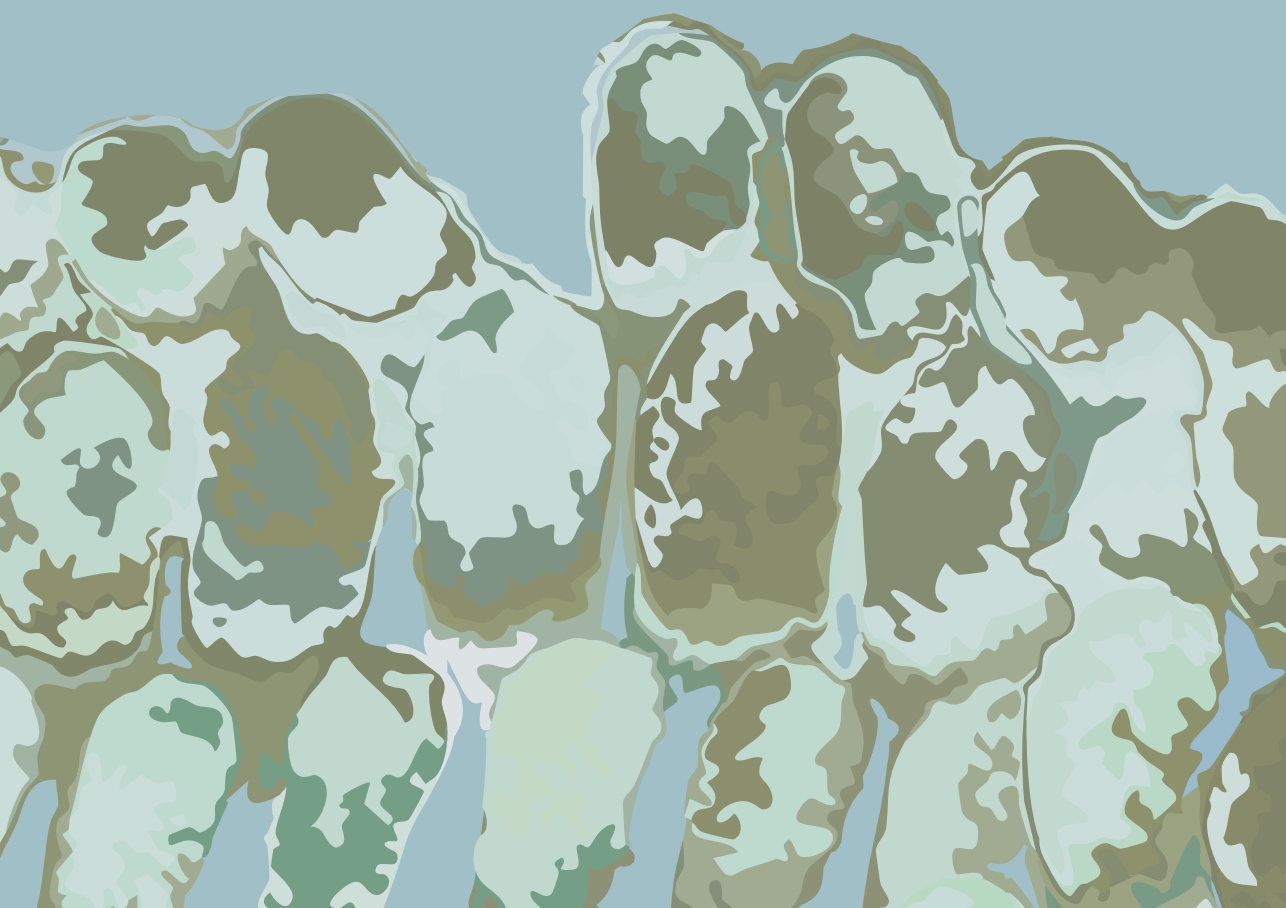
TOWARDS THE **PREVENTION** OF CANCER TREATMENT-RELATED **HEARING LOSS**

CHARLOTTE DUINKERKEN



Towards the Prevention of Cancer Treatment Related Hearing Loss

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Towards the Prevention of Cancer Treatment-Related Hearing Loss

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Aan mijn ouders

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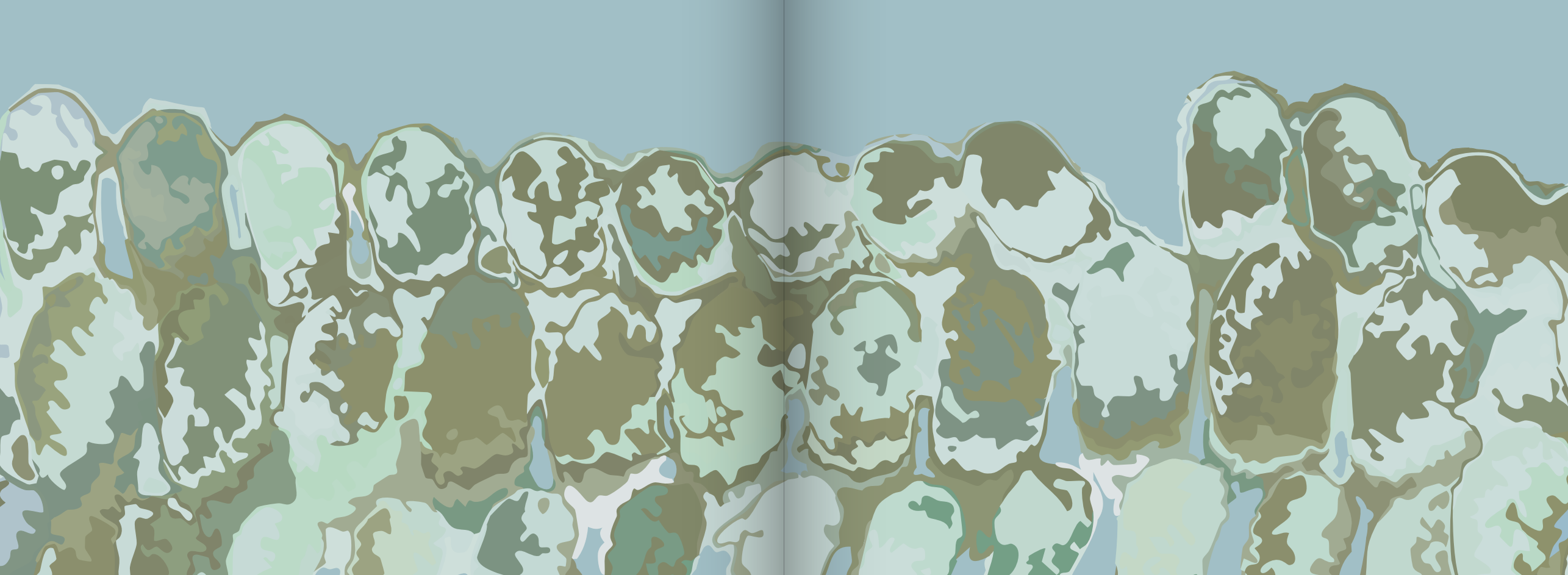
1

