psycho-social implications and may affect body image and acceptance of treatment.[1-4] In the past two decades, a considerable amount of intensive research has been conducted into other chemotherapy related side-effects, such as nausea and fatigue. The treatment of these symptoms has since improved. CIA remains an issue that is difficult to resolve. For some patients CIA is a reason to refuse certain types of chemotherapy.[5;6] Despite the importance of hair loss for patients, alopecia is infrequently mentioned in phase II and III oncology trials. Although CIA is not life threatening and rarely leads to rejection of chemotherapy, it should be incorporated as an important patient-reported outcome measure in pharmaceutical trials. When there are no survival benefits between cytotoxic treatments, quality-of-life issues such as CIA might be decisive.[7]

Scalp cooling is used to prevent CIA (figure 1).[8] It is assumed that it reduces skin temperature, thereby affecting the exposure and metabolism of cytotoxic agents in the hair follicles.[9;10] The hair-preserving effects of scalp cooling are variable, mainly depending on type and dose of chemotherapy and probably on degree and duration of cooling.[10-12] In theory, the scalp should remain cooled until the level of active drug or its metabolites is reduced to sufficiently low levels in the plasma.

Figure 1: Paxman (PSC-1) cooling device equipped with a thermostat



Docetaxel is a semi-synthetic taxoid and is effective against various cancers.[13;14] It is usually administered as a 1-h intravenous infusion repeated every 21 days, at a recommended dose of 75 mg/m2 or 100 mg/m2 depending on tumour type and use as a single or combined agent. Docetaxel-induced alopecia is common at doses higher than 55 mg/m^2 and has been observed in over 80% of patients at doses higher than 70 mg/m^2.[13-17] The pharmacokinetics of docetaxel fit a tri-exponential curve: the α, β and γ half-lives with a 115 mg/m^2 1-h infusion are 4 min, 36 min and 22 h, respectively.[14] As data regarding the concentration and duration of exposure of docetaxel that causes alopecia are lacking, the optimum scalp cooling time remains unclear. In addition, there is considerable intra-individual variation in half-life times among patients who are treated with docetaxel.[14] Consequently, recommendations for scalp-cooling times are often based upon past experience or are arbitrary.[18;19] In daily practice, post-infusion cooling times range from 15 minutes to 4 hours.[11] Scalp cooling has a hair-preserving result in 61-94% of docetaxel-treated patients with a post-infusion cooling time of 15-90 minutes and depends on dose and treatment schedule.[12;19-22]

The duration of post-infusion cooling implies a prolonged stay on the chemotherapy ward; this is potentially a disadvantage both for patients and for the logistics of the clinic. As a previous study on post-infusion cooling time in patients treated with docetaxel chemotherapy found no difference between 90 and 45 minutes, we investigated whether hair-preserving results could be maintained with an even shorter post-infusion time of 20 minutes.[22]

PATIENTS AND METHODS

Patients were enrolled in this prospective multi-centre randomised study between October 2009 and May 2013. The study was approved by an independent ethics committee and institution review board. All procedures were conducted in accordance with the 1964 Helsinki Declaration and its subsequent amendments. Specialised oncology nurses informed patients about the study. Written informed consent was obtained from all individual participants included in the study. Inclusion criteria were that patients were undergoing docetaxel-containing schedules at 3-weekly intervals and were aged 18 or over. Exclusion criteria were treatment with docetaxel in sequential schemes (docetaxel monotherapy after 5-fluouracil, epirubicin, cyclophosphamide (FEC) or after adriamycin, cyclophosphamide (AC)), treatment with docetaxel combined with adriamycin and cyclophosphamide (TAC), alopecia before the start of the study, haematological malignancies and rare disorders like cold sensitivity, cold agglutinin disease, cryoglobulinaemia, cryofibrinogenaemia and cold posttraumatic dystrophy. Eligible patients who chose scalp cooling were randomly assigned to a post-infusion cooling time

of 45 minutes or 20 minutes in a 1:1 ratio. The random sequence was kept by an external centre (Comprehensive Cancer Organisation Netherlands, IKNL, location Eindhoven).

All sixteen participating hospitals used the Paxman one-person cooling machine (PSC-1). The cap was applied according to the instructions for use in the nursing protocol. The temperature of the coolant in the refrigeration tank was -10°C. This temperature is a standard set-up installed by the manufacturer. The pre-cooling time was 30 minutes before the start of the chemotherapy infusion. The cool cap remained on the scalp during the infusion period, 60 minutes being the standard. Scalp cooling was applied during all planned cycles of chemotherapy, unless the patient decided to stop the cooling procedure based on hair loss, side-effects or for other reasons.

The success of scalp cooling was defined in terms of the patient's self-determined need to wear a wig or other head covering (e.g. hat or scarf) to mask visible hair loss after docetaxel treatment. Patients additionally evaluated hair loss on the 4-point scale for alopecia (0=no change, 1=minimal hair loss, 2=moderate, 3=patchy alopecia, 4=complete alopecia) of the World Health Organisation (WHO, offset Publication No. 48).[23] Patients were considered eligible for evaluation of hair preservation if they were treated with at least two cycles of docetaxel chemotherapy or if they discontinued scalp cooling after one cycle due to severe hair loss. Tolerance of scalp cooling was measured by a (self-adapted) Visual Analogue Scale (VAS) of 0-10, in which 0 represented 'not tolerable at all' and 10 meant 'very tolerable'. Patients were also asked whether they experienced other side-effects such as headaches.

Statistical analysis

The primary end-point was the need to wear a wig or other head covering as assessed by the patient. Nominal variables like hair loss, gender, chemotherapy, etc. were analysed using a Chi-square test. Ordinal variables, like the 10-point VAS-scale, WHO and pre- and post-infusion cooling times were analysed using the Mann-Whitney test. Age and follow-up were analysed using a t-test. The analyses were carried out on all randomised patients on an intention to treat (ITT) basis while a secondary analysis was performed on the subgroup of patients receiving at least 2 cycles of chemotherapy and scalp cooling. The power of the test was estimated as 80% with a two-sided α value of 0.05, which indicated a sample size of 40 evaluable subjects in each arm. Based on the number of patients who were not completely evaluable in an earlier multi-centre scalp cooling trial[22], we considered a sample size of 60 patients per arm. Finally 97 out of 134 included patients were evaluable for hair preservation. Data were collected using standard forms, which were compiled into a SPSS database. All tests of significance were

two-sided, and differences were considered statistically significant when P<0.05. All tests were performed using SPSS software (version 20.0) for Windows XP.

RESULTS

In this study a total of 134 patients treated with docetaxel chemotherapy were entered and randomised to a post-infusion cooling time of 45 minutes or 20 minutes. The study was ended when the pre-determined number of enrolled subjects was obtained. Patient characteristics are listed in Table 1. The median age of patients was 64 years. There were no significant differences between the 45 and 20 minute group with respect to clinical characteristics and treatment. Most patients were treated for prostate cancer (43%) and received docetaxel monotherapy (84%). All patients were treated in accordance with the assigned randomisation. The median pre-infusion cooling time was 33 minutes (IQR 15).

Table 1. Patient characteristics

	20 minutes post infusion cooling time (n=64)	45 minutes post infusion cooling time (n=70)	*P* value
Mean age, years (range)	64 (43-82)	64 (25-83)	1.0
Gender			0.3
Male	36 (56%)	46 (66%)	
Female	28 (44%)	24 (34%)	
Cancer			0.6
Breast	17 (27%)	20 (28%)	
Lung	13 (20%)	9 (13%)	
Prostate	26 (41%)	32 (46%)	
Gastro-intestinal	1 (1%)	1 (1%)	
Other	2 (3%)	2 (3%)	
Missing	5 (8%)	6 (9%)	
Chemotherapy			0.7
Docetaxel monotherapy	55 (86%)	58 (83%)	
Docetaxel combination therapy*	6 (9%)	8 (11%)	
Missing	3 (5%)	4 (6%)	
Setting			0.8
Curative	5 (9%)	6 (10%)	
Palliative	53 (91%)	54 (90%)	
Median number of cycles with scalp cooling	5	5	

* Docetacel combined with doxorubicin, cyclophosphamide, gemcitabin, carboplatin or capecitabine
** Chi-square results are invalid. because of cell counts less than 5

At the time of data analysis (April 8, 2014), the median follow-up for patients in the 20 minute group was 6.5 months and 7.5 months for the 45 minute group (Table 2). Thirty-seven patients were not evaluable for hair preservation. Five patients stopped due to intolerance during the first chemotherapy cycle, 21 patients stopped chemotherapy before completing the second cycle, one patient died before hair loss could be reported, three patients decided to leave the study after randomisation, one patient was not

treated with docetaxel, one patient withdrew informed consent and five questionnaires could not be retrieved.

Table 2. Tolerance, side-effects and follow up

	20 min minutes post infusion cooling time (n=64)	45 min minutes post infusion cooling time (n=70)	*P* value
Tolerance (VAS 0-10*) ± SD	8.6 ±1.4	8.0 ±9.6	0.1
Headache (any grade)	19 (31%)	19 (29%)	1.0
Reasons to stop scalp cooling other than hair loss			**
Intolerance	1 (2%)	5 (7%)	
Chemotherapy finished or interrupted	46 (72%)	50 (71%)	
Other	8 (13%)	10 (14%)	
Median follow up, months (IQR)	6.5 (6.3)	7.5 (6.9)	0.7

*0 represents 'not tolerable' and 10 means 'very well tolerable'
**Chi-square results are invalid because of cell counts less than 5

In this study 97 patients were evaluable for hair preservation (Table 3). There was no significant difference in need to wear head covering in the 20 minute group compared to the 45 minute group (20 min, n=33/45 (73%) no head covering; 45 min, n=41/52 (79%) no head covering; p=0.5). A significant difference in need to wear head covering was seen when the dosages of docetaxel were compared (75 mg/m2, n=5/65 (8%); 100 mg/m2, n=14/25 (57%); p<0.0001) and gender (male, n= 59/62 (95%); female, n= 15/35 (43%); p<0.0001) (Table 4).

Table 3. Response to scalp cooling

	20 min minutes post infusion cooling time (n=64)	45 min minutes post infusion cooling time (n=70)	*P* value
Evaluable for scalp cooling	45	52	0.5
Patients with head covering	12/45 (27%)	11/52 (21%)	
Patients without head covering	33/45 (73%)	41/52 (79%)	
Not evaluable	19 (30%)	18 (26%)	
WHO* for alopecia			0.8
0	22 (46%)	24 (45%)	
1	18 (37%)	18 (34%)	
2	7 (15%)	8 (15%)	
3	1 (2%)	3 (6%)	

* WHO, offset Publication No.48[23]

Table 4. Efficacy of scalp cooling depending on type and dosage of chemotherapy and gender

	No head covering	Head covering	*P* value
Chemotherapy			*
Docetaxel monotherapy	67 (78%)	19 (22%)	
Docetaxel combination therapy	7 (70%)	3 (30%)	
Docetaxel/ gemcitabin	1	0	
Docetaxel/ carboplatin	5	2	
Docetaxel/ capecitabin	1	0	
Docetaxel/ cyclophosphamide	0	1	
Dosage			<0.0001
75 mg/m^2	60 (92%)	5 (8%)	
100 mg/m^2	11 (44%)	14 (56%)	
Gender			<0.0001
Male	59 (95%)	3 (5%)	
Female	15 (43%)	20 (57%)	

*Chi-square results are invalid because of cell counts less than 5

During the follow-up period information on hair status was not available while scalp metastases were not reported. Scalp cooling was well tolerated. A VAS score for tolerance of scalp cooling was performed 471 times, resulting in a mean score of 8.3 (Table 2). Information concerning headache was reported 488 times: in 417 (85%) sessions, patients reported no headache; in 55 sessions (11%) minimal; in 14 (3%) moderate; and in 2 (0.4%) sessions patients reported severe headaches. No other side-effects were reported.

DISCUSSION

In the present study 74 out of 97 patients (76%) treated with scalp cooling did not need a head covering to cover visible hair loss after docetaxel chemotherapy, while no difference was found between 45 and 20 minute post-infusion cooling time. As mentioned, a previous study on scalp cooling to prevent docetaxel-induced alopecia found no difference between 90 and 45 minutes post infusion cooling times.[22] Of all the patients in that study, 84% (n=129) did not wear a head covering. Twenty minutes can therefore be advised as a standard post-infusion cooling time for patients treated with 3-weekly docetaxel-containing chemotherapy. This is a major benefit for both patients and hospitals, as patients can be discharged earlier and since the stay in hospital is shorter, more patients can be treated.

Hair preservation was very good, which is in accordance with the results of other scalp-cooling studies. Results were better at lower docetaxel dosages as also observed in other such studies.[12;19-22;24-26] However, because there was no significant difference between the 45 and 20 minute group with respect to dosage, conclusions on shortening post-infusion cooling times remain valid.

Scalp cooling was very well tolerated (VAS= 8.3). Nevertheless 38 patients reported a (mostly mild) headache somewhere during at least one of their cycles. Five

patients (5.3%) stopped scalp cooling because of intolerance, which is comparable with findings in the literature.[10;18;22] This refutes the argument of some doctors and nurses who do not offer scalp cooling because it would be too hard to tolerate.[27]

Comparing studies concerning hair preservation is complicated by the lack of a standardised methodology for evaluating hair loss.[28-30] To assess hair loss, the authors chose from among several widely accepted scales, such as the World Health Organisation (WHO) classification of chemotherapy-induced alopecia, the Common Terminology Criteria for Adverse Events (CTCAE) and Visual Analogue Scales (VAS). In the present study we asked patients whether a wig or head cover was used and we measured the degree of hair loss according to the WHO criteria. Recently, an objective method has become available to measure hair quantity: the cross-section trichometer [31], a very promising technology for research purposes.[32] However, the use of a wig or head cover as a parameter for patient satisfaction should remain the most important clinical criterion for the success of scalp cooling.[8] In future studies concerning scalp cooling and hair preservation, the use of a cross-section trichometer combined with previously used methods is strongly recommended.

In this study 61% of patients were male. Contrary to prevailing assumptions, men also describe negative feelings about hair loss. Men's experiences have been largely ignored and healthcare professionals should spend more time assisting men with adjustment to CIA.[33] Men are often treated with docetaxel, a regimen in which the results of scalp cooling proved to be very good (figure 2). In this study 95% of male patients did not need a head covering, against 43% of female patients. This is in agreement with previous findings.[19] However, the result in males may be overestimated, since men are in general less inclined to wear a wig or head covering. As upfront chemo-hormonal therapy for metastatic prostate cancer seems to improve overall survival, even more men will be treated with docetaxel in future.[34] It would be a major improvement if all men undergoing docetaxel chemotherapy were informed about the highly protective effect of scalp cooling in preventing CIA.

Although the numbers are small, we can formulate a concrete recommendation on post-infusion cooling time for patients treated with docetaxel-containing schedules at 3-weekly intervals. The pre-cooling time should remain at 30 minutes before the start of the chemotherapy infusion. The post-infusion cooling time can be adapted from 45 minutes to 20 minutes.

Another interesting aspect for investigation might be scalp cooling without a post-infusion cooling time for cytotoxics with rapid clearance. Scalp cooling decreases the exposure of hair follicles to chemotherapy, but might also unnecessarily suppress the hair repair mechanism.[22;35]

Figure 2: Results of scalp cooling in a male patient who was treated with docetaxel

Cycle 1 Cycle 6

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Declaration of interest: the authors declare that they have no conflict of interest.

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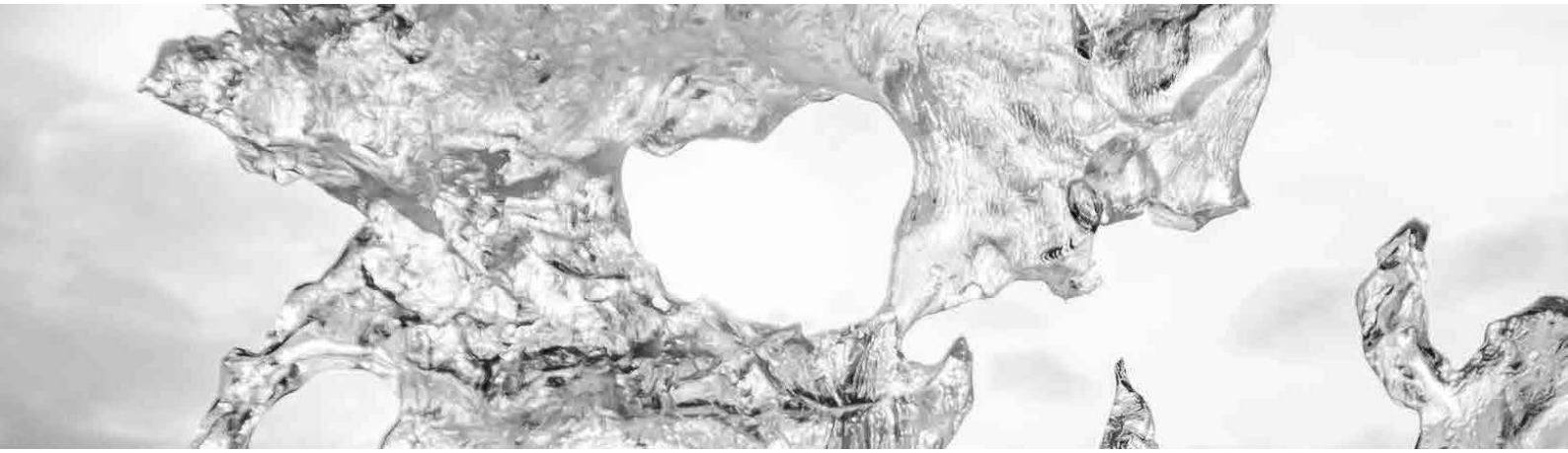
Chapter 5



Prolonging the duration of post-infusion scalp cooling in the prevention of anthracycline induced alopecia: a randomized trial in patients with breast cancer treated with adjuvant chemotherapy

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ABSTRACT

Purpose: Scalp cooling as a method to reduce the incidence of chemotherapy induced alopecia (CIA) is increasingly used in daily practice worldwide. However, in patients treated with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC), scalp cooling fails in 48-67% of patients. This study investigated the efficacy of extended duration of post-infusion scalp cooling in breast cancer patients treated with this regimen.

Methods: In this prospective multi-centre randomised study, 102 patients with early breast cancer treated with adjuvant FEC chemotherapy were randomly assigned in a 1:1 ratio to a post-infusion cooling time of 90 or 150 minutes. The primary endpoint was the need to wear a wig or other head covering to mask visible hair loss.

Results: Sixteen out of 48 patients (33%) treated with 90 minutes of post-infusion cooling did not need any head covering, compared with 21 out of 46 patients (45%) treated with 150 minutes of post-infusion cooling (p=0.2). WHO grade 2-3 (moderate-complete) alopecia was reported more often in patients treated with 90 minutes post-infusion cooling time (n= 25/51 (49%) versus n=17/51 (33%); p=0,02). Scalp cooling was well tolerated (mean Visual Analogue Score 7.4) and only three patients (3%) stopped due to intolerance during treatment.

Conclusions: Extending the duration of 90 minutes post-infusion scalp cooling to 150 minutes in patients treated with adjuvant FEC chemotherapy was well tolerated but did not significantly diminish the need for head covering. However, grade 2-3 alopecia was seen less often with prolonged post-infusion scalp cooling.

INTRODUCTION

As there is a growing awareness for optimal supportive care in patients with cancer, research increasingly focusses on minimizing side effects of chemotherapy to improve quality of life. [1] The social and psychological consequences of chemotherapy induced alopecia (CIA) are obvious to everyone and may affect body image and acceptance of treatment. [1-4] Scalp cooling as a method to reduce the incidence of CIA is increasingly being used in daily practice worldwide. [5-7] The mechanism of scalp cooling during treatment with chemotherapy is based on the theory that reducing the scalp skin temperature during the administration of chemotherapy affects the exposure to and metabolism of cytotoxic agents in the hair follicles. [8, 9] The hair-preserving effects of scalp cooling are highly variable, mainly depending on type and dose of chemotherapy and probably also on the temperature and duration of cooling. [9-12] Scalp cooling has only limited beneficial effect in patients who are treated with anthracyclines.[13]

Epirubicin, a frequently used anthracycline, is a semisynthetic derivate of doxorubicin and has a wide range of antitumor activity. [14, 15] Being effective in the treatment of breast cancer it is frequently used as adjuvant therapy in patients with early breast cancer or palliative chemotherapy for metastatic disease. [15] The drug may be administered alone or in combination with other agents. In the adjuvant setting for breast cancer, a commonly used anthracycline-containing combination chemotherapy regimen is 5-fluorouracil together with epirubicin and cyclophosphamide (FEC). [14] Standard dose of the FEC regimen consists of fluorouracil 500-600 mg/m^2, epirubicin 90-100 mg/m^2 and cyclophosphamide 500-600 mg/m^2 administered intravenously once every 3 weeks. A very common side effect of this regimen is complete alopecia. [14] Theoretically, the duration of scalp cooling after the infusion of chemotherapy should be related to pharmacokinetics of exposure to the cytostatic agent and its active metabolites. [9, 11] The pharmacokinetics of epirubicin fit a tri-exponential curve with half-lives for the initial (α), intermediate (β) and terminal (γ) elimination phases of approximately 3 minutes, 1 hour and 30 hours respectively [14], but show considerable inter-individual variation. [15] Consequently, recommendations for post-infusion scalp-cooling times are often based upon past experience or are arbitrary. [13, 16] Indeed, in daily practice post-infusion cooling times range from 15 minutes to 4 hours. [10] In the Netherlands a duration of 90 minutes post-infusion cooling time has been arbitrarily chosen as the standard post-infusion cooling time for any chemotherapy regimen. FEC chemotherapy is frequently used as adjuvant treatment in patients with breast cancer and scalp cooling is increasingly being used in this setting to prevent CIA. However, scalp cooling fails in 48-67% of patients treated with this chemotherapy regimen. [13] To investigate whether the efficacy of scalp cooling could be improved by a longer post-

infusion time we compared a post-infusion cooling time of 150 minutes versus 90 minutes in patients treated with adjuvant FEC chemotherapy.

METHODS

Patients

The study enrolled female patients with primary breast cancer, aged 18 or older. They were planned for a minimum of three cycles FEC chemotherapy with an epirubicin dose of 90-100 mg/m^2 at 3-weekly intervals and were willing to use scalp cooling to prevent CIA. Patients with alopecia before the start of the study were excluded from the study. Also excluded were patients with (concomitant) haematological malignancies or contraindications for scalp cooling such as cold sensitivity, cold agglutinin disease, cryoglobulinaemia, cryofibrinogenaemia or cold posttraumatic dystrophy.

Study design

We conducted a prospective multi-centre randomised study in seven hospitals in the Netherlands. The primary endpoint of this study was the need to wear a wig or other head covering to mask visible hair loss. The severity of hair loss was evaluated on the 4-point scale for alopecia (0=no change, 1=minimal hair loss, 2=moderate, patchy alopecia, 3=complete alopecia) of the World Health Organisation. [17] Tolerance of scalp cooling was measured on a 1-10 Visual Analogue Scale (VAS), with 10 being the most tolerable. Other side-effects such as headaches were also recorded. Patients were considered eligible for final analyses if they were treated with at least two cycles of FEC chemotherapy or if they discontinued scalp cooling after one cycle due to severe hair loss. Patients were randomly assigned to a post-infusion cooling time of 90 minutes or 150 minutes with the allocation ratio of 1:1 (Figure 1). The random sequence was carried out following a predefined randomization schedule by an external independent centre (Netherlands Comprehensive Cancer Network (IKNL)). Each institutional review board approved the study before participants were enrolled. All procedures were conducted in accordance with the 1964 Helsinki Declaration and its later amendments. Patients were informed about the study by specialised oncology nurses. All patients gave written informed consent prior to enrolment and randomization.

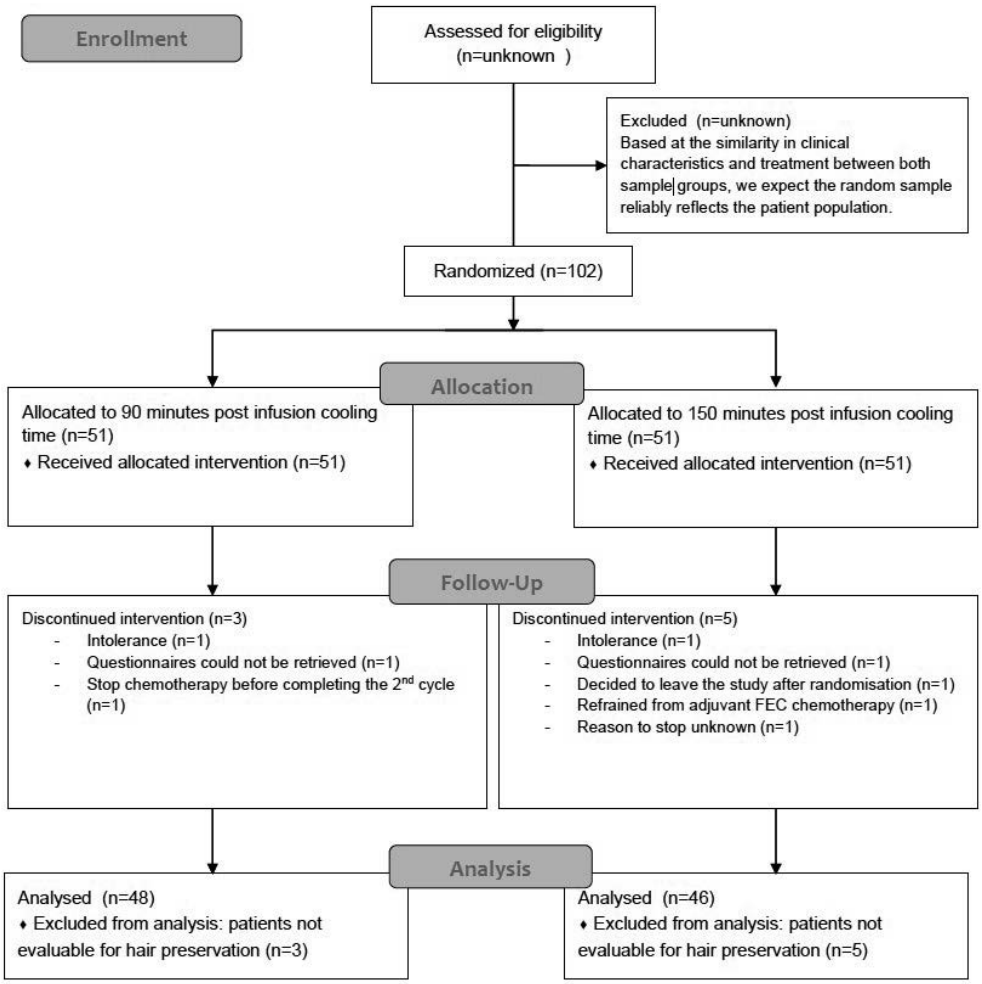

Figure 1. Consort 2010 flow diagram

Intervention

The Paxman one-person cooling machine (PSC-1), with a standard temperature of -10°C was used in this study by all participating hospitals. Oncology nurses applied the cool cap according to the instructions for use in the nursing protocol. The pre-cooling time was 30 minutes before the start of the chemotherapy infusion and the cool cap remained on the scalp during the infusion period of 60 minutes. Scalp cooling was applied during all planned cycles of chemotherapy, unless the patient decided to stop the cooling procedure because of hair loss, side-effects or for patients' preference.

Sample size and statistical analysis

The sample size was calculated for the different cooling times. A power and sample size program was used to estimate the sample size (PS: Power and sample size calculation). [18] The primary endpoint was the need to wear a wig or other head covering to mask visible hair loss. Based on data from our registration study, the risk of hair loss for patients treated with the standard scalp cooling was approximately 50%. We assumed

that an improvement of at least 30% in the outcome of scalp cooling would be clinically relevant to justify the burden of a prolonged post infusion scalp cooling time of 150 minutes. With a power set at 80%, 30% difference could be detected by 44 patients in each randomisation arm. Based on the expected drop-out after inclusion, 51 patients per arm were included. All outcomes were analysed with two-tailed tests at $\alpha=0.05$, and differences were considered statistically significant when $p<0.05$. Analyses were performed using SPSS software (version 20.0) for Windows XP. Patient and treatment characteristics were analyzed using a Chi-square test. Response to scalp cooling and tolerance were analyzed using the Mann-Whitney test. Age and follow-up were analysed using a t-test. The analyses were carried out on all randomised patients on an intention-to-treat (ITT) basis, while a secondary analysis was performed on the subgroup of patients receiving at least 2 cycles of chemotherapy and scalp cooling.

RESULTS

Patient and treatment characteristics

Between March 2007 and July 2015 a total of 102 female patients were randomised to a post-infusion cooling time of 90 or 150 minutes. All patients were treated in accordance with the assigned randomisation of post-infusion duration of scalp cooling. Patient and treatment characteristics are listed in table 1. The mean age was 52 years. Thirty-six out of 102 patients (35%) were treated with 5 cycles FEC with an epirubicin dose of 90 mg/m^2, 35 patients with 6 cycles FEC with an epirubicin dose of 100 mg/m^2, and 31 patients with 3 cycles FEC (epirubicin dose 100 mg/m^2) followed by 3 cycles docetaxel. There was no significant difference between patients in the 90 and 150 minutes group with respect to treatment characteristics. All patients were treated conform the study protocol with scalp cooling during FEC chemotherapy, for a median of 4 cycles. At the time of the data cut-off (December 7, 2015), the median follow-up of patients was 73 months.

Table 1. Patient and treatment characteristics

	90 minutes post infusion cooling time (n=51)	150 minutes post infusion cooling time (n=51)	*P* value
Mean age, years (range)	51 (30-72)	52 (40-69)	
Chemotherapy (type)			1.0
5x FEC	18 (35%)	18 (35%)	
6x FEC	17 (33%)	18 (35%)	
3x FEC followed by 3x TXT	16 (32%)	15 (30%)	
Chemotherapy (dose)			0.7
F(500[a])E(90[a])C(500[a])	21 (41%)	19 (37%)	
F(500[a])E(100[a])C(500[a])	30 (59%)	32 (63%)	
Median number of cycles with scalp cooling ± SD	3 ± 0,2	5 ± 0,3	

[a] mg/m^2

Efficacy analysis

In this study eight patients (7.8%) were not evaluable for hair loss. Two patients stopped scalp cooling due to intolerance before the second cycle was completed, one patient refrained from adjuvant FEC chemotherapy, one patient stopped chemotherapy before the second cycle was completed, one patient decided to leave the study after randomisation, two questionnaires could not be retrieved and in one patient the reason to stop was unknown.

Finally, a total of 94 out of 102 included patients was evaluable for hair preservation (Table 2). Thirty-seven out of 94 evaluable patients (40%) did not need to wear a wig or other head covering to mask hair loss during their therapy. There was no significant difference in the proportion of patients who wore a wig or head cover between the 90-minutes group and the 150-minutes group (n= 16/48 (33%) versus n= 21/46 (45%); p=0.2). WHO-score for alopecia grade 2-3 (moderate-complete) was reported significantly more often in patients treated with 90 minutes post-infusion cooling time (n= 25/51 (49%) versus n=17/51 (33%); p=0,02). The planned number and type of adjuvant chemotherapy (5x FEC, 6x FEC or 3x FEC followed by 3x docetaxel) did not correlate with the need to wear head covering (p= 0.08). The need to wear head covering was 47% in patients treated with epirubicin at a dose of 90 mg/m2 compared to 69% in patients treated with 100 mg/m2 (p=0.04) (Table 3).

Table 2. Response to scalp cooling

	90 minutes post infusion cooling time (n=51)	150 minutes post infusion cooling time (n=51)	*P* value
Evaluable for scalp cooling efficacy	48	46	
Head covering			0.2
Patients with head covering	32 (67%)	25 (55%)	
Patients without head covering	16 (33%)	21 (45%)	
Not evaluable	3 (6%)	5 (10%)	
WHO[a] for alopecia			0.02
0-1	10 (20%)	23 (45%)	
2-3	25 (49%)	17 (33%)	
missing	16 (31%)	11 (22%)	

[a] WHO, offset Publication No.48 [17]

Table 3. Efficacy of scalp cooling depending on type and dosage of chemotherapy in 94 evaluable patients

	No head covering (n=48)	Head covering (n=46)	*P* value
Chemotherapy (type)			0.08
5xFEC	18 (55%)	15 (45%)	
6xFEC	10 (32%)	21 (68%)	
3x FEC followed by 3x TXT	9 (30%)	21 (70%)	
Chemotherapy (dose)			0.04
90 mg/m^2	19 (53%)	17 (47%)	
100 mg/m^2	18 (31%)	40 (69%)	

Tolerance and safety analysis

Scalp cooling was well tolerated, irrespective of the post-infusion duration (Table 4). Tolerance of scalp cooling, recorded by a VAS score was performed after 322 cooling procedures, and resulted in a mean score of 7,4 (SD: 2,1) There were 3 patients (3%) who stopped scalp cooling because of intolerance. Side effects such as headaches were recorded during 327 cooling procedures: in 238 sessions (73%) no headaches were reported; while headache was reported as mild, moderate or severe in 66 (20%), 20 (6%) and 3 (1%) sessions, respectively. There were no scalp metastases reported during the follow up period.

Table 4. Tolerance, side-effects and follow up

	90 minutes post infusion cooling time (n=51)	150 minutes post infusion cooling time (n=51)	*P* value
Tolerance (VAS 0-10[a]) ± SD	7,5 ±2,0	7,3 ±2,2	0.8
Reasons to stop scalp cooling other than hair loss			[b]
Intolerance	1 (2%)	2 (4%)	
Chemotherapy finished or interrupted	25 (49%)	27 (53%)	
Other	1 (2%)	5 (10%)	
Median follow up, months (range)	74 (15-106)	72 (8-108)	1.0

[a]0 represents 'not tolerable' and 10 means 'very well tolerable'
[b]Chi-square results are invalid because of cell counts less than 5

DISCUSSION

In this randomized study performed in patients with early breast cancer treated with adjuvant chemotherapy with the FEC regimen, prolonging of the duration of 90 minutes post-infusion scalp cooling to 150 minutes was well tolerated but did not significantly diminish the need for head covering. However, grade 2-3 alopecia was observed less often with prolonged post-infusion scalp cooling.

Thirty-seven out of 94 evaluable (40%) patients were successfully treated with scalp cooling to prevent CIA, corresponding with the results (33-52%) of FEC high dose chemotherapy in a large registry study on scalp cooling. [13]

Our study has some limitations and may have been underpowered. At the start of the study, the standard chemotherapy regimen in the Netherlands for patients with primary breast cancer was 5 cycles FEC at an epirubicin dose of 90 mg/m^2. Based on international guidelines, treatment with 6 cycles FEC at a dose of 100 mg/m^2 was also given in some centers. However, there was no data available on the positive effect of scalp cooling at this higher dose. Therefore, the effect of scalp cooling in the FEC regimen at a dose of 90 mg/m^2, as was available from our registration data (50% efficacy), was used for power calculation. At present, after extension of our registry, the estimation of scalp cooling efficacy in the FEC regimen with epirubicin 90 mg/m^2 proved to be correct (52%

efficacy). However, we found that the efficacy of scalp cooling in the FEC regimen with epirubicin 100mg/m^2 is 33%.[13] Therefore, a significant difference in efficacy might have been missed.