INTRODUCTION



Head and neck squamous cell carcinoma

Over 3000 patients were diagnosed with head and neck squamous cell carcinoma (HNSCC) in the Netherlands in 2022. (1) HNSCC includes tumors of mucosal epithelium origin, involving different anatomic subsites, i.e. the oral cavity, nasal cavity, paranasal sinuses, pharynx (oropharynx, nasopharynx, hypopharynx) and larynx, see Figure 1. Due to potential interference of tumor growth with vital functions like speech and swallowing, choices of treatment for patients with HNSCC need to be individualized. According to national guidelines, treatment decisions are made in a multidisciplinary team with expert professionals from different specialties, as amongst others head and neck (reconstructive) surgery, radiation oncology, medical oncology, radiology, nuclear medicine, speech therapy, physical therapy, and pathology. To this respect, several factors are taken into account, including tumor (sub-)site, TNM classification, Human Papilloma Virus status[1], and patient specific characteristics, such as age and comorbidities. (2) In broad terms, the main therapies for lower staged HNSCC are surgery or radiotherapy (RT). Locally advanced disease is generally treated with definite RT, which functions both as an organ preservation strategy and as a treatment modality for non-resectable disease. (2) In patients younger than 70 years old, RT is often combined with concomitant chemotherapy (CRT) using high-dose cisplatin (with a cumulative dose of ≥ 200 mg/m^2).

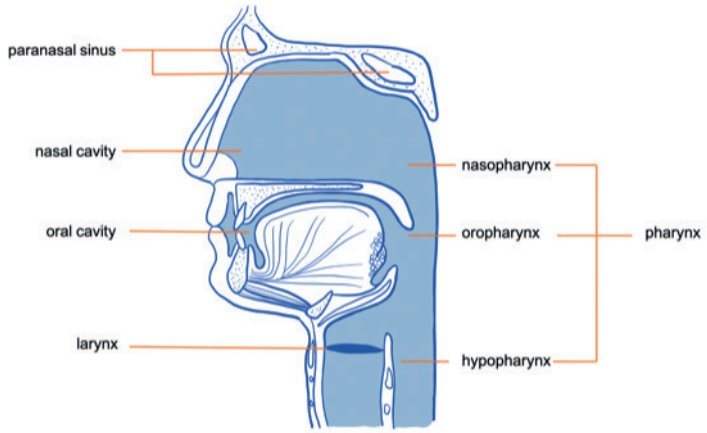


Figure 1. Anatomic sub-sites of the head and neck area.
Created by C.W. Duinkerken[2]

1 HPV tumors have a better prognosis. (2)

2 Maxillary and ethmoid sinuses are not depicted in this midsagittal view.

Cisplatin

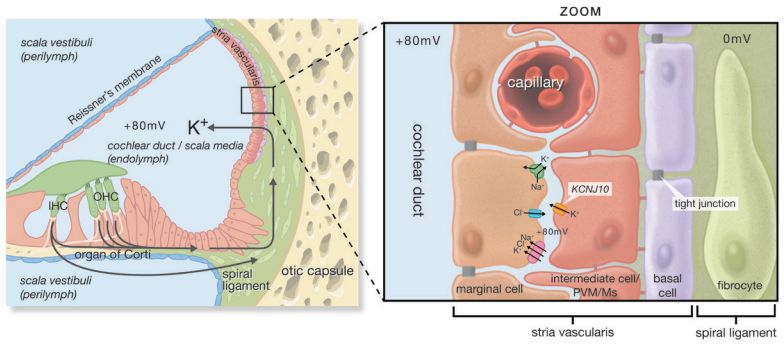
Platinum-derivates, like cisplatin and carboplatin, play an essential role in the treatment of several solid cancers since the 1970's. (3-5) Platinum causes cancer cell-death via intra- and inter-strand crosslinking with the tumour's DNA purine bases. (4) In locally advanced HNSCC, concurrent cisplatin CRT gives an absolute 6.5% overall survival benefit compared to RT alone (6), in which a cumulative cisplatin dose of ≥ 200 mg/m^2 is needed to achieve a significantly increased anticancer efficacy when compared to RT alone. (7, 8) However, both the number of cycles of intravenous cisplatin administration and the cumulative cisplatin (or carboplatin) dose are limited due to dose-limiting toxicities. These include nephrotoxicity, nausea, vomiting, myelosuppression, neuropathy, and ototoxicity. (3, 9, 10) Nephrotoxicity can be prevented by intravenous hyperhydration. However, for ototoxicity and neuropathy (peripheral nerve toxicity) no standardized preventive or curative options are available yet.

Cisplatin-induced hearing loss

The clinical presentation of cisplatin-induced hearing loss (CIHL) is comprised of irreversible, dose-dependent, and symmetrical hearing loss. (3, 4, 11) It typically starts in the (ultra)high frequencies, but as treatment continues, hearing loss may progress to lower frequencies vital for the perception of speech [1 to 4 kHz hearing level (HL)]. As there is heterogeneity amongst studies in the used cisplatin dose and criteria for the definition of CIHL, it is hard to report on the exact incidence of CIHL. A meta-analysis, assessing over a million patients treated with high-dose cisplatin for various solid cancer types, reported that 43.17% of patients develops CIHL. (12) Nevertheless, also higher incidences up to 80% have been reported. (3, 13-15)

At molecular level, multiple processes are involved in the development of CIHL. (16, 17) Cisplatin can enter the cochlea via the stria vascularis, which is a vascularized tissue that functions as a blood-labyrinth barrier, see Figure 2 (*left and right*). (17, 18) The most widely known mechanism of CIHL is the destruction of the outer hair cells within the organ of Corti, see Figure 2 (*left*). (16, 17) Typically, this begins in the hair cells located at the basal cochlear windings, resulting in hearing loss at ultrahigh frequencies. With ongoing platinum therapy (and higher cumulative dose), the lower frequencies will be affected too, due to involvement of the apical windings. (3, 19, 20) Next, other cochlear cells may be damaged, including the inner hair cells, spiral ganglion, and stria vascularis. (16) The stria vascularis is responsible for cochlear homeostasis, which is needed for normal hearing, by generating the endolymphatic potential (+ 80mV) via potassium (K^+) recycling, see Figure 2 (*left*). (17, 18) Furthermore, cisplatin induces hearing loss by the release of toxic reactive oxygen species (ROS). (16, 17) In addition, cisplatin can negatively impact the

cochlea's own defense mechanism against ROS, as cisplatin reduces the cochlear availability of normally otoprotective endogenous antioxidants. (3, 4, 17, 21-23)



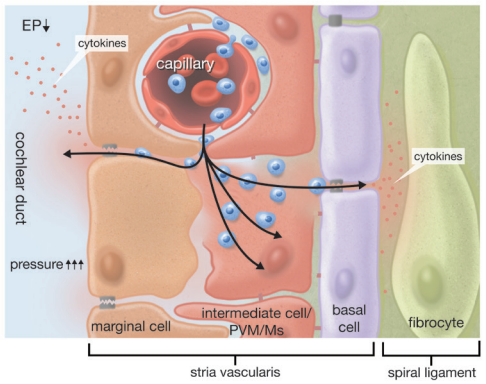


Figure 2. Anatomy of the inner ear.
Left: a schematic cross-section through the human cochlea, depicting the different inner ear structures and the route of potassium (K^+) recycling. *Right (zoom):* a schematic model of the cochlear capillaries, the three cell types of the stria vascularis, the spiral ligament, and the most important ion-transporters for K^+-recycling. (24) Abbreviations: IHC: inner hair cell; OHC: outer hair cell; PVM/Ms: perivascular-resident macrophage-like melanocytes.

Detection of (cancer-)therapy related hearing loss

In this thesis, pure tone audiometry was used for the detection of hearing loss. With this technique, pure tones at different sound intensities for the frequencies 0.125 to 8 kHz (HL) are presented to the subject. Because CIHL usually starts at the ultrahigh frequencies, ultrahigh frequency audiometry - measuring the frequencies from 8 up to 20 kHz (in sound pressure level (SPL)) - was also performed. Audiometry is performed in a soundproof room. At each individual frequency, signals are presented in different sound intensities (from -10 dB up

to +120 dB). The threshold (in dB) at which the person is able to hear the signal is plotted in an audiogram, see Figure 3. To detect therapy-related hearing loss, audiometry needs to be performed pre-treatment (baseline) and post-treatment (follow-up). In the studies included in this thesis, Pure Tone Averages (PTAs) were calculated. The average hearing threshold at 1, 2, and 4 kHz in dB HL was used for the PTA relevant for the perception of speech in noise (further referred to as PTA 1-2-4 kHz). For the perception of ultra-high frequencies (needed for e.g. high-pitched ring tones or high tones in music) we used the average hearing threshold at 8, 10, and 12.5 kHz in dB SPL (further referred to as PTA 8-10-12.5 kHz).

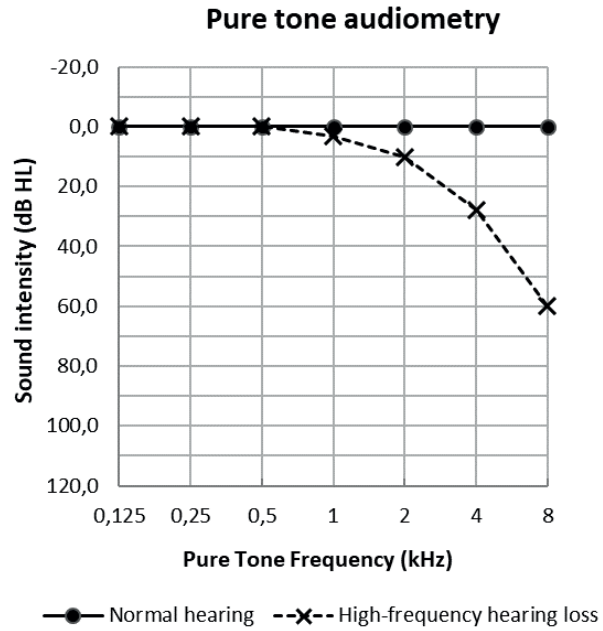


Figure 3. Pure tone audiometry showing normal hearing (uninterrupted line) and hearing loss at high frequencies as seen in presbycusis or cisplatin-related hearing loss (dotted line). In this audiogram, hearing thresholds (in dB HL) for frequencies 0.125 up to 8 kHz are plotted. Abbreviations: dB: decibel; HL: hearing level; kHz: kilohertz.

Prevention of platinum-induced hearing loss

While cure remains unquestionably the cornerstone of treating patients with HNSCC, there is growing focus on the improvement of post-treatment quality of life. As CIHL is characterized by irreversible hearing loss for which no treatment options are yet available, several research groups are still trying to find a preventive strategy to protect against CIHL. Different approaches, including both systemic and topical (transtympanic) administration, have been explored in attempts to prevent from CIHL with varying successes. (12, 25-27) Among these, antioxidants have

arose as particularly promising, with the ability to counteract the damaging release of ROS by cisplatin. The antioxidant sodium thiosulfate (STS) also has the capacity to inactivate cisplatin by binding to its active form. (4, 18, 28) Encouraging effects of intravenous STS against CIHL were demonstrated in two phase III trials in children. (29, 30) Nevertheless, the clinical application of intravenous STS is limited due to its potential interference with cisplatin's antitumor efficacy and potential side effects. (4, 29) The development of a topical approach is therefore needed.

Identification of patients that may suffer from CIHL

In order to select patients that may benefit from prophylaxis against CIHL, the identification of patient groups at risk for the development of CIHL is most important. Apart from cisplatin dose, several co-occurring risk factors for the development of CIHL have been reported. For patients with HNSCC, one of the most important risk factors is RT: a radiation dose to the cochlea of ≥ 30 Gray is known to cause clinically relevant hearing loss, which can be both sensorineural and conductive of origin. (13, 31)

Another risk factor for the development of CIHL is a favorable pre-treatment hearing level). (12, 14, 19, 20, 26, 32) Obviously, excellent hearing is mainly observed in younger patients who do not suffer from age-related hearing loss.

In addition, patients may have a genetic vulnerability for CIHL, as several single-nucleotide polymorphisms (SNPs) have been associated with increased CIHL. (33-37) At a molecular level, cochlear melanoma antigen expression seems also to be involved in cancer therapy related hearing loss in patients treated with T cell receptor gene therapy against metastatic cutaneous melanoma. (38)

From a systemic point of view, vulnerability for cisplatin toxicity may vary pending on interindividual variabilities in the distribution of cisplatin into the tissues. As cisplatin mainly distributes to the fat-free mass, patients with a low skeletal muscle mass (sarcopenia) might experience higher peak dosages of cisplatin. Therefore, they are potentially at risk for platinum-related toxicities, including CIHL. (39)

At last, CIHL depends on the dose intensity of cisplatin. In the Netherlands, the standard of care CRT for HNSCC uses 3-weekly 100 mg/m^2 cisplatin (on days 1, 22, and 43). Approximately 30% of cisplatin-treated patients suffer from dose-limiting toxicities. (40-42) In order to reduce toxicity and increase compliance to cisplatin therapy, recently, some Dutch centers have adapted their schedule and now employ weekly cisplatin infusions of 40 mg/m^2 (during seven consecutive CRT weeks).

Remaining issues in cancer therapy related hearing loss

Based on preclinical models, topical application of STS as an otoprotector against CIHL seems promising. However, this approach needs to be investigated to learn whether transtympanic application of STS is safe and feasible in humans too. In addition, the pharmacokinetics of systemically available cisplatin after topical STS application needs to be assessed, to ensure that the anticancer effect of cisplatin is not compromised by transtympanic STS. Next, it would be desirable to identify which patients are most at risk for the development of clinically relevant CIHL, as these patients may require prophylaxis against CIHL in the future. Also, given the substantial developments in the field of cancer treatments, one should be aware of the option that hearing loss may also arise after new forms of cancer therapy, especially when inner ear structures are being targeted.

AIM AND OUTLINE OF THIS THESIS

The general aim of this thesis is to move forward in the development of preventive strategies against CIHL.

To this respect, our first aim was to assess whether it is safe and feasible to use transtympanic STS injections to avoid systemic anti-cisplatin effects in a phase I randomized clinical trial (**Chapter 2**). The efficacy of this intervention will be studied in an upcoming multicenter phase III randomized clinical trial (protocol in **Appendix II**).