In our study, grade 2-3 alopecia was seen less often with prolonged post-infusion scalp cooling. Therefore, we cannot exclude a clinical meaningful difference between the two post-infusion cooling times. This should be explored further in studies with a larger sample size and quantitative methods to measure the degree of hair loss. However, even if the effect of 150 minutes post-infusion cooling would indeed be superior in a larger study, one should consider the long stay in the hospital which is less feasible for both patients and nurses and goes with increased costs for hospitals due to extended duration of nursing time and stay at the chemotherapy unit.

The follow up in this study is very long (73 months, range 8-108 months), and can be explained by the long inclusion period of the study. Contrary to our expectations, it was difficult to motivate patients to be randomized between 90 and 150 minutes post infusion cooling time. This extended the period of inclusion in which the dose of FEC initially changed from 90 mg/m^2 to 100 mg/m^2 and later changed to a sequential schedule of three courses FEC with 100 mg/m^2 epirubicin, followed by three courses docetaxel mono-therapy of 100 mg/m^2.

In recent years the Hair Check method has become available for objective hair loss measurement. [19] An objective and more sensitive method of measuring hair loss may especially be of value in the research on refining scalp cooling techniques to prevent CIA. However, patient reported outcome as a parameter for patient satisfaction remains the most important clinical criterion for the efficacy of scalp cooling. In order to compare scalp cooling outcomes, preferably a combination of a subjective clinical scale and an objective method like the Hair Check should be used, at least in clinical studies.

Several reports on the anthracycline pharmacokinetics point out the large inter-individual variations and the need for individualization of the doses based on measured plasma concentrations. [20, 21] Eksborg et al. measured that an increase in maximum plasma concentration of epirubicin was associated with an increasing degree of alopecia. [22] Therefore it might be interesting to investigate whether adapting the post-infusion cooling time to the maximum plasma concentration could improve scalp cooling outcomes.

The procedure of scalp cooling was very well tolerated (VAS=7.4) and independent of duration of post-scalp cooling. Nevertheless, 48 patients reported any grade headache (mostly mild) during at least one of their cycles. Only 3 patients stopped scalp cooling because of intolerance, both in line with the literature. [6, 9, 12, 16, 23, 24]

In conclusion, our study did not significantly contribute to an overall favourable clinical effect of scalp cooling in reducing the use of head covering. However, it demonstrated that prolonging the post-infusion cooling time of the FEC (90-100mg) regimen did show some reduction of grade 2/3 alopecia after 150 minutes.

Conflict of interest

The authors declare that they have no conflict of interest.

The authors have full control of all primary data and agree to allow the journal to review their data if requested.

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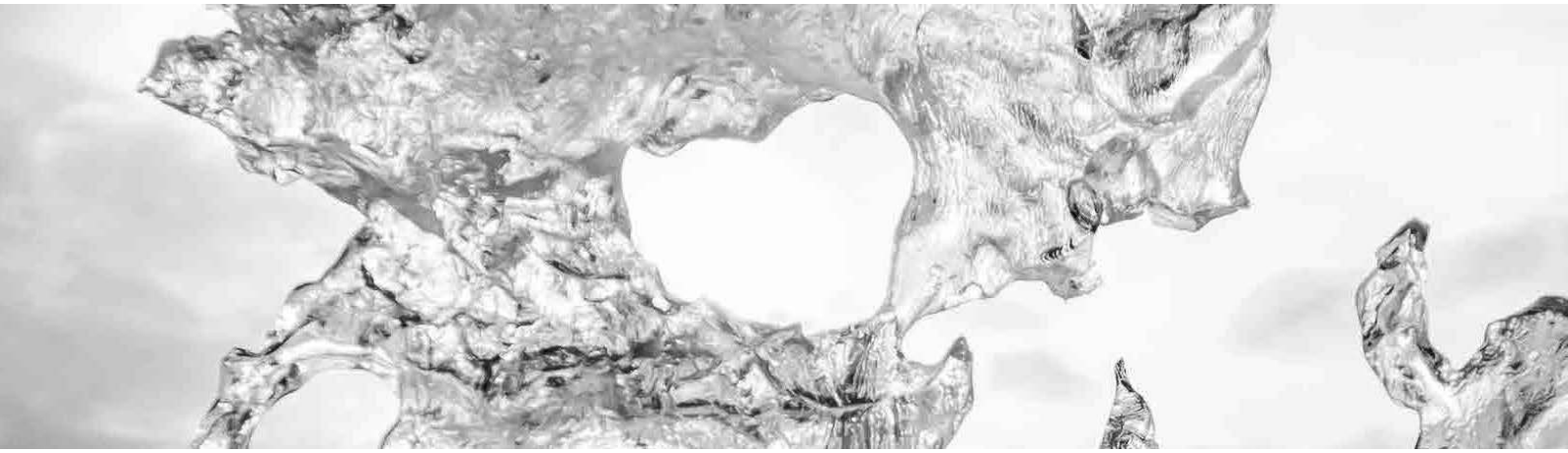
Chapter 6



Patient-reported outcome assessment and objective evaluation of chemotherapy-induced alopecia

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ABSTRACT

Purpose: Alopecia is one of the most distressing side effects of chemotherapy. Evaluating and comparing the efficacy of potential therapies to prevent chemotherapy-induced alopecia (CIA) has been complicated by the lack of a standardized measurement for hair loss. In this study we investigated the correlation between patient-reported outcome assessments and quantitative measurement with the hair check to assess CIA in clinical practice.

Method: Scalp cooling efficacy was evaluated by patients by World Health Organisation (WHO) of CIA, Visual Analogue Scale (VAS) and wig use. The Hair Check was used to determine the amount of hair (in mm^2) per unit of scalp skin area (in cm^2) (Hair Mass Index, HMI). CIA was also evaluated by doctors, nurses and hairdressers.

Results: Baseline HMI was not predictive for hair loss. HMI declined throughout all chemotherapy cycles, which was not reflected by patient-reported measures. HMI correlated with patient-reported hair quantity before the start of the therapy, but not with WHO and/or VAS during therapy. Patient's opinion correlated moderately with the opinion of doctors and nurses (ρ=0.50-0.56 respectively), but strongly with hair dressers (ρ=0.70).

Conclusions: The Hair check is suitable to quantify the amount of hair loss and could complement research on refining outcome of scalp cooling, but the patient's opinion should be considered as the best method to assess hair loss in clinical practice.

INTRODUCTION

Alopecia is one of the most distressing side effects of chemotherapy and may have an impact on treatment decisions. (Batchelor, 2001, Rosman, 2004, Hesketh et al., 2004, Mols et al., 2009) Scalp cooling is a treatment option to prevent chemotherapy-induced alopecia (CIA)[1]. (Nangia et al., 2017, Rugo et al., 2017) It is assumed that scalp cooling works by inducing vasoconstriction and reduction of metabolism. Vasoconstriction leads to reduced blood flow to the hair follicles during the time period of peak plasma concentrations of the relevant chemotherapeutic agent. In addition, reduced metabolic activity could make hair follicles less vulnerable to the damage of cytotoxic agents. Both randomized and nonrandomized studies proof that scalp cooling can prevent CIA. (Breed et al., 2011, Grevelman and Breed, 2005, Rugo et al., 2017, Nangia et al., 2017) However, comparing or pooling data on the efficacy of scalp cooling between studies has been complicated by the lack of a standardized methodology to evaluate hair loss. (Van Neste, 1999, Van Neste, 2002, Chamberlain and Dawber, 2003, van den Hurk et al., 2015)

Methods to measure the severity of chemotherapy-induced hair loss can be categorised as invasive, semi-invasive and non-invasive. Invasive and semi-invasive measurements like scalp skin-biopsies and hair root analysis are objective, but can be unpleasant for patients and are costly and time consuming. (Chamberlain and Dawber, 2003, Van Neste, 2002, Van Neste, 1999, Canfield, 1996, Donati et al., 2011) Non-invasive techniques like photography or counting shed hairs could also be useful in assessing the severity of hair loss. (Chamberlain and Dawber, 2003, Van Neste, 2002, Donati et al., 2011, Massey, 2004, Peck et al., 2000, Ridderheim et al., 2003) Photography may be used to compare the difference in visible hair loss during treatment, but it is subjective and does not generate a reliable estimation for hair loss on a localized area of the scalp. Hair counts do generate a quantitative value, although they also do not provide information about hair loss on a localized area of the scalp. (Cohen, 2008) In scalp cooling studies, several widely accepted subjective scales have been used to assess hair loss, such as the World Health Organisation (WHO) classification of chemotherapy-induced alopecia (World Health Organisation, 1979), the Common Terminology Criteria for Adverse Events (CTC-AE) (U.S.Department of Health and Human Services, 2009) or Visual Analogue Scale (VAS) (Ridderheim et al., 2003) In addition, other measurements like Dean's alopecia scale (grade 1: <25% hair loss; grade 2: 25%-50% hair loss; grade 3: 50%-75% hair loss, grade 4: >75% hair loss), various Likert scales (rating scales) and pictorial assessments have been described in literature on scalp cooling. (van den Hurk et al., 2015) For study purposes, there is a need for an operator- and patient friendly, inexpensive, and validated method for measuring hair quantity. Until recently

[1] CIA chemotherapy-induced alopecia

there was no reliable, simple method available to measure hair quantity in an objective way, but in recent years the Hair Check method has become available. (Cohen, 2008) The Hair Check is a mechanical device that compresses a bundle of hair in a disposable cartridge from a delineated area of the scalp and measures its cross-sectional area. In this way, the amount of hair (in mm^2) per unit of scalp skin area (in cm^2) (Hair Mass Index, HMI)[2] can be defined. (Hendriks et al., 2012) In a study to test the clinical utility of the Hair Check in healthy volunteers it was concluded that measurements were simple to perform, and data showed high reproducibility. (Hendriks et al., 2012) We therefore decided to investigate the correlation between patient reported outcome assessments and the quantitative method of HMI measurement to assess the amount of hair loss in patients treated with chemotherapy. In addition, we studied the correlation between the opinion of patients, doctors, nurses and hairdressers assessed with subjective methods.

PATIENTS AND METHODS

Patients

The study enrolled patients with primary invasive breast cancer without distant metastasis planned for treatment with three to six cycles of combination chemotherapy at 3-weekly intervals with FEC (5-fluorouracil, epirubicin, cyclophosphamide) or AC (adriamycin, cyclophosphamide). Subsequent chemotherapy cycles consisting of docetaxel monotherapy were allowed after 3 FEC cycles. Patients were excluded if they lacked basic proficiency in Dutch, if they were unable to understand the patient information brochure or if they suffered from cold sensitivity, cold agglutinin disease, cryoglobulinemia, cryofibrinogenemia or cold posttraumatic dystrophy.

Study design

We conducted an explorative prospective single-centre study between August 2010 and January 2014 at the department of Internal Medicine of the Medical Centre Alkmaar, the Netherlands. At baseline, patient characteristics were collected. Before the start of the chemotherapy, patients were asked to rate their hair quantity (much, moderate, little hair).

Objective hair quantity was measured with a Hair Check before each chemotherapy cycle (table 1). The mechanical device compresses a bundle of hair in a disposable cartridge from a delineated area of the scalp and measures its cross-sectional area. HMI incorporates both density and diameter and was measured at both temporal sides. (Cohen, 2008) The validity of the device was tested using bundles of hair and surgical silk fibres. It showed a high degree of precision and it was concluded that the device could be used as a reliable substitute for the methods that are presently used to

[2] HMI Hair Mass Index

measure hair loss.(Cohen, 2008) Hendriks et al. designed a study to test the clinical utility and reproducibility of the Hair Check. Data in this study showed high reproducibility. For intra-observer reproducibility, the mean difference was .2 (95% confidence interval (CI)= -4.7-5.1, correlation coefficient (*r*) =.99). For interobserver reproducibility, the mean difference was -.4, 95% CI = -8.0-7.2, *r*=.97. (Hendriks et al., 2012) To define the measuring location, a location strip was used and marked using a four-legged marking template moistened with red ink (figure 1). The hair bundle within this marked area was measured using the Hair Check.

Table 1. Methods to evaluate the amount of hair loss after scalp cooling to prevent CIA

Grading scale	Subjective/ objective	Scale	Measuring timepoints
HMI* 100 much hair 1 little hair	Objective	Scale:Continuous	Cycle 1-6
VAS** 0 → 10 No hair loss total hair loss	Subjective	Ordinal: 0-10	Cycle 2-6
WHO classification [19] 0 No change 1 minimal hair loss 2 moderate, patchy alopecia 3 complete alopecia, but reversible	Subjective	Ordinal: 0-3	Cycle 2-6
Head covering Yes/ no	Subjective	Nominal: Yes/ no	Cycle 2-6

*HMI: Hair Mass Index

**VAS: Visual Analogue Scale

Figure 1. The use of the hair check

Before cycle 2, patients evaluated the severity of alopecia on the 4-point scale (range 0-3) for alopecia of the World Health Organisation (WHO) (World Health Organisation, 1979) and by using a VAS for hair loss (range 0-10, 0 = 'No hair loss', 10 = 'Total hair loss'). The success of scalp cooling was defined in terms of the patient's self-determined need to wear a wig or other head covering to mask visible hair loss after chemotherapy treatment (table 1). To assess hair loss by photography, a protocol was designed to standardise camera settings to depict various degrees of hair loss. Five digital images at standard views from frontal, vertex, occipital and both temporal sides were made before start of chemotherapy and before the 4th and 6th or last chemotherapy cycle. Images were kept in the medical records. Doctors, nurses and hairdressers were asked to rate the visible hair loss as depicted on pictures of the patients according to the WHO and VAS scores. They assessed the same images twice. Mean scores of the two assessments were used and the mean scores of the doctors, nurses and hair dressers were calculated. End points were the mean VAS scores of cycle 4 and cycle 6.

Tolerance of scalp cooling was measured during all visits by a Visual Analogue Scale (VAS) of 0-10, in which 0 represented 'Not tolerable' and 10 meant 'Very well tolerable'. Patients were considered evaluable for hair preservation if they were treated with at least three cycles of chemotherapy or if they discontinued scalp cooling due to severe hair loss.

The study was approved by an independent ethics committee and institution review board. All procedures were conducted in accordance with the 1964 Helsinki declaration and its later amendments. Specialised oncology nurses informed patients about the study. Written informed consent was obtained from all individual participants included in the study.

Scalp cooling

All patients used the one-person cooling machine (PSC-1) of Paxman. The cap was applied according to the instructions for use in the nursing protocol. The temperature of the coolant in the refrigeration tank was -10°C. This temperature is a standard set up performed by the manufacturer. The pre-cooling time was 30 minutes before the start of

the chemotherapy infusion. The cool cap remained on the scalp during the infusion period, being 60 minutes as a standard, and during 90 minutes afterwards. Scalp cooling was applied during all planned cycles of chemotherapy, unless the patient decided to stop the cooling procedure based on hair loss, side-effects or for other reasons.

Statistical analysis

All tests of significance were two-sided, and differences were considered statistically significant when $p<0.05$. All tests were performed using SPSS software (version 20.0) for Windows XP. Data was collected using standard forms, which were compiled into a SPSS database. The analyses were carried out on all evaluable patients. Descriptive statistics were performed to describe the socio-demographic and clinical-related characteristics of the study sample. Spearman's rho rank correlation was used for measuring the association between the objective and subjective measurements and between the patients, doctors, nurses and hairdressers opinion.

RESULTS

Patient and treatment characteristics

Sixty-two female Caucasian patients with breast cancer were included in this study. Patient characteristics are listed in table 2. The median age of the patients was 60 years. All patients were treated conform the study protocol, for a median of three cycles of chemotherapy and scalp cooling (range 1-6). The median duration of scalp cooling was 195 minutes per cycle. All patients were evaluable for hair preservation and tolerance. At the time of data cut-off (January 1, 2016), the median follow-up of patients was 51 months.

Table 2. Patient characteristics (n=62)

	N (%)	No head covering *N* (%)	Head covering *N* (%)
Patients included	62	13 (21%)	49 (79%)
Median age, years (range)	60 (32-74)		
Epirubicin	50 (81%)	8 (16%)	42 (84%)
6x F500/E100/C500	28 (45%)	5 (18%)	23 (82%)
3x F500/E100/C500 followed by 3xT100	22 (36%)		
Overall 3xFEC, 3xT		3 (14%)	19 (86%)
Result after 3xFEC		8 (36%)	14 (64%)
Adriamycin	12 (19%)	5 (42%)	7 (58%)
4x A60/C600	10 (16%)	4 (40%)	6 (60%)
6x A60/C600dd	2 (3%)	1 (50%)	1 (50%)
Median number of cycles with scalp cooling (range)	3 (1-6)		

F: 5-fluorouracil; E: Epirubicin; C: Cyclofosfamide; A: Adriamycin; T: Docetaxel

dd: every two weeks

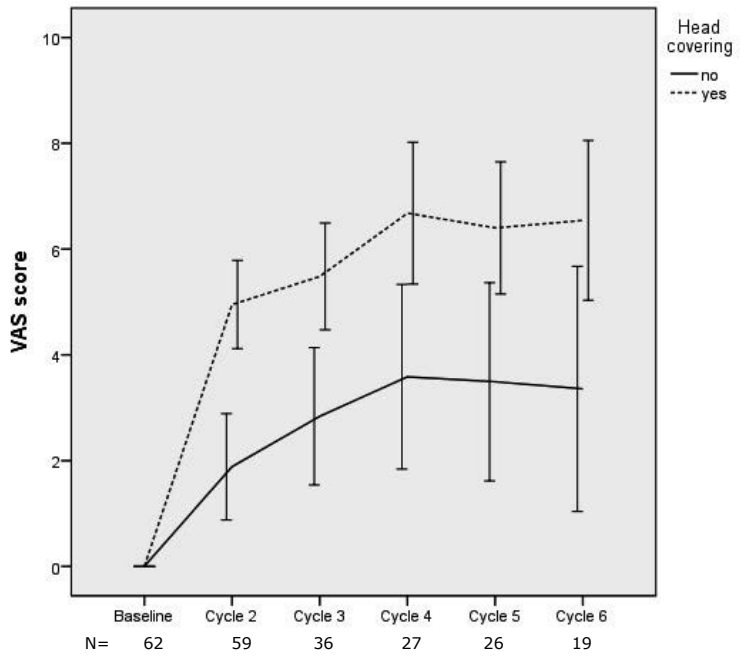
Baseline hair quantity

Forty-one percent of the patients reported their hair quantity prior to chemotherapy as moderate (scale: much, moderate, little).