Our second aim was to identify patient-groups that may benefit from preventive strategies in the future: which patients are particularly at risk for the development of CIHL? To gain more insight into CIHL risk profiling the following studies were performed:

- Assessment of the extent of CIHL in young men with testicular cancer treated with high dose cis- or carboplatin for primary or recurrent disease (**Chapter 3**);
- Research into SNPs which might be related to the development of CIHL in patients treated for HNSCC (**Chapter 4**);
- HNSCC patient cohort data analysis to study whether pre-treatment sarcopenia is correlated to increased CIHL after treatment with cisplatin-based CRT (**Chapter 5**);
- Investigation of the difference in CIHL between two different cisplatin dose-intensity CRT schedules for HNSCC (**Chapter 6**).

In the last part of this thesis, we present a novel form of cancer-therapy related hearing loss. We provided a rationale for severe sensorineural hearing loss with unilateral deafness that may occur during T-cell receptor gene therapy applied for metastatic melanoma (**Chapter 7**).

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2

TRANSTYMPANIC SODIUM THIOSULFATE FOR PREVENTION OF CISPLATIN-INDUCED OTOTOXICITY

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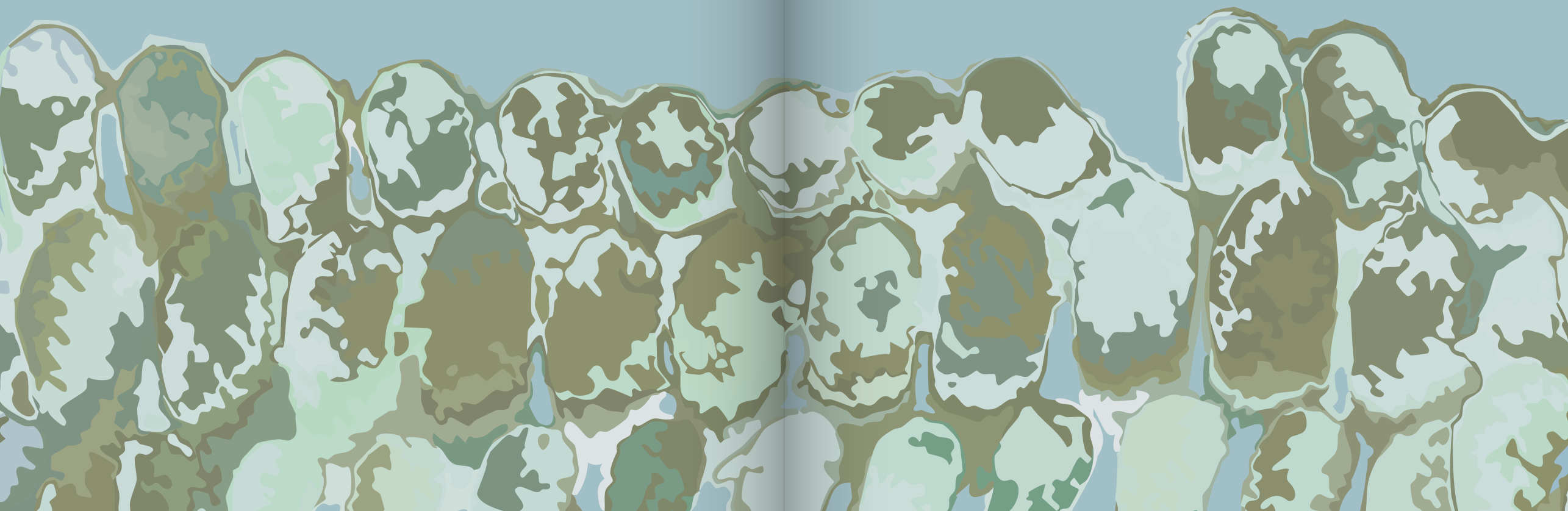

ABSTRACT

Objectives

To determine safety, feasibility, and preliminary activity of transtympanic injection of sodium thiosulfate (STS) against cisplatin-induced hearing loss (CIHL).
DESIGN: Randomized controlled trial.
SETTING: Tertiary cancer hospital.
PATIENTS: Adults to be treated with high-dose cisplatin (≥ 75 mg/m2).
INTERVENTION: Selected by randomization, 0.1 M STS gel on one side and placebo gel on the other side was transtympanically applied to the middle ear 3 hours before cisplatin administration. After amendment, the placebo ear was left untreated.

Main Outcome Measure

Primary outcome was safety and feasibility. Secondary outcomes included pharmacokinetic analysis of systemic cisplatin and preliminary activity of STS. Clinically relevant CIHL was defined as a ≥ 10 dB threshold shift at pure-tone average 8-10-12.5 kHz (PTA8-12.5). Response to STS was defined as a threshold shift at PTA8-12.5 in the STS-treated ear of ≥ 10 dB smaller than the untreated ear.

Results

Twelve patients were treated. Average CIHL at PTA8-12.5 was 12.7 dB in untreated ears and 8.8 dB SPL in STS-treated ears (p = 0.403). Four patients did not develop CIHL. Four out of eight patients with CIHL responded to STS: CIHL at PTA8-12.5 in STS-treated ears was 18.4 dB less compared to untreated ears (p = 0.068). Grade 1 adverse events were reported. Pharmacokinetic results were available for 11 patients.

Conclusion

Transtympanic application of STS was safe and feasible. Based on our pharmacokinetic analysis, we postulate that transtympanic STS does not interfere with the systemically available cisplatin. Our results provide a preliminary proof of concept for transtympanic application of STS in preventing CIHL and warrants further evaluation on a larger scale.

INTRODUCTION

Cisplatin-induced hearing loss (CIHL) occurs in 75-80% of the cisplatin-treated patients. (1-4) CIHL is dose-dependent and characterized by symmetric, bilateral, and irreversible sensorineural hearing loss (SNHL), starting shortly after treatment. (1, 5) Cisplatin destructs the hair cells (HCs) within the organ of Corti. (1, 5, 6) First, the outer HCs located at the basal cochlear turns are first affected, leading to SNHL at the ultrahigh frequencies. After subsequent doses also the apical windings are involved and CIHL progresses to lower frequencies. (1, 7, 8) Furthermore, cisplatin may damage the inner HCs, spiral ganglion, and stria vascularis. (1, 5)

There is an increasing interest in the research field of (preventive) strategies against SNHL, including CIHL. (9) The pathophysiology of CIHL consists of the formation of toxic levels of reactive oxygen species (ROS) and the depletion of otoprotective antioxidants. (1, 4, 5, 7) Distinct antioxidants protect the cochlea from ototoxic stress, but are incapable of dealing with cisplatin-induced cochlear damage. (10) The use of antioxidants that aim to reduce damage caused by ROS may therefore prevent CIHL. Various antioxidants, including sodium thiosulfate (STS) and N-acetylcysteine, have been shown to scavenge ROS and reverse endogenous antioxidant depletion. (5, 11-13) Furthermore, they inactivate cisplatin by binding to its active form. (5, 12, 13)