Patient-reported success of scalp cooling

- Head covering yes/no
  Thirteen out of 62 patients (21%) did not wear a wig or head cover at the end of anthracycline chemotherapy, despite some slight hair loss.
- VAS
  Median VAS scores for hair loss increased from 4.1 (range 0-10) after the 1st cycle to 5.8 (range 0-10) at the 6th cycle. Patients who did not need a head covering had a significantly lower median VAS score for hair loss (VAS: 2.3 versus 5.8; $p<0.0001$) (Figure 2).

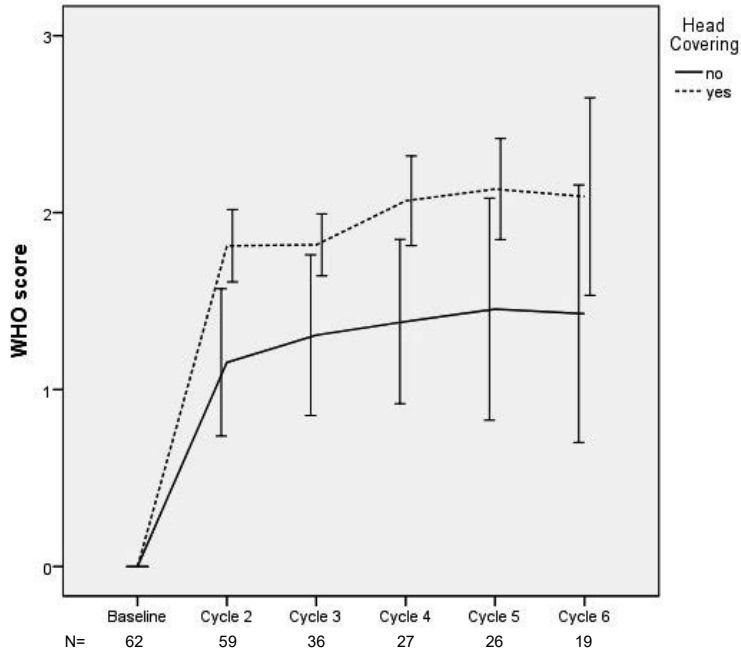
Figure 2. Visual Analogue Scale (VAS) scores for patients with and without head covering during 6 cycles chemotherapy (P<0,0001)



- WHO

  Median WHO scores for hair loss were 2 (range 0-3) after cycle 1 and at cycle 6. Overall, the median WHO score in patients who did not need a head covering was significantly lower than in patients who needed a head covering (WHO: 1 versus 2; p<0.0001) (figure 3). Hair loss assessed with VAS and WHO demonstrated a strong correlation (ρ= 0.7).

Figure 3. World Health Organisation (WHO) scores for patients with and without head covering during 6 cycles chemotherapy (P<0,0001)



HMI

Hair Check measurements at the left and right side of the head did not show significant differences, except for cycle 4. Since there is no rationale for these differences, they were reported as the mean of the two temporal measures. The mean baseline HMI was 64 (range 24-100) and decreased to 25 (range 6-53) in the 17 patients who were evaluable after 6 cycles (table 3). HMI decreased throughout all cycles of chemotherapy, but the highest decrease was seen after the second cycle (figure 4). Baseline HMI correlated with patient-reported baseline hair quantity, but was not predictive for hair loss (HMI no head covering 61; HMI head covering 64; p=0.7). HMI did not correlate with VAS and WHO during therapy (HMI-WHO: ρ= -0.4; HMI-VAS: ρ= -0.4). Patients who did not need a head covering had a higher median HMI score (HMI: 51 versus 43; p=0.034) (Figure 5).

Table 3. Mean HMI before each chemotherapy cycle

Cycle of chemotherapy	No. of patients	Mean HMI	SEM	Range of HMI
1	59	64	2.5	24-100
2	41	54	3.2	13-95
3	28	44	4.0	15-84
4	26	39	3.9	9-73
5	25	35	4.0	10-80
6	17	25	3.7	6-53

Figure 4 Hair Mass Index (HMI) during six cycles chemotherapy

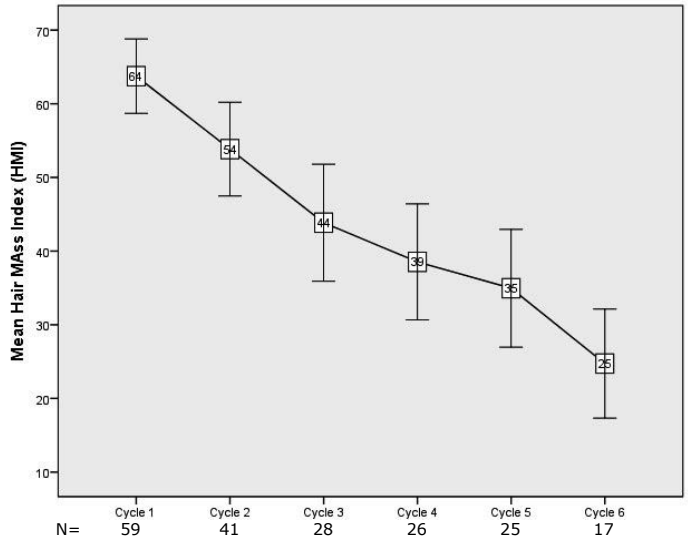
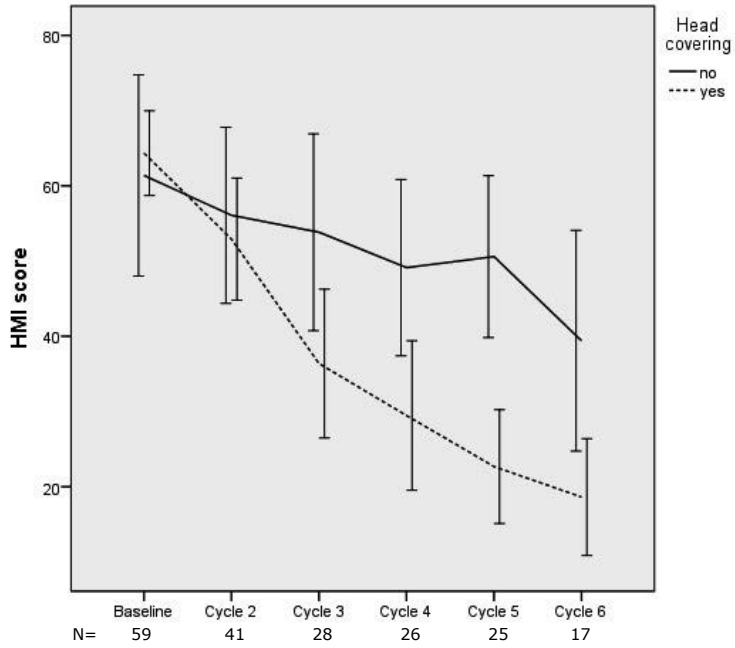


Figure 5. Hair Mass Index (HMI) scores for patients with and without head covering during 6 cycles chemotherapy (P=0,034)



Healthcare professional evaluation of hair loss-photography

There was a strong correlation of the VAS scores of hair loss, as measured by doctors and nurses (ρ=0.84). The correlation between the opinion of health care professionals (doctors and nurses) and the opinion of the patients was moderate (VAS: ρ=0.50-0.56 respectively). However, the opinion of the hair dressers matched strongly with the opinion of the patients with respect to hair loss measured with VAS (ρ=0.70) (table 4).

Table 4. The association between the VAS-scores of patients and professionals

	Nurse	Doctor	Hair dresser	Patient
Nurse	-	0.84**	0.83**	0.56**
Doctor	-	-	0.80**	0.50**
Hair dresser	-	-	-	0.70**
Patient	-	-	-	-

** = sig. at 0.01 level

Tolerance and safety analysis

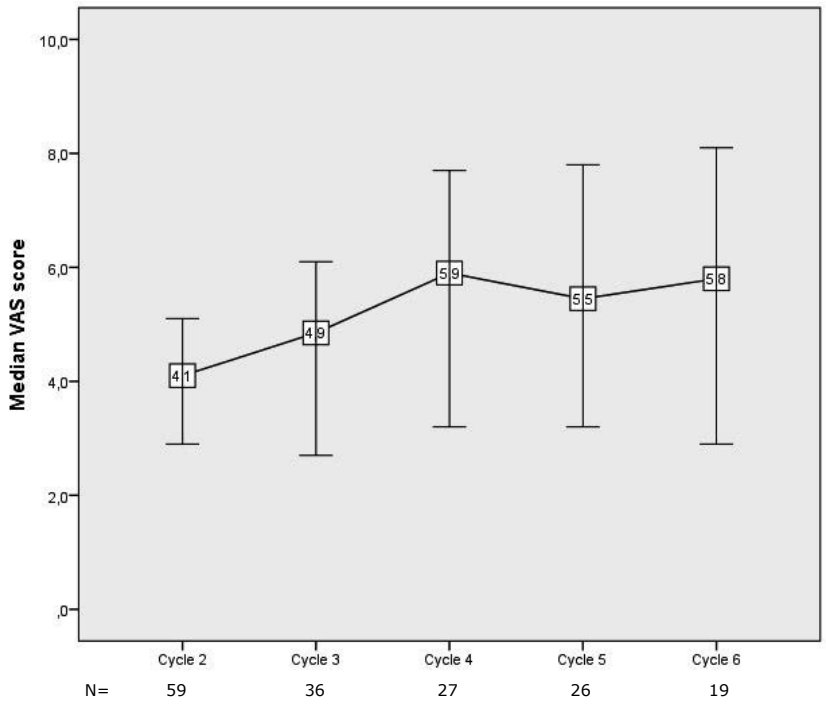
Scalp cooling was very well tolerated. The VAS score for the tolerance of scalp cooling was performed over 192 cooling sessions, resulting in a mean score of 8 (SD: 1.9). Only one patient stopped scalp cooling because of intolerance after cycle 4. No scalp metastases were reported during follow up.

DISCUSSION

This study investigated the value of the Hair Check to measure chemotherapy-induced hair loss in patients treated with anthracycline-containing combination chemotherapy. Initial hair mass as measured by the Hair Check correlated with patient-reported hair quantity before the start of the chemotherapy, but was not predictive for the severity of hair loss during scalp cooling. Thus, it seems that the efficacy of scalp cooling in preventing CIA is independent of having either thin or thick hair. This adds to our knowledge that the efficacy of scalp cooling mainly depends on the type and dose of chemotherapy, and the degree and duration of scalp cooling. (Komen et al., 2013) Unfortunately, we do not have data on the Hair Check during scalp cooling in Asian or Afro-hair patients.

It is noteworthy that, according to the Hair Check, hair loss continued throughout six chemotherapy cycles, although patients themselves did not report any increase in hair loss (as measured with VAS for hair loss) after cycle 4 (figure 6). Obviously, HMI is more sensitive for detecting subtle changes in hair loss than subjective measurements. Therefore, to improve the efficacy of scalp cooling, efforts should be made to increase its efficacy throughout the complete time span in which all cycles of chemotherapy are administered. Hair mass as measured by the Hair Check did not correlate with patient-reported outcome assessments such as WHO and VAS score. Therefore, it is not useful as a clinically relevant endpoint for hair loss, while it is time consuming in daily practice as well (about 15 minutes per measurement).

Figure 6 Visual Analogue Scale (VAS) score during six cycles chemotherapy



The opinions of hair loss of health care professionals correlated only moderately with those of patients, but the opinion of hair dressers was more in line with the patients. Apparently, due to their professional experience hairdressers are better capable to estimate hair loss than healthcare professionals are. The weak correlation between patients and health care professionals demonstrates that the patients' opinion of hair loss should be considered as the best subjective method to assess the efficacy of scalp cooling. In some clinical studies on CIA hair loss is measured by clinicians (Nangia et al., 2017) or nurses (Lemenager et al., 1997, Massey, 2004), but our study confirms the findings of Mulders et al. (Mulders et al., 2008) that healthcare professionals underestimate the severity of hair loss.

This study has some limitations. Firstly, the sample size was limited. The study may have been underpowered to detect differences between the methods of evaluation. Secondly, we did not prescribe a standardized hair care at the day of measuring HMI. Possibly, the use of styling products could have slightly influenced HMI, but before standardized hair care might be prescribed, the exact influence has to be examined. Thirdly, we delineated the measured location by using a location strip. The use of this strip can cause slight deviations in retrieving the exact measuring location every cycle. However, Vleut et al. (Vleut et al., 2013) showed that a slight deviation of the measurement area caused no significant deviations in HMI.

In a large registry study on scalp cooling 33% and 39% of patients treated with FEC or AC chemotherapy, respectively, did not need a head covering. (van den Hurk et al., 2012) In this study, only 21% of patients was successfully treated with scalp cooling to prevent CIA. The low efficacy in the present study might be explained by the fact that patients decided to stop scalp cooling because they were more aware of the continuous hair loss during chemotherapy as measured by the Hair Check and subjective registries.

We measured HMI at both sides of the head, because the gradation of hair loss in a balding individual was found to be much higher along the sagittal axis than the coronal axis. (Cohen, 2008) According to our results, HMI measurements at the left and right side of the head did not show significant differences; therefore we recommend HMI measurement at one side for future research. However, in daily practice we advise to preferably use a generally accepted and practical subjective scale for hair loss such as WHO or VAS.

Although it was confirmed that WHO and VAS correlated strongly ($\rho = 0.7$), the latter is more sensitive for small changes in hair loss and therefore more valuable to use when comparing different groups of patients. In addition, to evaluate hair loss in clinical practice, the use of a wig or head cover can be considered as a parameter for patient satisfaction. (Breed et al., 2011)

CONCLUSIONS

Initial hair mass index (HMI) as measured by the Hair Check correlated with patient-reported hair quantity before the start of the chemotherapy, but was not predictive for the severity of hair loss during scalp cooling. It seems that the efficacy of scalp cooling in preventing CIA is independent of having either thin or thick hair. According to the Hair Check, hair loss continued during all chemotherapy cycles. Therefore, to improve the efficacy of scalp cooling, efforts should be made to increase its efficacy throughout the complete time span in which all cycles of chemotherapy are administered. The weak correlation between patients and health care professionals demonstrates that the patients' opinion of hair loss should be considered as the best method to assess the efficacy of scalp cooling.

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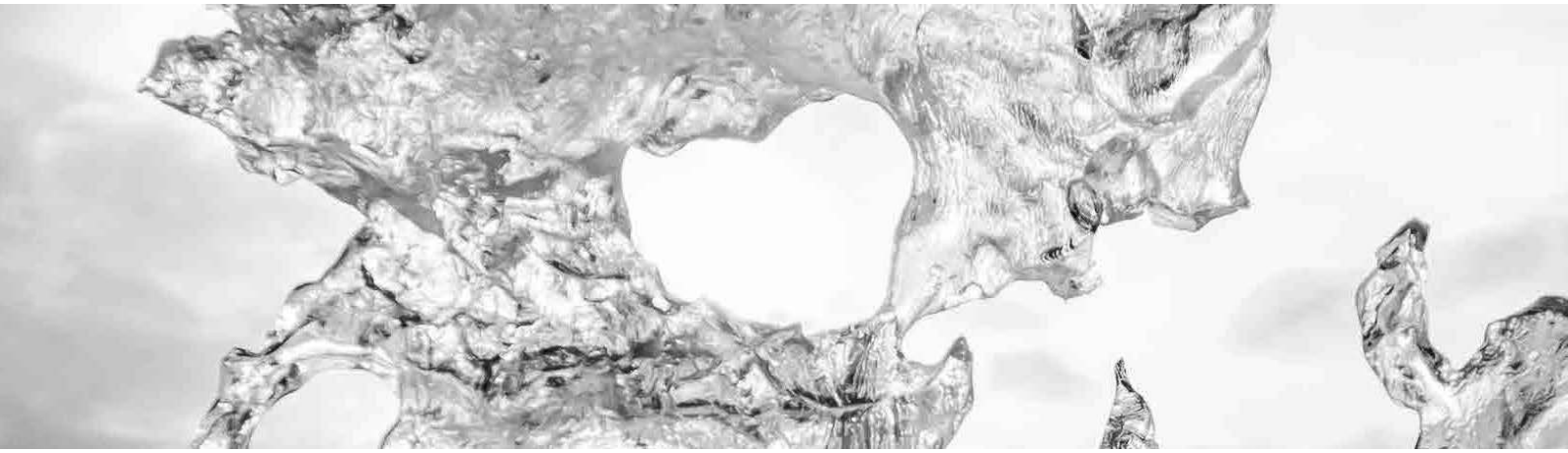
Chapter 7



Translational study with collected hair samples in chemotherapy induced alopecia

Submitted

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ABSTRACT

Purpose: Chemotherapy-induced alopecia (CIA) is generally considered as one of the side-effects with the most impact for patients. To explore the pathobiology of CIA and to improve the efficacy of scalp cooling in preventing CIA, it is important to analyse the molecular damage-response pathways in human hair follicles during and after administration of chemotherapy. We report a single centre explorative pilot study.

Objectives: The objective of this study was to explore damage-response pathways in hair follicles after chemotherapy.

Methods: Hair follicles were collected from ten patients with breast cancer who were planned for treatment with anthracycline-based combination chemotherapy without scalp cooling. Hair follicles were collected before and at different time points after chemotherapy administration and subsequently embedded in methyl-methacrylate. Haematoxylin and eosin (H&E) staining was used to analyse the sections for apoptotic cells. These sections were stained with immunohistochemical antibodies.

Results: Hair follicles were successfully processed according to the protocol. Morphological signs of apoptosis were detected at different time points. Immunohistochemical staining could be successfully carried out in most of the sections. Cells staining positive for p53, caspase-3 and ki-67 could be detected in the hair shaft and the hair bulb. We were not successful in obtaining sufficient numbers of evaluable hair bulbs and therefore could not generate reliable data on markers of apoptosis in the ten treated patients.

Conclusions: These findings stimulate further development of methods to use hair follicles collected from patients as a human-based model for exploring the working mechanism of CIA and to test new potential interventions for hair loss prevention.

INTRODUCTION

Cancer treatment with cytotoxic agents is associated with severe side effects. Chemotherapy induced alopecia (CIA) is one of the most distressing side effects for many patients(1,2) and begins days to weeks after the administration of chemotherapy.(3,4) Chemotherapy interferes with proliferating cells causing cell death or preventing cell growth, but the exact underlying pathobiology of CIA remains insufficiently understood.(3-12) In normal life, the growth cycle of a hair follicle consists of three phases: the anagen, catagen and telogen phase. The hair growth (anagen) phase involves the growth of a hair from a hair follicle and lasts for three to seven years. During the transitional (catagen) phase (2-3 weeks), the hair follicle atrophies and migrates upwards in the skin. During the resting (telogen) phase (3-4 months), the hair does not grow but stays attached to the hair follicle. The telogen phase ends when the old hair is shed and a new hair is regenerated in the anagen phase.(13,14) Since 90% of all hair is in anagen phase and rapidly proliferates, hair follicles are highly at risk to be affected by chemotherapy, resulting in CIA. The most characteristic response of a hair follicle to chemotherapy is an anagen effluvium (shedding of hair follicles in the growth phase). Telogen effluvium also occurs, characterized by increased proliferative activity, presumably as a repair strategy, resulting in diffuse CIA.(15)

Research models for investigating molecular mechanisms of CIA are depicted in Table 1. At present, research in rodent and ex-vivo models is used to explore the pathobiology of CIA and to test novel management strategies in the prevention of CIA. However, these models have limitations in studying the direct effect of interventions on human hair follicles treated with chemotherapy. In rodent models, such as mouse or neonatal rat models, it was found that hair follicle apoptosis largely depended on p53, a key mediator of cellular damage caused by a stress response.(15) However, the clinical relevance of rodent hair follicle models is limited because they do not reflect the cycling rhythm of hair follicles in human beings. Research models with ex vivo cultures of human hair follicles enable direct testing of the damaging effects of defined cytostatic agents.(16-18) Al-Tameemi et al. confirmed with their ex-vivo model that cooling reduced or completely inhibited drug cytotoxicity in human keratinocytes exposed to chemotherapy, supporting the hypothesis of scalp cooling.(17) However, keratinocytes in this model are not in cycle and begin to degenerate after 1-2 weeks in culture. Moreover, it is difficult to imitate the application schedule of standard chemotherapy regimens as human hair follicles damaged by chemotherapy will still be in the recovery phase when they are exposed to the next cycle of chemotherapy. Botchkarev demonstrated that in contrast to wild-type mice, p53-deficient mice show neither hair loss nor apoptosis in the hair follicle keratinocytes after cyclophosphamide treatment.(13) Hendrix et al. and Paus et al.(15,19) showed in mice that hair follicles undergo two distinct pathways of

dystrophy (dystrophic anagen and dystrophic catagen pathway) after chemical damage. During the so-called dystrophic anagen pathway (induced mainly by a lower dose of chemotherapy), the hair follicle undergoes an incomplete primary recovery followed by a retarded secondary recovery during which a normal hair shaft is generated. Hair follicles that undergo the dystrophic catagen pathway (in response to a higher dose of chemotherapy) immediately enter into a dystrophic catagen stage, followed by an abnormally shortened telogen phase. Scalp cooling, possibly forces hair follicles into the dystrophic anagen phase, thereby enabling patients to keep their hair. Two hair follicle damage response pathways (dystrophic anagen and dystrophic catagen pathway) were also reported in a research model of surgical grafting of human hair follicles on immunodeficient mice .(13,15,20) Unfortunately, this technique still does not demonstrate the direct effect of treatment with chemotherapy in hair follicles of patients. Randall et al.(21) reported a method for the fixation, processing, sectioning and immunohistochemical staining on plucked human hair, which allows monitoring of the effects of anti-cancer drugs on cell proliferation Ki -67) and on the upstream signaling molecules that control cell proliferation (p53/ Caspase-3).

So far, there is no effective preventive pharmacological therapy for CIA. Management of CIA primarily consists of counseling, professional psychological support and the recommendation to use a wig when hair loss occurs.(14,22,23) Scalp cooling is the only intervention to prevent CIA, its efficacy depending on the type and dose of chemotherapy. It is assumed that scalp cooling reduces skin temperature, thereby affecting the exposure and metabolism of cytotoxic agents in the hair follicles.(9,24) In vitro models showed that cooling markedly reduced cytotoxicity, in agreement with clinical observations.(17) Sakurai et al. examined the effects of low temperature (32 degrees C) on cells exposed to cytotoxic agents in vitro. (25) Mild hypothermia suppressed induction of apoptosis by p53-dependent and p53-independent mechanisms. P53 activates downstream apoptotic events, leading to a cascade of activation of caspases, including Caspase-3, which induces apoptosis.(26) Ki-67, a cellular marker for proliferation, is down regulated in hair follicles showing apoptotic changes(20), and is therefore an interesting marker in research discovering the mechanism of scalp cooling.

Plucked human hair may offer a minimally-invasive, easily sampled and well-tolerated source of hair follicle cells for the investigation of damage response pathways in patients treated with chemotherapy. In order to improve scalp cooling and to develop new treatment strategies for the prevention of CIA, we set out to explore molecular damage-response pathways in plucked human hair from patients treated with chemotherapy.

Table 1. Research models for investigating the molecular mechanisms of chemotherapy-induced alopecia

Model of chemotherapy-induced alopecia	Strengts	Limitations
Rodent models (neonatal rats and adult mice)(19,27,28)	Rodent models permit investigation of how chemotherapy affects the hair follicle cycling. Similar to in human CIA, hair loss in rodent models generally follows specific patterns.	In neonatal rats chemotherapy affects hair follicles that are still in the final stages of postnatal morphogenesis. In adult mice hair cycling is more or less synchronized across all follicles, whereas in human beings each hair follicle follows its own cycling rhythm.
Organ cultured hair follicles(16,17,20,29)	Enables direct testing of the damaging effects of cytostatic agents and provide the most clinically relevant surrogate model for CIA	Not possible to investigate how chemotherapy affects the hair follicle cycling. No information about the hair loss pattern.
Surgical grafting of human scalp skin on to mice(20,28,30)	Combines benefits of human and rodent models. Resembles key features of CIA that occurs in patients with cancer.	Human hair follicles are exposed to a different milieu of the mouse hormones and local growth regulators.
Hair collection from patients	Minimally invasive technique which enables direct testing of the damaging effects of cytostatic agents in human hair follicles.	No available surrounding scalp tissue to provide more insight in the mechanism of action. Very time consuming and delicate process.

MATERIALS AND METHODS

We conducted an explorative single centre pilot study at the department of Medical Oncology of Northwest Clinics, the Netherlands. Patients with breast cancer who were planned for treatment with at least one cycle of anthracycline-based combination chemotherapy (docetaxel-doxorubicin-cyclophosphamide(TAC), fluorouracil-epirubicin-cyclophosphamide(FEC) or doxorubicin-cyclophosphamide(AC)) without scalp cooling

were enrolled. Patients were excluded if they lacked basic proficiency in Dutch or if they were unable to understand the patient information brochure. The hair follicle analyses were conducted at the department of pathology of the University Medical Centre Utrecht. The study was approved by an independent ethical committee. All procedures were conducted in accordance with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all patients included in the study.

Sampling and fixation

Patients were asked to collect approximately 30 scalp hairs at six time points after the first cycle of chemotherapy. Collection time points were T0= before chemotherapy, T3=day 3, T5=day 5, T9=day 9, T13=day 13, T17=day 17 after chemotherapy. The first sample collection (T0) took place at Northwest Clinics and was conducted by a research nurse. The following five samples were collected by patients at home. Hairs were plucked from the parietal region of the scalp using a blunt-nosed forceps. Hairs were stored into a tube containing 10% neutral-buffered formalin which was brought home by the patient. The tubes were handed in at the next hospital visit. After collection, the hairs were checked by a research nurse for the presence of a bulb and stored in phosphate buffered saline (PBS) at room temperature at Northwest Clinics and sent to the department of Pathology of the University Medical Centre Utrecht for further analysis.

Processing and embedding

Hairs were trimmed to approximately 1 cm and attached to a small piece of photo sticker, enabling parallel embedding of the hairs and simultaneous sectioning of multiple hair bulbs. Per photo sticker, five hairs were embedded in 3% low melting point agarose. Hairs were horizontally embedded in one agarose block cast in silicone embedding molds. The agarose blocks were trimmed to fit in the processing baskets of the Leica EM tissue processor. The blocks were processed according to the steps as described by Randall et al.(21) After processing, the remaining photo stickers were carefully removed. The processed agarose blocks were embedded in 8 mm, flat-ended, embedding capsules filled with polymerization solution which consisted of catalyzed MMA plus 125 µl N,N-dimethylaniline. The blocks were orientated longitudinally at the bottom of the embedding capsules. To keep the blocks from floating upward after adding the polymerization solution, a horizontally placed shortened pipette tip was gently pushed onto the block. The capsules were then placed in a desiccator at room temperature to flush out the oxygen-free nitrogen for approximately 2,5h during the polymerization of the resin. A layer of water was added to dissipate the heat of the exothermic polymerization.

Sectioning

The polymerized blocks were kept in capsules for another 24 hours at room temperature. When removed from the capsules, the plastic blocks were stored at room temperature before sectioning. The blocks were cut in 2 µm-thick sections on a Leica Ultracut Microsystems microtome (Cell Microscopy Core, Department of Cell Biology at the UMCU) using a glass knife. The sections were picked up with a fine-nose forceps and spread out on a droplet of water on a glass slide. The slide was then transferred to a hotplate to flatten the section out. A toluidine blue staining was performed to confirm the presence of hair follicles in the section. After sectioning, the slides were stored in a refrigerator.

Preparation of slides and Haeamatoxyline and Eosine staining (H&E)

Processed sections were microscopically analyzed and assessed for evaluability. Prior to H&E staining, the slides were incubated at 37°C for approximately 3 hours, followed by overnight deplasticising in xylene at room temperature. Sections were rinsed twice in xylene, three times in 10% methylated spirit, once in 100% ethanol and once in 70% ethanol, before hydration in distilled water. The sections were H&E stained by incubating in haeamatoxylin for 10 minutes and in eosin for 2 minutes. After dehydration, slides were mechanically cover slipped in pertex. H&E stained hair bulbs were analyzed for the presence of apoptotic cells at the Departments of Internal Medicine and Pathology of Northwest Clinics.

Immunohistochemistry

Slides were treated by blocking in 3% hydrogen peroxide for 15 minutes. After being rinsed in distilled water, heat-mediated antigen retrieval took place in an autoclave at 125 ◦C in EDTA, pH 9.0, and cooled down for 20 minutes. IHC staining was performed using the following antibodies: Ki-67 (Dako, clone MIB-1, 1/100), P53 (Dako, clone Do7, or 1/500) and anti-active caspase 3 (Pharmingen Purified rabbit 1/500). Primary antibodies were incubated at room temperature for 1 hour. For p53 and Ki-67 staining, slides were treated pre-and post- antibody incubation with the Novolink kit (Leica, NL). Active caspase 3 staining was treated with the Novolink kit, Bright Vision poly AP-Anti rabbit IgG and Dako liquid permanent red. After counterstaining with haeamatoxylin for 10 seconds, the sections were dehydrated and mechanically cover slipped in pertex. The material was assessed microscopically for evaluability of apoptotic cells. Appropriate positive and negative controls were taken along each staining and consisted of formalin fixed paraffin embedded control sections- and/ or MMA embedded positive control material.

Statistical analysis

Data were collected using standard forms, which were compiled into a database. Due to the small sample size of the pilot study, it was not possible to perform statistical analysis.

RESULTS

Patient characteristics

Between January 2013 and November 2014, ten patients treated with anthracycline-based chemotherapy were included to explore the best time points for hair collection and to optimise the fixation, embedding and staining protocol. Patient characteristics are shown in table 2. The median age of patients was 48 years and all patients were treated for breast cancer. Eight patients were evaluable for hair root analysis after T0. Two patients had not collected hairs at home because of sickness.

Table 2. Patient characteristics

	N
Patients included	10
Patients evaluable for hair root analysis	8
Median age, years (range)	48 (35-57)
Chemotherapy	
FEC	1
TAC	7
AC	2

Hair samples

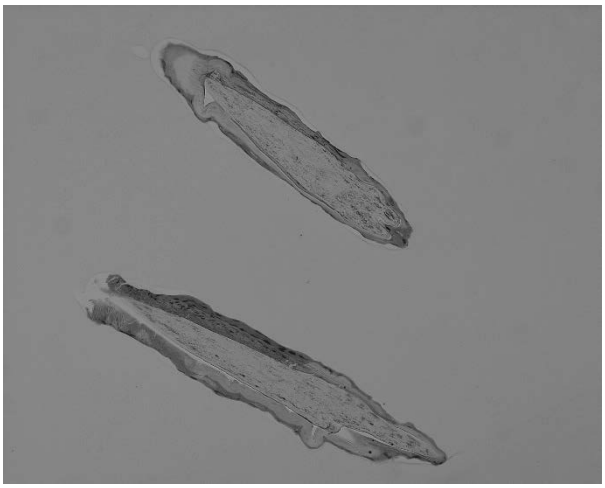
Processed sections were microscopically analyzed and assessed for evaluability. Hair follicles collected are shown table 3. At T0 a total of 61% of collected hair samples was evaluable for analysis. Non-evaluable sections were seen in 39% of the samples and contained hair remnants with or without attached connective tissue or without the presence of any hair follicle epithelium (Figure 1). Unfortunately, a sharp decrease of evaluable hairs was detected over time. At T17 none of the hair follicle samples were evaluable, only hair shafts or connective tissue could be identified.

Table 3. Hair follicles collected at various time points

	T0	T3	T5	T9	T13	T17
No. Patients collecting hairs	10	8	7	6	6	2
No. H&E tissue slides (Each slide containing 1-5 hair follicles)	20	18	14	12	12	6
% Evaluable hair follicles	86%	94%	57%	42%	17%	0%
No. Apoptotic cells in evaluable hair follicles	8	0	2	2	1	0

T0: Before the start of chemotherapy
T3: Three days after chemotherapy
T5: Five days after chemotherapy
T9: Nine days after chemotherapy
T13: Thirteen days after chemotherapy
T17: Seventeen days after chemotherapy

Figure 1. Non-evaluable sections containing connective tissue without the presence of epithelium



Apoptosis

Results of hair root analysis demonstrated the presence of a low frequency of apoptotic cells, characterized by cellular shrinkage, eosinophilic cytoplasm and nuclear fragmentation. Apoptotic cells, found at different time points, seemed to be preferentially located in the root sheath. Isolated apoptotic cells were found as well as areas with several apoptotic cells (Figure 2). The highest amount of apoptotic cells was observed at

T0 and decreased after chemotherapy. In some sections, pink artefacts were present (Figure 3), probably representing dislocation of parts of the inner root sheath resulting from overstretching of the hair follicle when pulling out.

Figure 2. Apoptotic cell in H&E stained section

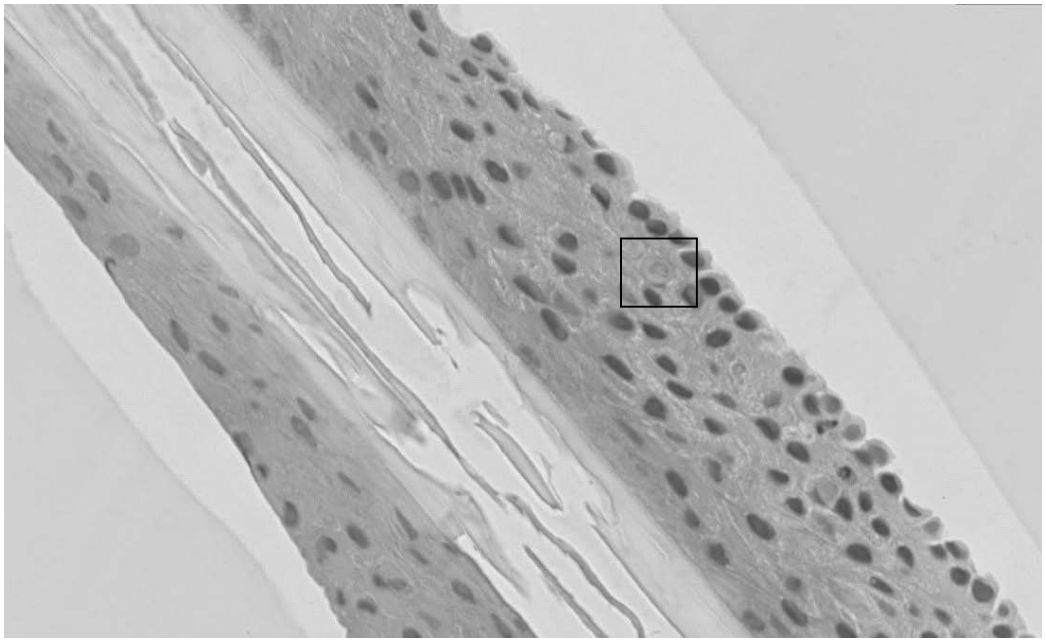
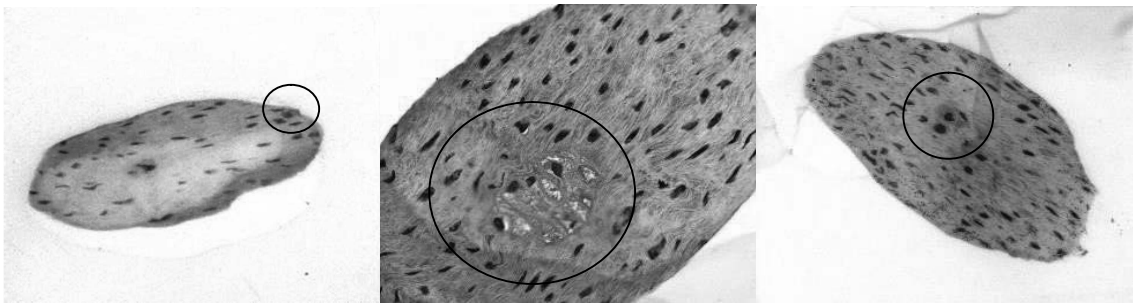


Figure 3. Abnormalities that might be apoptotic cells in H&E stained sections



Immunohistochemical analysis

Immunohistochemical staining was exploratively performed in samples of four patients in whom histochemical sections showed the best quality. P53 and Ki-67 staining showed positive nuclei for p53 (figure 4) and Ki-67 (figure 5) at T0, mainly in the hair shaft and bulbus.