Two recent phase III trials on the prevention of CIHL in children showed promising otoprotective effects of the antioxidant sodium thiosulfate (STS) when administered intravenously during cisplatin chemotherapy. (14, 15) Brock et al. (15) showed that the incidence of CIHL was 48% lower in children treated with cisplatin plus intravenous STS compared with cisplatin alone (relative risk, 52%; 95% confidence interval, 0.33–0.81, p = 0.002). Similarly, in the study of Freyer et al. (14) CIHL occurred in 56% of the children treated with cisplatin alone and in 29% when treated with simultaneous STS (p = 0.00022). Clinical application of intravenous STS may however be restricted by its side-effects and potential interference with cisplatin's antitumor activity. (5, 15) Accordingly, Freyer et al. (14) reported lower overall survival in disseminated disease when treated with additional STS (45%) compared with cisplatin alone (84%) (p = 0.009). Adverse events have been reported that were likely attributed to intravenous STS administration, including tumor progression, grade 3 infection, neutropenia, electrolyte disturbances, and anemia. (14, 15) A topical approach of STS application may be advantageous in preventing CIHL while preserving cisplatin's antineoplastic effect. Several proof-of-principle studies showed that transtympanic application of antioxidants is safe and feasible. (11, 16, 17) Interestingly, in guinea pigs, higher perilymph STS concentrations were achieved after transtympanic application when compared to intravenous infusion. (12) This phase I study evaluated the safety and feasibility

and aimed to determine preliminary activity of transtympanic application of STS gel in adults treated with cisplatin dosed ≥ 75 mg/m2 for advanced solid tumors.

METHODS

Study design

This proof-of-concept phase I trial consists of two cohorts. Cohort A was a single-blind, placebo-controlled study. One ear was treated with STS gel and the other with placebo gel. Cohort B was a nonblinded, non-placebo-controlled study. Here, one ear was treated with STS gel and one was left untreated.

Setting

The study was performed at the Netherlands Cancer Institute in Amsterdam, the Netherlands. The protocol was approved by the institutional medical research ethics committee and registered in the European Clinical Trials Database (EudraCT: 2012-004653-80).

Patients

Patients of 18 years or older who were to be treated with cisplatin at a dose of ≥ 75 mg/m2 for lung or head and neck (HNSCC) cancer were eligible. If patients were to receive concomitant radiotherapy, the maximum cochlear dose was 30 Gray as to avoid radiotherapy-induced hearing loss. (2, 18, 19) Exclusion criteria were symptomatic brain or leptomeningeal metastases and relevant otological history (e.g., conductive hearing loss). All patients gave written informed consent. Patients were considered evaluable after the completion of one cycle of cisplatin including study medication.

Intervention

The ear to be treated with the STS gel was assigned by simple unstratified randomization in both cohorts at the institutional trial center using ALEA Clinical (Forms Vision BV). Patients were enrolled by their treating physician. In cohort A, two syringes with 2.0 ml 0.5% sodium hyaluronate (HYA) based gels were used: one without STS (placebo) and one with 0.1 M STS. In cohort B only the STS gel was prepared. Syringes with study medication were warmed up to 37 °C for 30 minutes in an incubator (CULTURA M, Almedica AG) to prevent caloric symptoms during injections. The syringe was connected to the needle (Braun, Pencan 25G) via a 10 cm infusion line (BD Becton Dickenson Connecta). The

needle was bended to approach the eardrum perpendicularly under sight. Topical anesthesia was applied by 3x3 mm gauzes soaked in xylocaine 10% (lidocaine 100 mg/ml, AstraZeneca) applied on the eardrum before the placement of the grommet and injections. In cohort A, the gels were administered through a grommet, which was placed for venting air to prevent barotrauma while injecting. During this procedure there was backflow of gel along the infusion needle into the external ear canal. A different protocol was chosen for cohort B: the grommet was still placed for ventilation, but STS was injected directly through the posterior part of the eardrum (Fig. 1). During administration of the gel the patient was positioned with the upper body 30 degrees upward. After injection the patient's head was turned 45 degrees contra-laterally to allow the gel to reach the round window. Patients remained in this position for 30 minutes and were instructed to keep swallowing and talking to a minimum. Cisplatin was given 3 hours after STS administration. Concomitant chemotherapy or radiotherapy was administered as per local protocol. Follow-up was performed within 7 days before start of each cisplatin cycle, and within 1 and 3 months after the last cycle. This consisted of audiometry, physical examination, registration of adverse events, and laboratory assessments (hematology, chemistry).

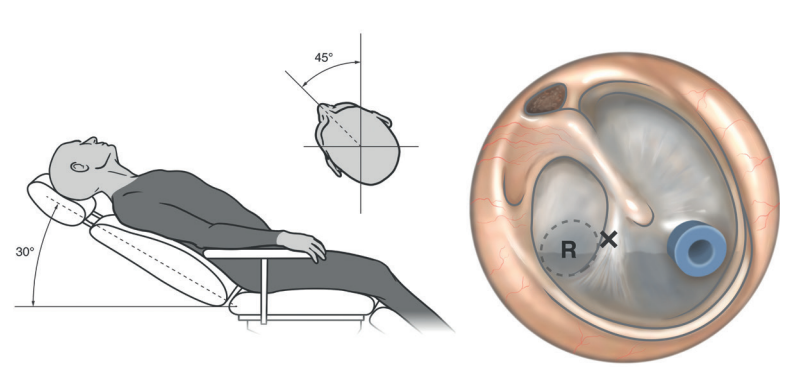

Figure 1: The position of the patient during transtympanic injection (left) and the eardrum (right).
Right: The patient is lying with the upper body positioned 30 degrees upwards and the patient's head is turned 45 degrees contralaterally. *Left:* The grommet is placed for ventilation of the middle ear and the sodium thiosulfate containing gel is injected via the needle directly through the posterior part of the eardrum (X). The gel fills the middle ear, which enables exposure of the round window (R) to the drug.

The ear to be treated with the transtympanic STS gel was assigned by randomisation in both cohorts using a randomisation program at the institutional trial centre. Patients were enrolled by their treating physician.

Cisplatin infusion was started three hours after STS administration. If concomitant chemotherapy or radiotherapy was to be administered, this was done as per local

protocol. Follow-up was performed within 7 days prior to start of each cisplatin cycle, and within 1 and 3 months after the last cycle and consisted of audiometry, physical examination, registration of adverse events and safety laboratory assessments consisting of haematology and serum chemistry.

Outcome measures

We aimed to determine safety and feasibility and the preliminary activity of transtympanic injection of STS against CIHL. Safety and feasibility were evaluated using adverse events registered according to the Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). (20) To evaluate whether the transtympanically administered STS does interfere with systemically available cisplatin, pharmacokinetic (PK) sampling of cisplatin was performed for comparison with previously published data. Samples were drawn predose, at the end of cisplatin infusion and 1, 2, 3, 4, and 18 hours thereafter. Blood was collected in a 10.0 ml heparin tube, which was centrifuged at 1,500 g for 10 minutes at 4 °C. Of the plasma 2.0 ml was transferred to a 2.0 ml Eppendorf tube and stored at -20 °C (total platinum). A plasma ultra-filtrate tube (Centrifree ultra-filtrate tubes, Merck Millipore Ltd.) was filled with plasma and centrifuged at 1,800 g for 10 minutes. The ultra-filtrate was transferred to a 2.0 ml Eppendorf and stored at -20 °C (unbound platinum). Platinum levels were measured using a validated inductively coupled plasma mass spectrometer method (ICP-MS).(21) The lower level of quantification was 7.50 ng/L. (21) PK parameters were calculated using validated scripts in the software package R version 3.0.1. The maximum observed plasma concentration (C_{max}) and area under the plasma concentration time curve from start of the cisplatin infusion (time = 0) to 22 hours ($AUC_{0\text{-}22h}$) were reported.

Efficacy was assessed using standard audiometry, including air conduction (AC) and bone conduction (BC) thresholds, performed in a sound-proof booth using the Decos Audiology Workstation. If thresholds at 8 kHz were not available for ultrahigh frequency audiometry, we converted them from pure-tone audiometry thresholds into dB SPL following ISO 389-1**.** (22) If the threshold level was beyond the audiometer's maximum output, we computed the threshold by adding 5 dB to this maximum. Audiometric testing was performed pretreatment (baseline), after each cisplatin cycle (posttreatment) and after 3 months (follow-up). We marked a conductive component if the average AC threshold at 0.5, 1, 2, and 4 kHz was ≥ 10 dB poorer than the average BC threshold. If there was a conductive component during or after therapy, we took BC thresholds for analysis.

Hearing thresholds of < 10 dB are accepted to indicate (sub)-normal hearing, according to the CTCAE and ASHA guidelines. (20, 23) Since cisplatin first affects the ultrahigh frequencies, clinically relevant CIHL was defined as ≥ 10 dB SNHL

at pure-tone average 8-10-12.5 kHz ($PTA_{8\text{-}12.5}$). Clinically relevant response to STS was defined as a SNHL in the placebo (cohort A) or untreated (cohort B) ear exceeding SNHL in the STS-treated ear by ≥ 10 dB at $PTA_{8\text{-}12.5}$. Next, the patients were divided into three groups: 1) patients without CIHL, 2) patients with CIHL who responded to STS, and 3) patients with CIHL but no response to STS.

Statistics

The low standard errors for audiometric differences before and after cisplatin that were shown in a previous study allowed us to use a small number of patients. (24) To study whether there was a significant difference in $\Delta PTA_{8\text{-}12.5}$ between the STS ears and the untreated ears, an exact Wilcoxon signed-rank test for paired samples was used. *p* values of ≤ 0.05 were considered statistically significant.

RESULTS

Sixteen patients were enrolled. Four patients withdrew consent, of whom three did not start therapy: one due to pain from bone metastases, one considered logistics to be troublesome, and one without a formal reason. Another patient with a narrow ear canal withdrew consent after a painful grommet insertion and was not available for evaluation. Patients were treated between June 2013 and October 2018. For baseline characteristics see Table 1. Eight patients were male (67%). The median age was 60 (range 46–67) years. Cisplatin was discontinued in two patients due to nephrotoxicity**.**

In total, 34 STS injections were given in 12 patients. In cohort A there was backflow of gel into the external meatus after application through the grommet. An average volume of 0.2 ml (range, 0.1–0.3 ml) was injected. The technique was improved in cohort B: The gel was injected by direct transtympanic puncture of the eardrum. A mean volume of 0.37 ml (range, 0.3–0.5 ml) could be administered.

After the insertion of the grommet and application of the gel(s) temporary, modest adverse events (AEs) were reported. After placement of the grommet and upon request, patients reported modification of sound perception in quality (not quantity), which could not be objectified by audiometry. One patient needed to receive subcutaneous local anesthesia with lidocaine 2% before grommet insertion and experienced grade 1 vertigo, which resolved within 4 hours. Only grade 1 AEs were reported for the transtympanic injections, which resolved within few hours. One patient with a narrow ear canal reported grade 1 pain, but continued therapy. Some patients reported fullness of the middle ear after the application of the gel (grade 1), which resolved within 1 hour. No persistent otitis media as

a result of gel application occurred. Four patients reported grade 1 tinnitus after therapy (three bilateral and one in the placebo-treated ear). AEs to be attributed to cisplatin doublet treatment or malignancy and unrelated to STS injection, were renal failure, electrolyte disturbances, anorexia, dermatitis, and dysphagia. No grade ≥ 2 neither serious AEs (SAEs) related to STS injections were observed. The six reported SAEs occurred in patients treated with concomitant radiotherapy and 100 mg/m^2 cisplatin and included hospitalization due to renal failure, neutropenia, and dehydration.

	Cohort A (n = 6)	**Cohort B** (n = 6)	**Total** (n=12)
Age			
Median (range), years	60 (46-67)	59 (46-63)	59 (36-67)
Sex			
Male	5 (83%)	3 (50%)	8 (67%)
Female	1 (17%)	3 (50%)	4 (33%)
WHO Performance Score			
0	5 (83%)	6 (100%)	11 (92%)
1	1 (17%)	0	1 (8%)
Tumor type			
NSCLC	3 (50%)	1 (17%)	4 (33%)
Mesothelioma	3 (50%)	0	3 (24%)
Thymus carcinoma	0	1 (17%)	1 (8%)
HNSSC	0	4 (67%)	4 (33%)
Number of cycles cisplatin			
Median (range)	3 (2-4)	3 (1-4)	3 (1-4)
Cisplatin dose			
75 mg/m^2	6 (100%)	2 (33%)	8 (67%)
Number of cycles, median (range)	3 (1-4)	4 (4)	3.5 (1-4)
100 mg/m^2	0	4 (67%)	4 (33%)
Number of cycles, median (range)	-	2 (1-3)	2 (1-3)

Table 1: Baseline characteristics. Abbreviations: HNSSC = head and neck squamous cell carcinoma; NSCLC = non-small cell lung cancer; WHO = World Health Organization.

For all 12 patients, the mean thresholds for the STS-treated ears and the untreated/placebo ears as measured at baseline, posttreatment and at follow-up are depicted in Figure 2A and Table 2. The mean threshold shift at PTA 8-10-12.5 kHz ($\Delta PTA_{8\text{-}12.5}$) was 12.7 dB in the untreated ears and 8.8 dB in the STS-treated ears (p = 0.402). Four patients did not develop CIHL. Their platinum PK curves were comparable to the rest of the group. Eight patients developed CIHL, of whom four (50%) responded to STS. The average difference in $\Delta PTA_{8\text{-}12.5}$ between STS-treated ($\Delta PTA_{8\text{-}12.5}$ of 14.1 dB) and untreated ears ($\Delta PTA_{8\text{-}12.5}$ of 20.2 dB) in these eight patients was 6.1 dB in favor of the STS-treated ears (p = 0.141) (Fig. 2B). Regarding the four responders, the average difference in $\Delta PTA_{8\text{-}12.5}$ between the STS-treated ears ($\Delta PTA_{8\text{-}12.5}$ of 6.8 dB) and untreated ears ($\Delta PTA_{8\text{-}12.5}$ of 25.2 dB) was 18.4 dB (p = 0.068) (Fig. 2C).