Figure 4. P53 staining (a: T=1, b: T=5)

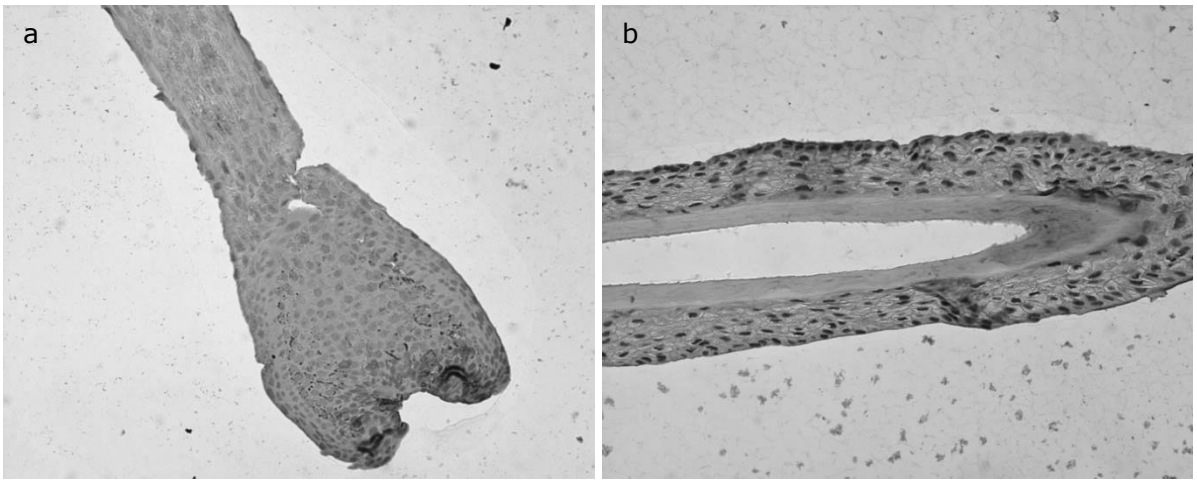
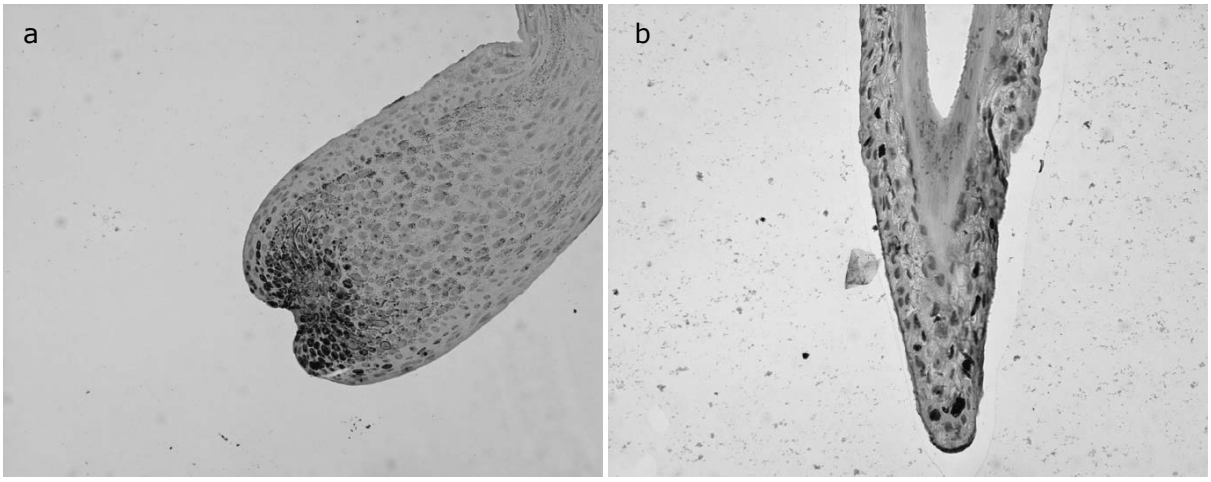
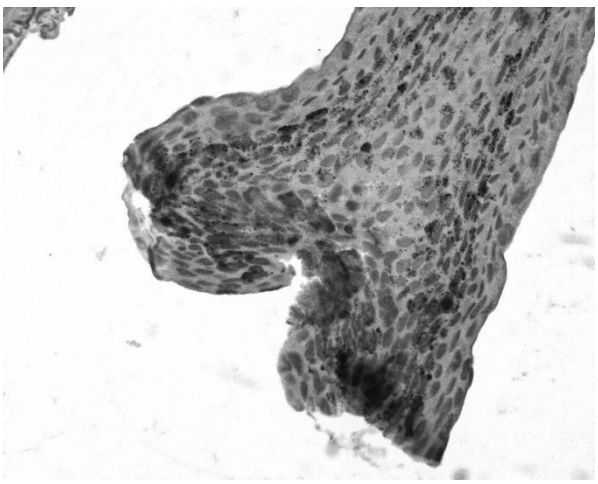


Figure 5. Ki-67 staining (a: T=1, b: T=5)



Ki-67 IHC showed a decreasing brown diaminobenzidine (DAB) precipitate from core to shaft, following the normal proliferation rate of cells in hairs. Samples stained with active caspase 3 antibody showed little positive cytoplasm at T0, mainly in the bulbus section (Figure 6).

Figure 6. Caspase-3 staining (T=1)



DISCUSSION

This study shows results of hair follicle analyses in patients treated with chemotherapy. We optimised the fixation, embedding and staining protocol of hair follicles from patients and explored signs of apoptosis at various time points after chemotherapy. We hypothesized that we could detect signs of apoptosis after chemotherapy administration and it was our intention to confirm this hypothesis in a larger study.
Unexpectedly, as is depicted in table 3, we found a decrease of evaluable sections of hair follicles and shafts over time after administration of chemotherapy. Therefore, we decided to shorten the time interval between hair root analysis in a second group of patients.

Between March 2017 and April 2018 another twenty patients with or without scalp cooling were studied in a similar way to explore the molecular damage-response pathways in human hair follicles after chemotherapy. However, the process of embedding, cutting and coloring was very complicated and time consuming, and there were not enough evaluable hair bulbs for analysis in these 20 patients.
We identified several reasons which can explain why we were unable to confirm our hypothesis (Table 4).

Table 4. Limitations and recommendations

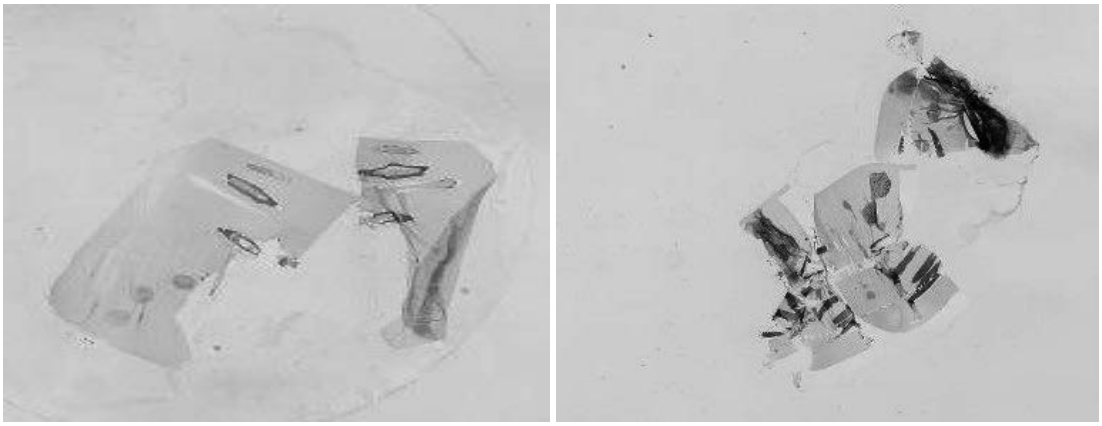
Influencing factor	Limitation	Recommendation for future research
Collection of hairs	Only hair follicles were collected	Take skin biopsies to collect surrounding scalp tissue
Selection of hairs	Hairs were collected by the patient themselves	Controlled collection by an experienced nurse and preselection of hairs with a preparation microscope
Fixation period	Long fixation period could affect the material	Fixation of hair samples maximal 4 hours
Sectioning protocol	Complicated protocol and very delicate sectioning process	3-D scanning or Microscopy with Ultraviolet Surface Excitation (MUSE)

Firstly, hair follicles were collected using a blunt nose forceps. Preferably we would have taken scalp skin biopsies from patients treated with chemotherapy. By collecting surrounding scalp tissue, the effect of chemotherapy on the surrounding skin could have given us more insight into the mechanisms of action. However, when patients have no direct treatment benefit, performing biopsies presents a difficult ethical issue. Due to

these ethical problems, we explored the possibilities to perform immunohistochemical staining on hair follicles directly collected from the patient's scalp. Secondly, the yield of hairs could have been improved by a controlled method for pulling out hairs, and by a preselection of hairs with sufficient follicle epithelium for analysis with a preparation microscope.

Thirdly, the long fixation period of the hair follicles might have caused a disadvantage. According to Randall et al.(21) the optimal period of fixation appears to be 4 hours at room temperature. To prevent patients from daily travelling to the hospital to deliver their collected hair samples, the samples were brought to the hospital with the next scheduled appointment. This considerably delayed the period of fixation but had no negative effect on the morphology of the hair follicles. However, immune histology had to take this into account in the degree of antigen retrieval. Unfortunately, hair follicle research turned out to be a very delicate process and depended too much on the availability of dedicated people. The method of fixation according to Randall et al. was difficult to reproduce and we failed in making a robust model for large scale analysis of hair bulbs. The procedure was too time consuming and despite all effort, too much tissue was not evaluable (figure 7). For a better understanding of the working mechanism of CIA, the method as described by Randall et al. may not be the best option. Solid methods which consistently and reliably can demonstrate molecular damage response pathways are needed.

Figure 7. Non-evaluable hairs due to complicated sectioning



There are some important developments which could be helpful. Multiple hairs could be embedded in an upright position and close to each other to be cut in series and to create a 3-D reconstruction through scanning. This technique enables visualizing the entire hair follicle and suffers less from different levels of depth. Another possibility is the use of Microscopy with Ultraviolet Surface Excitation (MUSE), which uses ultraviolet light to illuminate tissue samples. Commonly used bright-field microscopy requires prior preparation of tissue sections mounted on glass slides, a process that can require hours

or days. The UV microscope removes the need for performing traditional histology and produces high-resolution images of biopsies and other fresh tissue samples within minutes.

A dedicated analytical staff is needed to handle the hair bulbs during the whole process of plucking, preservation, sectioning and staining in order to obtain sufficient slides to perform analysis and to draw conclusions.

CONCLUSION

The aim of this study was to explore molecular damage-response pathways in hair follicles from patients treated with chemotherapy. We found that H&E and immunohistochemical staining was possible on a number of embedded hair follicles. Unfortunately, we were not successful in obtaining sufficient numbers of evaluable hair bulbs and therefore could not generate data on markers of apoptosis in treated patients. However, better and standardized techniques for sampling and analyzing of hair bulbs are needed to study the damaging effects of cytostatic agents and to test new potential interventions for hair loss prevention.

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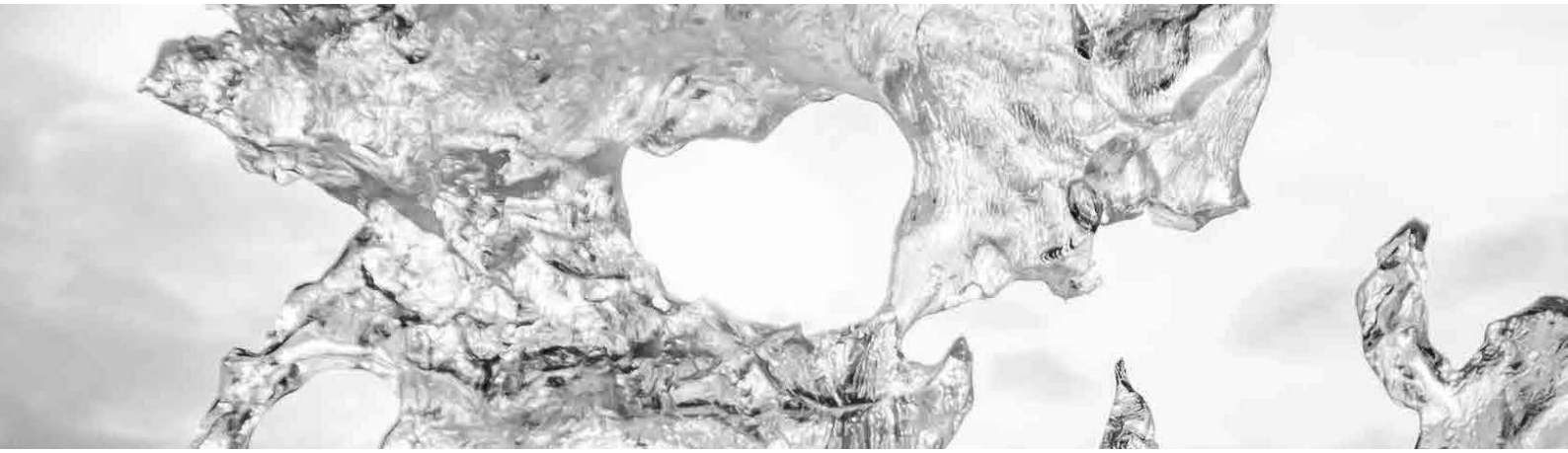
Chapter 8



General discussion and future research perspectives

GENERAL DISCUSSION

Efficacy of scalp cooling

Scalp cooling is increasingly being used in the prevention of chemotherapy-induced alopecia (CIA) in patients treated for cancer. Although a substantial number of patients does not suffer from CIA, scalp cooling is still not effective in all chemotherapy regimens and there is a subset of patients who has no beneficial effect from scalp cooling. Improvement of scalp cooling is therefore necessary as well as a better selection of patients who will likely benefit from it. To improve scalp cooling we focused on post-infusion cooling time and scalp skin temperature, two possible factors to improve outcomes. We also tried to analyse the molecular damage-response pathways in human hair follicles during and after administration of chemotherapy. Clarifying the working mechanism could help to improve scalp cooling and could be used to develop new treatment strategies for the prevention of CIA.

Adapting post-infusion cooling times

Determining the most optimal post-infusion cooling time has practical implications for scalp cooling in clinical practice. The most optimal post-infusion cooling time is unknown for many chemotherapy regimens. Is it 'the longer the better', or could it be shortened for patient comfort and for logistic reasons? The optimal cooling time for scalp cooling has not been studied extensively.(1,2)

We decided to investigate the post-infusion cooling times after treatment with low dose docetaxel (75 mg/m2 every three weeks) and after combination therapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC). In the low dose docetaxel regimen, scalp cooling is very effective (73% of patients do not require a wig)(3) and a short post-infusion cooling time (20 minutes) turned out to be just as effective as a long time (45 minutes).(2) In contrast, prolonging cooling time the FEC regimen (150 versus 90 minutes), in which scalp cooling is less effective (56%)(3), did not improve outcome significantly.(4) As the procedure of scalp cooling has to be feasible in daily practice, we decided that the small non-significant difference in effectiveness in our study with cooling times until 150 minutes did not justify further studies with even longer post-infusion cooling times.

Whereas shortening of the post-infusion scalp cooling time did not show differences in scalp cooling effectiveness in patients treated with low dose docetaxel, better results of scalp cooling were observed with the lower weekly docetaxel dose in comparison with the higher three weekly docetaxel dose. These findings are in line with the findings of Al Tameemi et al. who showed dose-dependent cytotoxicity in their in vitro model.(5) Apparently, the peak concentration of a cytostatic agent is more

important in determining toxicity than the exposure over time to chemotherapy. It is likely that individual variation in the efficacy of scalp cooling is due to interpatient variability in chemotherapy pharmacokinetics and peak drug concentrations.

It would be interesting to investigate short (20-minute) post-infusion cooling times in other chemotherapy regimens in which scalp cooling is used successfully (for example, all regimens with >70% effectiveness in preventing CIA). The so-called standard of 90-minute post-infusion cooling time has been chosen arbitrarily with the introduction of scalp cooling in the Netherlands. A possible explanation for protecting hair follicles even with shorter cooling times could be that the drug is flushed away from the hair follicles more rapidly during scalp cooling. Considering this, one might even question whether it is possible to omit the post-infusion cooling time.

Scalp skin temperature

In addition to the differences between individual patients with respect to pharmacokinetics of chemotherapy, the optimum scalp skin temperature during scalp cooling could also contribute to a better scalp cooling effect. When one wants to investigate this, an easy accurate temperature measurement of the scalp skin during scalp cooling is necessary. We used temperature probes to record scalp skin temperatures during cooling.(6) These measurements were accurate, but they were very time consuming and not useful in daily practice. It has been suggested that a scalp skin temperature $\leq$18°C is necessary for optimal scalp cooling results.(6) It would be interesting to investigate whether it is possible to decrease the scalp skin temperature below this level in those patients who do not reach this temperature.