Four patients did develop CIHL but did not respond to STS. This group included one patient treated in cohort A who received an estimated volume of 0.1 ml STS gel during all three injections due to backflow of the gel through the grommet into the ear canal. Another nonresponder was unable to stay in the desired position for 30 minutes due to grade 1 vertigo after anesthetics with subcutaneous lidocaine 2% injection. His movements may have troubled absorption of the gel by the round window. PK parameters Cmax and $AUC_{0\text{-}22h}$ are shown in Table 2. PK data of one patient treated with 100 mg/m^2 are missing, as PK samples were not taken due to logistic reasons.

		Cisplatin 75 mg/m^2 (n=8)		**Cisplatin 100 mg/m^2** (n=3)	
		Total platinum	**Unbound platinum**	**Total platinum**	**Unbound platinum**
C_{max} ug/ml	Mean ± SD (CV)	2.0 ± 0.57 (27.1%)	0.77 ± 0.18 (22.8%)	1.91 ± 0.31 (16.1%)	1.10 ± 0.52 (4.7%)
$AUC_{0\text{-}22h}$ ug*h/ml	Mean ± SD (CV)	29.77 ± 5.90 (19.7%)	3.02 ± 0.59 (19.5%)	22.39 ± 6.64 (28.4%)	4.29 ± 1.85 (43.2%)

Table 2: Pharmacokinetic parameters. Abbreviations: C_{max} indicates maximum observed concentration; $AUC_{0\text{-}22}$, area under the plasma concentration time curve from t = 0 to 22 hours; n, number of patients; SD, standard deviation; CV, coefficient of variation.

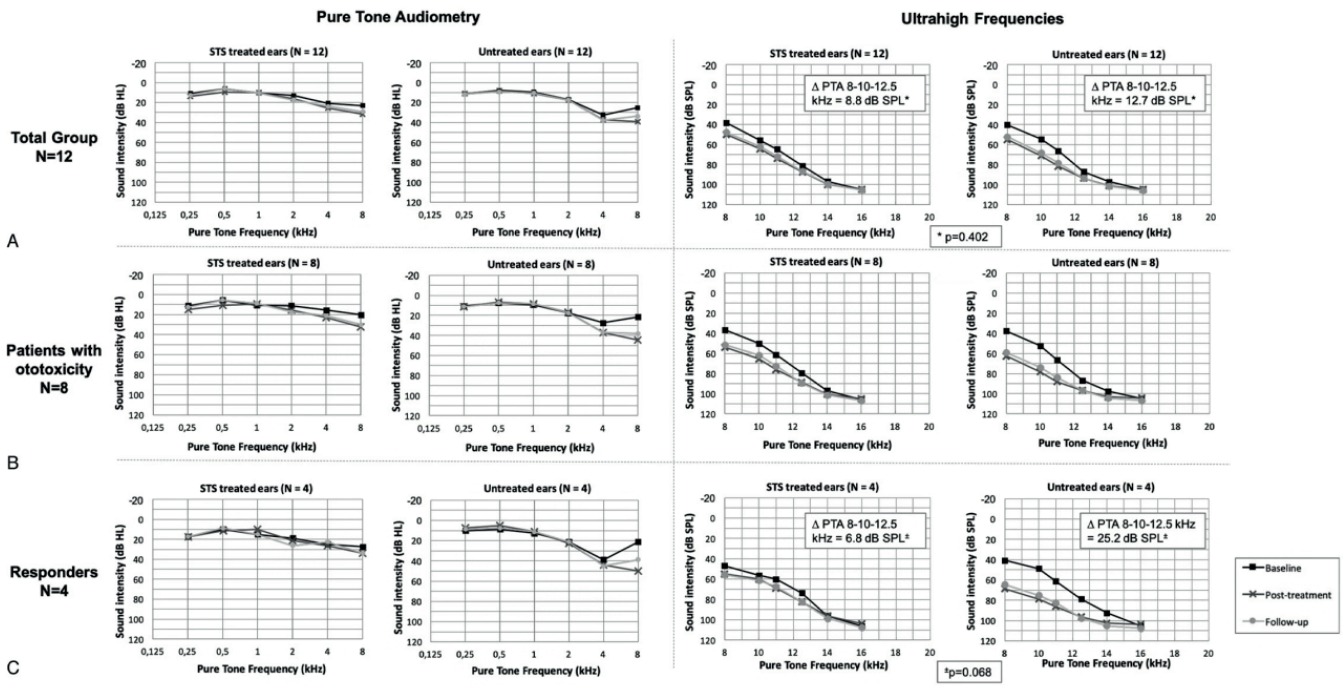

Figure 2. Audiometric results.

The curves show air conduction thresholds at baseline, directly after the last cycle of cisplatin (posttreatment) and at follow-up. *Left*: Pure-tone audiometry, mean of the thresholds in dB hearing level (HL). *Right*: Ultrahigh frequency audiometry, mean of the thresholds in dB sound pressure level (SPL). In some patients the thresholds at 8 kHz were not available for ultrahigh frequency audiometry. These values have been converted from the threshold measured with regular pure-tone audiometry into dB SPL following ISO 389-1. **A)** Audiometric results of the 12 patients in both cohorts A and B. The difference between the posttreatment pure-tone average (PTA) 8-10-12.5 kHz and baseline PTA 8-10-12.5 kHz is 3.9 dB SPL (12.7–8.8 dB, p = 0.402, Exact Wilcoxon test for matched pairs). **B)** Results of the eight patients who developed ototoxicity. Ototoxicity is defined as a shift of ≥ 10 dB at ΔPTA 8-10-12.5 kHz (ΔPTA 8-10-12.5 kHz = post-treatment PTA 8-10-12.5 kHz minus baseline PTA 8-10-12.5 kHz). **C)** Audiometric results of the four patients who developed ototoxicity and responded to transtympanic sodium thiosulfate (STS) injection. Response is defined as patients with ototoxicity with ΔPTA 8-10-12.5 kHz in the untreated ear exceeding Δ PTA 8-10-12.5 kHz in the STS-ear by ≥ 10 dB. The difference between posttreatment PTA 8-10-12.5 kHz and baseline PTA 8-10-12.5 kHz is 18.4 dB SPL (25.2–6.8 dB, p = 0.068, Exact Wilcoxon test for matched pairs).

	STS-treated ears	Untreated ears	*p*-value[a]
Total group (N = 12)			
Δ PTA 0.5-1-2 kHz	2.1 dB HL	0.7 dB HL	
Δ PTA 1-2-4 kHz	2.5 dB HL	2.2 dB HL	
Δ PTA 8-10-12.5 kHz	8.8 dB SPL	12.7 dB SPL	0.402
Responding patients (n = 4)			
Δ PTA 0.5-1-2 kHz	-0.4 dB HL	-1.3 dB HL	
Δ PTA 1-2-4 kHz	-0.4 dB HL	1.7 dB HL	
Δ PTA 8-10-12.5 kHz	6.8 dB SPL	25.2 dB SPL	0.068

Table 3: The threshold shifts of the pure-tone averages (PTAs) of all 12 patients (*up*) and the 4 patients that developed ototoxicity and responded to the STS gel (*down*).
Ototoxicity is defined as Δ PTA 8-10-12.5 ≥ 10 dB. Response is defined as patients with ototoxicity in which Δ PTA 8-10-12.5 kHz in the untreated ear exceeds Δ PTA 8-10-12.5 kHz in the STS-ear with ≥ 10 dB. Δ PTA is measured as the PTA directly after the last cycle of cisplatin minus the baseline PTA.
[a]Exact Wilcoxon test for matched pairs.
HL indicates hearing level; kHz, kilohertz; PTA, pure-tone average; SPL, sound pressure level; STS, sodium thiosulfate.