Failing to reach a temperature $\leq$18°C did not explain all differences in efficacy in our patients. We observed patients with a scalp skin temperature of 13-14°C, without a beneficial effect from scalp cooling. Apparently, not all patients will benefit from adapting the temperature, but it will obviously be worthwhile to try this in some of them as a next step to improve scalp cooling results.

Individualizing scalp cooling

To improve scalp cooling, it is important to understand the exact mechanism by which cooling reduces the risk of chemotherapy induced alopecia. It has been suggested that vasoconstriction and reduced cell metabolism are important factors. In addition, the process of hair loss during and after chemotherapy might be mediated by specific molecular pathways. Understanding these mechanisms enables evaluation of the influence of various factors and might be of value to predict the outcome of scalp cooling. Van den Hurk et al. concluded in 2012 that type and dose of chemotherapy, infusion time, age, gender and type of hair significantly influenced the proportion of head cover

use.(3) Schaffrin et al. concluded in 2015 that menopausal status, systemic comorbidities, medication, nicotin abuse and hair density could also influence the outcome of hair loss prevention.(7) However, determinants of scalp cooling results vary considerably.(3,7-9)

Predicting the outcome of scalp cooling with these or other factors could be meaningful in counseling patients in the future. However, at present a prediction can only be made based on the type, dose and schedule of chemotherapy. Patients should be properly informed on the risk of alopecia and other possible side-effects to decide with their oncologist which treatment they will receive, ideally in the process of shared decision making. It has been shown that patients who lose their hair despite scalp cooling experience additional stress.(10) Therefore, it is important to select patients who will likely benefit from scalp cooling and provide them with reliable information about what to expect in their specific situation.

Limitations and confounding factors

The many publications reporting scalp cooling contain conflicting data. The influence of various factors such as menopausal status or liver function is unknown(7,11), but could have influenced the results. This also applies for the chosen method to measure CIA. In research focusing on scalp cooling, it is difficult to put the results in a broader perspective, because of the lack of a standardized method to measure the amount of hair loss. Scalp cooling studies in The Netherlands generally measure CIA by reporting the need to wear a wig or other head covering to mask visible hair loss. In other trials the use of the World Health Organization (WHO) classification of chemotherapy-induced alopecia(12), the Common Terminology Criteria for Adverse Events (CTC-AE)(13) or Visual Analogue Scale (VAS) is reported.(14) We concluded that the patient's opinion of hair loss should be considered as the best subjective method to assess the efficacy of scalp cooling. It is recommended to register both the patient's opinion and the VAS or WHO classification to facilitate the comparison of the efficacy of scalp cooling in the various scalp cooling publications.(15)

An important limitation in our research was the small sample size in our studies. Therefore, the studies might have been underpowered. This particularly applies to our study investigating a longer post-infusion cooling time in the FEC-regimen. It was difficult to motivate patients to randomize between 90 and 150 minutes, because patients thought 150 minutes post-infusion cooling time would not be tolerated. Although we could not exclude a clinical meaningful difference between the two post-infusion cooling times, we are inclined to state that it would be more interesting to investigate a shorter post-infusion cooling time in regimens with good scalp cooling results (>70%) than to repeat studies with longer post-infusion cooling times in a larger sample size.

Safety information

For a long time, there was no reliable information on the safety of scalp cooling in clinical practice. This prohibited broad scale implementation of scalp cooling, in particular in the United States. Besides, clinicians and nurses doubted the effect of scalp cooling, because information from well-organized, properly performed randomized scalp cooling studies was lacking.

Since 2009 important safety data have become available.(16-19) A major concerns with scalp cooling was that its use would increase the risk of scalp skin metastases. Several reports and multiple reviews (16-19) provided evidence to refute any potential risk of worse cancer outcome associated with scalp cooling.

In 2017 Nangia et al. provided evidence for the positive effect of scalp cooling in a randomized clinical trial.(20) Women with stage I to II breast cancer receiving chemotherapy with either a taxane an anthracycline or both, were randomized between the use of scalp cooling or not. The patients who received chemotherapy with scalp cooling experienced significantly more often $\leq$50% hair loss after the fourth chemotherapy cycle compared with those patients who were randomized to chemotherapy without scalp cooling. The trial was stopped early after advice from the safety monitoring board because of superiority of hair retention in the participants who received scalp cooling. Another publication in 2017 of Rugo et al. also provided evidence for less hair loss due to the use of scalp cooling in a prospective cohort study among women undergoing non-anthracycline-based adjuvant chemotherapy for early stage breast cancer. The results of both studies, lead in 2017 to FDA approval for scalp cooling in The United States.(20,21) Since then the use of scalp cooling worldwide has increased and the need for improvement of the efficacy of scalp cooling with better selection of patients has been emphasized.

FUTURE RESEARCH PERSPECTIVES

We have shown that shortening the post-infusion cooling time after the administration of docetaxel does not negatively influence the outcome of scalp cooling. This is advantageous for the patient who can leave the hospital earlier and for the logistics of the nursing staff in planning patients for chemotherapy in the outpatient unit. Shortening the post-infusion cooling time in other chemotherapy regimens with reasonable scalp cooling results would be worthwhile to investigate in future studies. If the outcome is positive, it will overcome the disadvantage of a longer stay in the outpatient clinic, since we know that this is one of the reasons why not all oncological outpatient clinics offer their patients scalp cooling.

Adapting the cooling temperature could possibly improve outcomes in a subgroup of patients using scalp cooling. This should be one of the areas to explore further in the future.

Another interesting area is the use of topicals in combination with scalp cooling. Anagen protective agents like ciclosporin or tacrolimus might reduce visible hair loss.(22) However, administration of anagen protecting agents is complicated because the effect must be limited to the hair-follicle epithelium to avoid favoring intracutaneous micro metastasis.(22) Despite this limitation, this is an unexplored area which is interesting to investigate.

Improving cap fitting or adapting the scalp cooling technique will probably not lead to major improvements of the efficacy of scalp cooling. To move the technology forward and improve efficacy, the biological mechanism behind scalp cooling needs to be better understood.(23) From rodent and ex-vivo models we know that hair follicle apoptosis largely depends on p53, a key mediator of cellular damage caused by a stress response.(22) Development of hair follicle research enables direct testing of the damaging effects of cytostatic agents in human hair follicles and allows real-time assessment of the efficacy of potential preventive treatments.(23) Results of such studies could guide our thoughts for larger studies in which for example the addition of topicals to scalp cooling can be investigated in a randomized study. To explore molecular damage-response pathways in plucked human hair, it is important to select the most suitable method. We used the method of Randall et al.(24) Unfortunately, hair follicle research is a very delicate process and depends too much on the availability of dedicated people. We failed in making a robust model for large scale analysis of hair bulbs. The procedure was too time consuming and despite all effort, too much tissue was not evaluable. For a better understanding of the working mechanism of CIA, the method as described by Randall et al. may not be the best option. Solid methods which consistently and reliably can demonstrate molecular damage response pathways are needed. Microscopy with Ultraviolet Surface Excitation (MUSE), which produces high-resolution images of tissue samples within minutes, could be helpful. 3-D scanning, to visualize the entire hair, is another important development which is promising. Hopefully, research in this area will expand and lead to a better understanding of scalp cooling and ways to improve.

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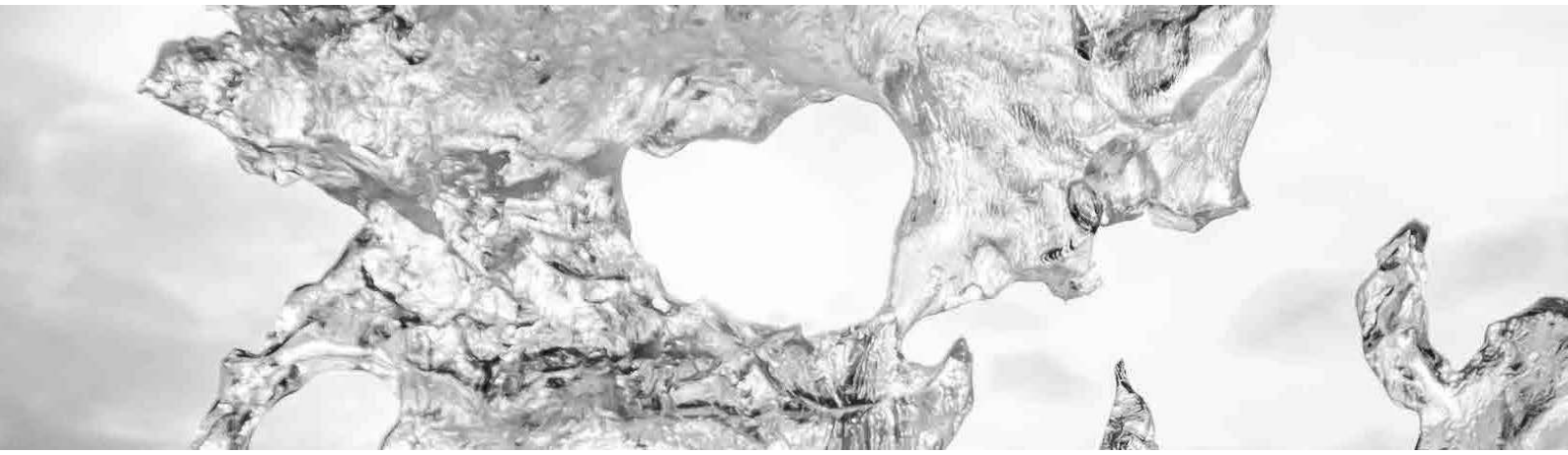
Summary



SUMMARY

Approximately 30,000 patients start chemotherapy each year (Nederlandse Kankerregistratie). Although many side effects of chemotherapy can be controlled, hair loss is still a major problem.(1,2) Every year 15,000 patients are at risk for hair loss as a result of chemotherapy treatment (Nederlandse Kankerregistratie). To prevent chemotherapy induced alopecia, scalp cooling can be used.(3)

Unfortunately, scalp cooling is not effective in every patient. A review in The Oncologist **(Chapter 2)** showed that type and dose of chemotherapy are the most important factors which can influence the outcome of scalp cooling. The influence of patient-related factors (age, gender and hair type) is less convincing or evidence is lacking.(4-7) Decreased liver function and menopausal status may also effect the outcome of scalp cooling, but so far, there is no convincing evidence.(4-7)

Gregory et al. carried out a study in 1982 to search for a threshold temperature, below which hair preservation was likely.(8) Although this study was performed with outdated cooling techniques and a small sample size, it showed that an epicutaneous scalp temperature below 19° C was needed for hair preservation. Nowadays, both cooling technology and chemotherapy regimens have changed. Therefore, a new study was performed to investigate the scalp skin temperature in relation to scalp cooling outcomes. Patients with hair preservation had significantly lower scalp skin temperatures compared to patients who lost their hair. A precise cutoff point could not be detected, but the best results seemed to be obtained when the scalp temperature decreases below 18° C **(Chapter 3)**.

Cooling time was another important factor that could influence scalp cooling outcomes. Scalp cooling is applied before, during and after chemotherapy administration. The pre-cooling time was easy to determine. A temperature measurement of the scalp skin during scalp cooling showed a temperature plateau after 30 minutes of cooling.(9) Determining the best post-infusion cooling time is more complicated. Theoretically, the half-life time of cytostatics should be considered. However, there are large differences between the half-lives of cytostatics, and the pharmacokinetics show considerable interindividual variation. Therefore, the results of different post-cooling times have been investigated. **(Chapters 4 and 5)** In a study investigating a shorter post-infusion cooling time, patients treated with docetaxel were randomized between 20 and 45 minutes after-cooling. The results for both patient groups were similar. **(Chapter 4)** In contrast, in the FEC chemotherapy regimen, a prolonged post-infusion cooling time was investigated. In this study, breast cancer patients were randomized between 90 and 150 minutes post-infusion cooling time. Prolonging the post-infusion cooling time did not significantly reduce hair loss. **(Chapter 5)**

To compare the results of scalp cooling research, it is important to standardize hair loss measurements **(Chapter 6)**. Scalp cooling studies report the use of various measurement scales. Beside the use of these subjective scales, there also exists a method to objectify hair loss with a Hair Check. Therefore, the correlation between subjective measurement scales and an objective measurement with the Hair Check to measure CIA was investigated in clinical practice. The Hair Check proved to be suitable to quantify the amount of hair loss. However, the best method to assess hair loss in clinical practice should be the patient's opinion.

The molecular damage caused by chemotherapy in hair follicles was also investigated (Figure 1) **(Chapter 7)**. It is thought that the mechanism of action is based on vasoconstriction and changed cell metabolism, but the exact mechanism is not known.

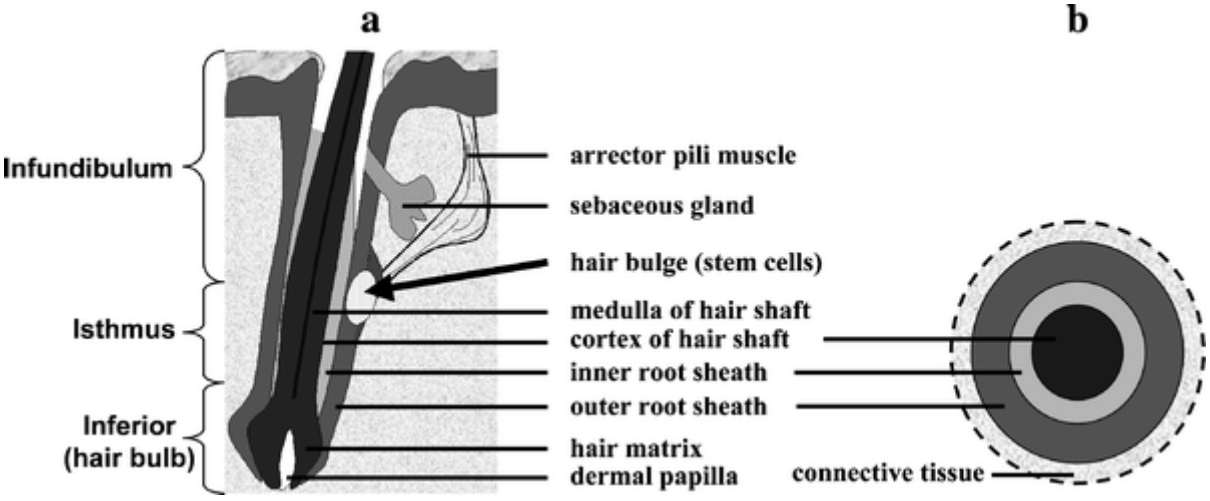


Figure 1. Hair follicle, (a) cross section length, (b) cross section. From: Protection against chemotherapy-induced alopecia. Wang J, Lu Z, Au JL. 2006 Pharm Res.Nov;23(11):2505-14.

Unfortunately, hair follicle research turned out to be a very delicate process. Better and standardized techniques are needed to study the damaging effects of cytostatic agents and to test new potential interventions for hair loss prevention.

Conclusion and future

The Netherlands have made considerable progress in improving scalp cooling and worldwide there is also more attention. Scalp cooling research has provided important implications for clinical practice and there are many opportunities for improvement. Future research should primarily focus on explaining the working mechanism at a molecular level and on individualizing scalp cooling: Would it be possible to easily measure the scalp skin temperature of the scalp during scalp cooling? Would it be possible to identify patients who will benefit from scalp cooling? And additional research to reduce the post-infusion scalp cooling time: Would it be possible to reduce the post-

infusion scalp cooling time for all types of chemotherapy to 20 minutes? Or could scalp cooling perhaps even be stopped immediately after chemotherapy administration? It is important to improve scalp cooling outcomes and to minimize the burden for the patient. Registration of data remains important because the treatment with chemotherapy is constantly changing.

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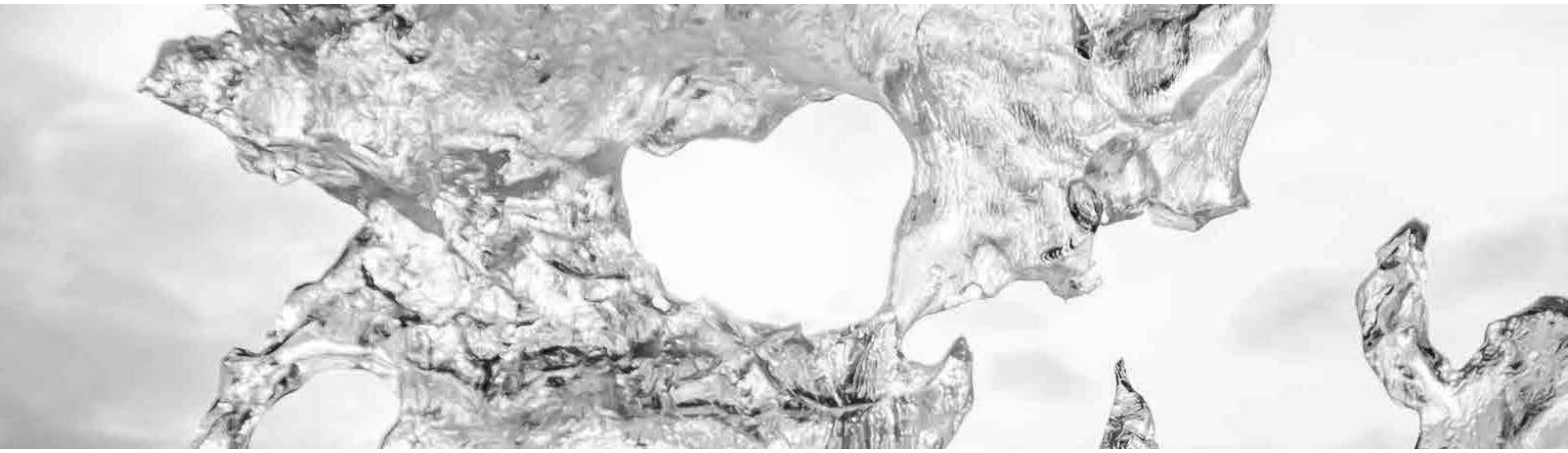
Samenvatting



SAMENVATTING

Jaarlijks starten ongeveer 30.000 patiënten met chemotherapie (Nederlandse kankerregistratie). Hoewel de bijwerkingen van chemotherapie steeds beter worden ondervangen, is haaruitval nog steeds een groot probleem.(1,2) Ieder jaar lopen 15.000 patiënten het risico hun haar te verliezen door chemotherapie (Nederlandse Kankerregistratie). Om haaruitval ten gevolge van de behandeling met chemotherapie te voorkomen kan hoofdhuidkoeling worden toegepast.(3)

Helaas werkt hoofdhuidkoeling niet bij iedereen. Een review in The Oncologist (**hoofdstuk 2**) liet zien dat type en dosering van chemotherapie de belangrijkste factoren zijn voor het effect van hoofdhuidkoeling. De invloed van allerlei patiënt gebonden factoren (leeftijd, geslacht en haartype) is minder overtuigend of ontbreekt.(4-7) Verminderde leverfunctie en menopausale status zouden mogelijk ook van invloed kunnen zijn op het effect van hoofdhuidkoeling, maar ook hiervoor is nog geen overtuigend bewijs geleverd.(4-7).

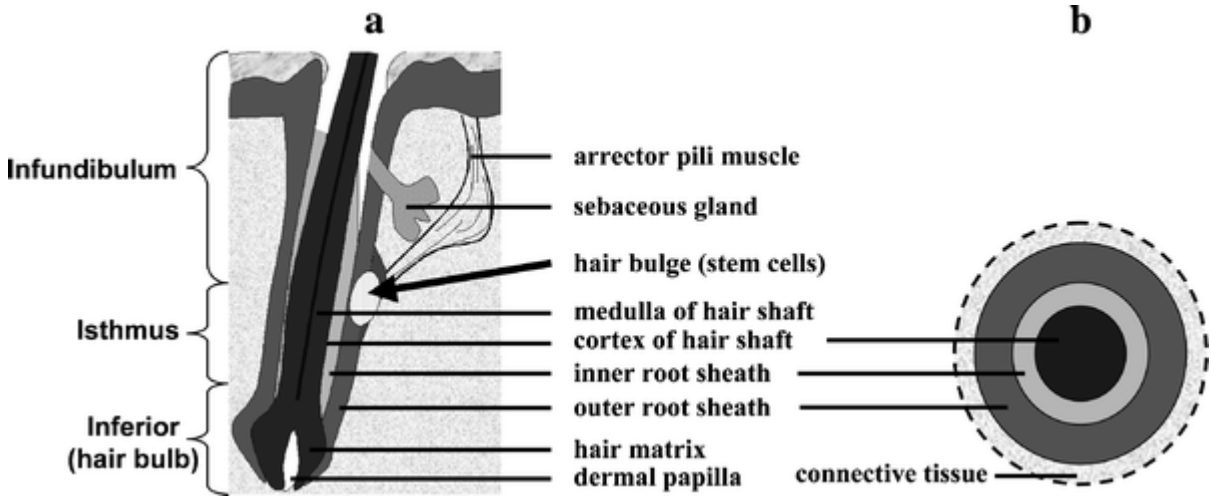
In 1982 verrichtten Gregory et al. onderzoek naar een mogelijk omslagpunt in temperatuur, waaronder hoofdhuidkoeling het beste werkt en waarboven niet.(8) Hoewel dit onderzoek werd uitgevoerd met verouderde koeltechnieken en een kleine steekproef, toonde dit onderzoek aan dat een epicutane hoofdhuidtemperatuur lager dan 19°C nodig was om het haar te behouden. Inmiddels zijn zowel de koeltechniek als de chemotherapie veranderd. Daarom werd opnieuw onderzoek verricht naar de hoofdhuidtemperatuur in relatie tot het effect van hoofdhuidkoeling. Patiënten van wie het haar behouden werd, bleken een significant lagere hoofdhuidtemperatuur te bereiken in vergelijking met patiënten die kaal werden. Er kon geen exacte omslagtemperatuur bepaald worden, maar de beste resultaten leken bereikt te worden met een temperatuur lager dan 18°C (**hoofdstuk 3**).

Een andere belangrijke factor die van invloed kon zijn op het effect van hoofdhuidkoeling was de koeltijd. Hoofdhuidkoeling wordt toegepast door voor, tijdens en na de toediening van chemotherapie een koelkap op het hoofd te plaatsen. De periode van voor-en nakoelen impliceert dat de patiënt langer op de dagbehandeling moet verblijven, wat zowel van de patiënt als van het ziekenhuis bij elke kuur een extra inspanning vergt. De voorkoeltijd was eenvoudig te bepalen. Een temperatuurmeting van de hoofdhuid tijdens hoofdhuidkoeling toonde na 30 minuten koelen een temperatuurplateau, waarna de temperatuur niet verder daalde.(9) Het bepalen van de optimale nakoeltijd is ingewikkelder. Theoretisch moet hierbij rekening gehouden worden met de halfwaardetijd van het cytostaticum. Er bestaan echter grote verschillen tussen de halfwaardetijden van cytostatica, maar ook tussen de farmacokinetiek van verschillende patiënten waardoor deze aanname toch minder waarschijnlijk is. Daarom

zijn de resultaten van verschillende nakoeltijden onderzocht. **(Hoofdstuk 4 en 5)** In een studie naar het verkorten van de nakoeltijd werden patiënten die behandeld werden met docetaxel gerandomiseerd tussen 20 en 45 minuten nakoeltijd. Analyse van de resultaten toonde een vergelijkbaar percentage patiënten dat geen gebruik hoefde te maken van een pruik. **(Hoofdstuk 4)** Wegens minder goede effecten van hoofdhuidkoeling bij FEC-chemotherapie werd bij deze soort chemotherapie juist onderzocht of het verlengen van de nakoeltijd van 90 naar 150 min een beter effect zou hebben. In dit onderzoek werden patiënten met borstkanker gerandomiseerd tussen 90 en 150 minuten nakoelen. Een verlenging van de nakoeltijd leidde echter niet tot een significante vermindering van haarverlies. **(Hoofdstuk 5)**.

Om de resultaten van onderzoek naar hoofdhuidkoeling goed te kunnen vergelijken is het belangrijk om haaruitval op een eenduidige manier vast te leggen (**hoofdstuk 6**). In de diverse onderzoeken naar hoofdhuidkoeling zijn de resultaten altijd subjectief vastgelegd door middel van uiteenlopende meetschalen. Er bestaat inmiddels ook een methode om haaruitval objectief vast te leggen met behulp van een trichometer. Daarom is onderzoek verricht naar de correlatie tussen subjectieve meetschalen en een objectieve meting met de Hair Check om CIA in de klinische praktijk te meten. De hair Check bleek een goede methode om de hoeveelheid haarverlies objectief vast te leggen. De mening van de patiënt zal echter altijd leidend moeten zijn bij de evaluatie van haarverlies in de klinische praktijk.

Er is ook onderzoek verricht naar de precieze werking van hoofdhuidkoeling **(hoofdstuk 7)**. Want hoewel we denken dat het werkingsmechanisme berust op vasoconstrictie en het verlagen van het cel metabolisme, is het exacte werkingsmechanisme nog niet bekend. Er werd onderzoek verricht naar de moleculaire schade die chemotherapie aanricht in haarfollikels (Figuur 1).



Figuur 1. Haar follikel, (a) lengte doorsnede, (b) dwarsdoorsnede. Uit Protection against chemotherapy-induced alopecia. Wang J, Lu Z, Au JL. 2006 Pharm Res.Nov;23(11):2505-14.

Helaas bleek de methode nog te fragiel. Er zijn betere en gestandaardiseerde technieken nodig om onderzoek op haarfollikels van patiënten te verrichten. Hoewel aanvullend onderzoek nodig is, speelt dit type onderzoek een belangrijke rol in toekomstig onderzoek naar de verbetering van hoofdhuidkoeling. Pas als het exacte werkingsmechanisme bekend is, kan onderzoek gerichter worden uitgevoerd en kan hoofdhuidkoeling meer worden toegespitst op de individuele patiënt.

Conclusie en toekomst

In Nederland is de afgelopen jaren een enorme vooruitgang geboekt in de verbetering van hoofdhuidkoeling en ook wereldwijd komt er steeds meer aandacht voor. Er is belangrijk onderzoek verricht en er zijn nog veel mogelijkheden voor verdere verbetering. Toekomstig onderzoek zou zich vooral moeten richten op het verklaren van het werkingsmechanisme op moleculair niveau en op het individualiseren van hoofdhuidkoeling: Hoe kan de temperatuur van de hoofdhuid tijdens hoofdhuidkoeling eenvoudig gemeten worden? Hoe kunnen patiënten geïdentificeerd worden die wel of juist geen baat hebben bij hoofdhuidkoeling? Maar ook aanvullend onderzoek naar het verkorten van de nakoeltijd: Kan de nakoeltijd voor alle soorten chemotherapie verkort worden naar 20 minuten? Kan hoofdhuidkoeling misschien zelfs direct na het inlopen van de chemotherapie gestopt worden? Het is belangrijk om de effectiviteit van hoofdhuidkoeling verder te verbeteren en de belasting voor de patiënt laag te houden. Registratie van gegevens blijft van belang, omdat de behandeling met chemotherapie voortdurend verandert.

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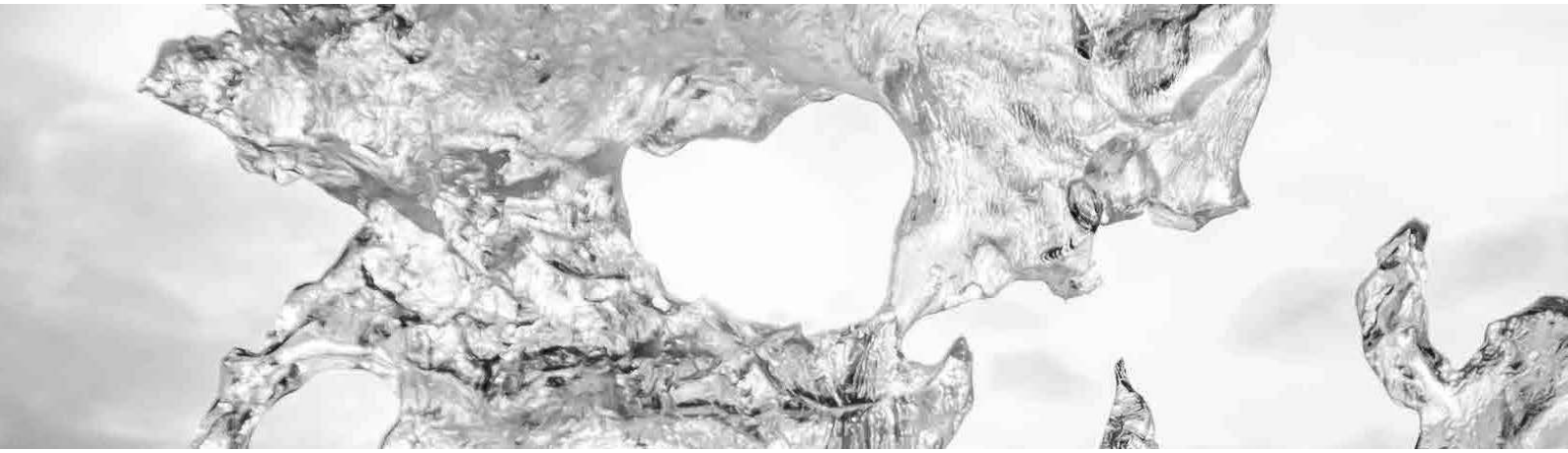
Curriculum Vitae



CURRICULUM VITAE

Manon Komen werd geboren op 19 februari 1981 in Alkmaar
In 1998 behaalde zij het HAVO diploma aan het Han Fortmanncollege in Heerhugowaard.

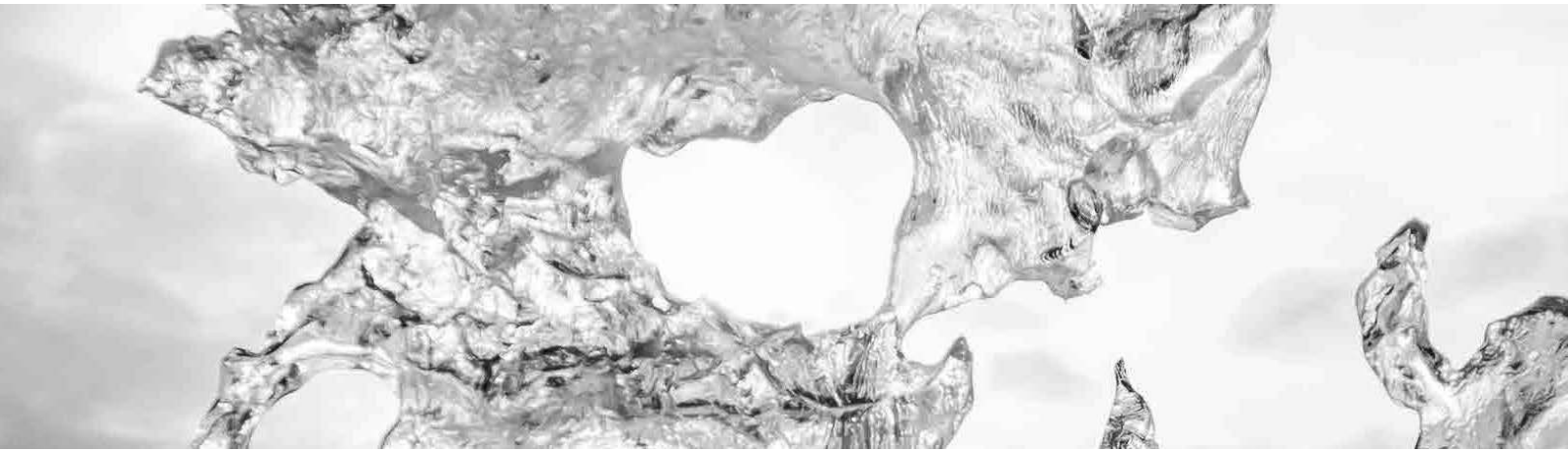
Vervolgens studeerde zij verpleegkunde aan de Hogeschool Alkmaar waar zij in 2002 haar diploma behaalde. Vanaf 2003 studeerde zij verplegingswetenschappen aan de Universiteit van Utrecht. In 2006 behaalde zij haar diploma.

Tijdens haar studie ging zij in dienst bij de oncologie afdeling in het Medisch Centrum Alkmaar. Op deze afdeling startte zij in 2010 met het onderzoek dat heeft geleid tot dit proefschrift.

Manon Komen is getrouwd met Allard Hanrath en samen hebben zij drie kinderen (Jet 2012, Jip 2014 en Saar 2016).

Manon M.C. Hanrath-Komen was born in Alkmaar, the Netherlands on February 19th, 1981. She finished secondary education at Han Fortmanncollege in Heerhugowaard in 1998. She continued to study nursing at Hogeschool Alkmaar. After completing nursing education in 2002 she studied Nursing Sciences at the Utrecht University and graduated 2006. During her study she worked as a research associate at the former Medical Center Alkmaar. In 2010 she started her PhD at Leiden University Medical Center and the Medical Center Alkmaar under the supervision of professor H. Nortier and professor J.J.M. van der Hoeven.

List of publications



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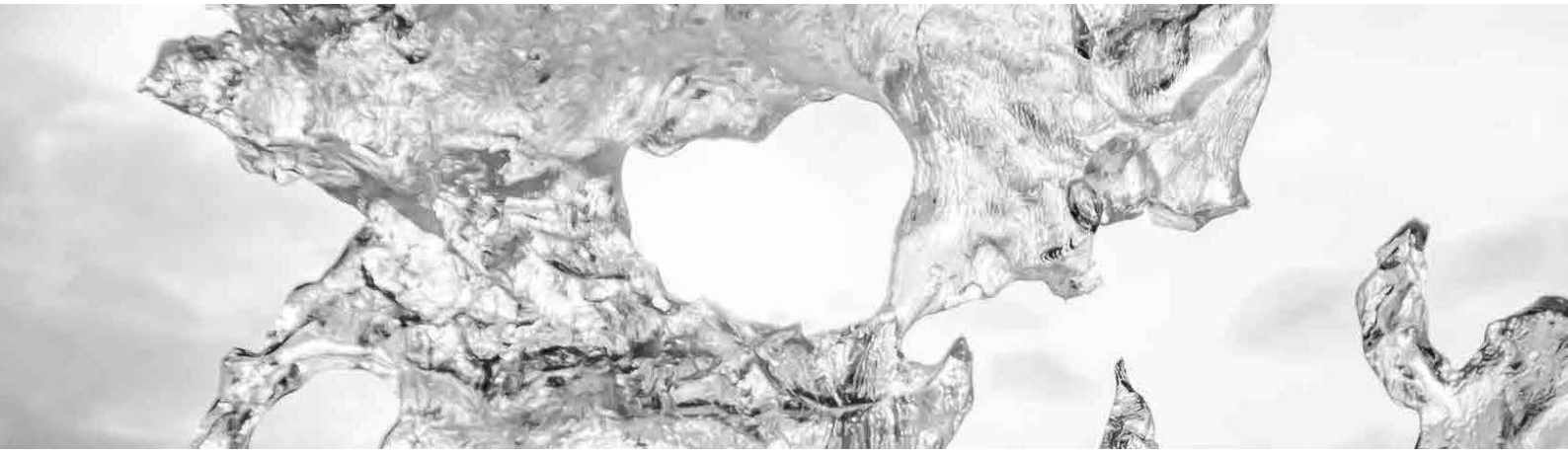
Dankwoord



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