DISCUSSION

Application of transtympanic STS was safe and feasible. Four out of eight patients with clinically relevant CIHL showed a relevant interauricular difference in posttreatment hearing capacity. In these four patients, the STS ear benefited compared with the other ear with an average difference in hearing loss of 18.4 dB at $PTA_{8\text{-}12.5}$. Although not significantly different, the results of the above-mentioned four patients rise above random observations in four single patients and suggest the first in-human clinically relevant efficacy of transtympanic application of STS to prevent CIHL in adults (Table 3).

The pathophysiology of CIHL is multifactorial. Cross-linking between platinum and DNA and the accumulation of cisplatin in cochlear structures induce the formation of toxic levels of reactive oxygen species (ROS). (1, 4, 7, 10) Excessive ROS leads to depletion of otoprotective cochlear antioxidants. (1, 4, 7, 10) This is followed by apoptosis of HCs and the stria vascularis. (1, 6, 7) Also, cisplatin is responsible for adenine dinucleotide phosphate oxidase 3 (NOX3)-mediated generation of ROS in the organ of Corti and spiral ganglion. (1, 10) Next, hydroxyl radicals are produced, causing HC damage by destructive calcium influx. (1) Cell death may also occur after calcium influx into HCs due to activation of the transient receptor potential vanilloid 1 channel (TRPV1). (1)

Preventive strategies aiming to reduce the production or activity of ROS within the inner ear can be of value to prevent CIHL. Antioxidants with a thiol group, including STS and N-acetylcysteine, have been shown to scavenge ROS and reverse endogenous antioxidant depletion. (5, 11-13) Furthermore, they inactivate cisplatin by binding to its active form. Preclinical in vivo studies showed that both intravenous and transtympanic administered antioxidants are able to prevent CIHL. (25, 26)

The current study was a phase I study, designed to assess safety and feasibility. Therefore, the study was not powered aiming to prove efficacy of transtympanic STS against cisplatin-induced hearing loss. Furthermore, ideally a double blinding and placebo-controlled procedure would have been used to assess efficacy. No placebo was used in cohort B as this was found too troublesome and time-consuming. One patient withdrew consent as the insertion of the grommet was too painful and was therefore not included in the analysis. In future studies an intention to treat analyses should be included. Also, a larger phase II trial is needed to adequately prove the efficacy of transtympanic STS against CIHL.

Several other clinical trials assessed transtympanic drugs for the prevention of CIHL. Two studies reported significant hearing preservation by transtympanic application of N-acetylcysteine. Riga et al. (11) showed that the threshold change at 8 kHz was 7 dB greater in patients treated with transtympanic N-acetylcysteine compared to untreated patients (p = 0.005). Sarafraz et al. (16) showed significantly better hearing preservation at 4 and 8 kHz when transtympanic N-acetylcysteine was injected compared with transtympanic dexamethasone. As both studies did not perform PK analysis, it remains uncertain whether transtympanic N-acetylcysteine interferes with the systemic exposure to cisplatin.

Rolland et al. (17) also evaluated transtympanic STS injections in 13 patients treated with concomitant radiotherapy and cisplatin for HNSCC. Hearing loss was 1.3 dB less in STS-treated ears compared to untreated ears at frequencies from 3 to 10 kHz. They injected a higher concentration of STS (0.5 M versus 0.1 M) in a smaller volume (0.1 ml versus 0.3–0.5 ml). We think that a larger volume results in improved exposure of the round window to the gel. Also, it seems important to use a high-viscosity gel that does not rapidly flow through the Eustachian tube. The timing of injection differed to ours: Rolland et al. injected mean 20.5 hours before cisplatin infusion, whereas we injected 3 hours before cisplatin infusion. We chose this timing since Berglin et al. showed impressive HC protection in guinea pigs when injecting 3 hours before cisplatin. (26) Preclinical PK results of transtympanic STS in guinea pigs are inconsistent: Berglin et al. showed stable perilymphatic STS concentrations between 1 and 3 hours after injection, while Schroeder II et al. (12) reported that perilymphatic STS has a short half-life of 44.4 minutes (dose, 250 mg/ml). Furthermore, Viglietta et al. recently published

their results of a phase I study evaluating transtympanic application of STS in 42 healthy volunteers. Application of different doses of STS (0.15 M, 0.5 M, 1.0 M, 1.5 M) was safe and feasible. (27)

Since cisplatin can be detected in the cochlea immediately after infusion and its elimination rate from the inner ear is slow, (28) one may advocate to inject STS directly before cisplatin infusion. However, these pre-clinical results cannot be translated directly to humans, as the human's otic capsule is thicker and the round window permeability is lower compared to guinea pigs. (5, 26) We think that adequate timing of STS administration is essential and demands future studying.

Furthermore, both the exposure of the gel to the round window and the uptake of STS in the perilymph may depend on anatomic variations, the patient's position and otologic pathology (e.g., otosclerosis, otitis media). These factors might explain the inter-individual differences in response to transtympanic STS that we found.

One of the strengths of the study is that we performed PK analysis of systemic cisplatin. When considering an average body surface area of 1.8 m^2, patients received about 0.43 mmol cisplatin (≥ 75 mg/m^2). The amount of STS administered ranged from 0.01 to 0.05 mmol. Since STS binds to platinum in a 1:1 ratio, < 10 % of the molar weight of cisplatin could be neutralized. However, the low oral bio-availability of STS restricts the amount of STS available in the systemic circulation. (29) A comparison of our PK results with literature is difficult, as STS-bound platinum may be detected as unbound platinum by the ICP-MS method. (30) A comparison of the unbound fraction is therefore not useful. The levels of unbound platinum were however in line with previously reported results. Interestingly, Viglietta et al. performed PK analysis of systemically available STS and state that the measured plasma STS levels are expected to be too low for interference with the antitumor effect of cisplatin. (31) Based on literature and the poor oral bio-availability of STS, we postulate that transtympanic STS does not interfere with the systemically available cisplatin

CONCLUSION

Transtympanic injection of STS was safe and feasible. In this small population of 12 patients, we were able to show hearing preservation by transtympanic STS in 4 of 8 patients enduring clinically relevant CIHL. Our PK data indicate that transtympanic STS does not interfere with the antineoplastic activity of cisplatin. Future research is needed to confirm the efficacy of transtympanic STS aiming to prevent CIHL. Variables including the optimal dose, viscosity, and timing require further investigation.

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3

PLATINUM-RELATED HEARING LOSS IN TESTICULAR CANCER PATIENTS UNDERGOING PRIMARY OR SALVAGE TREATMENT

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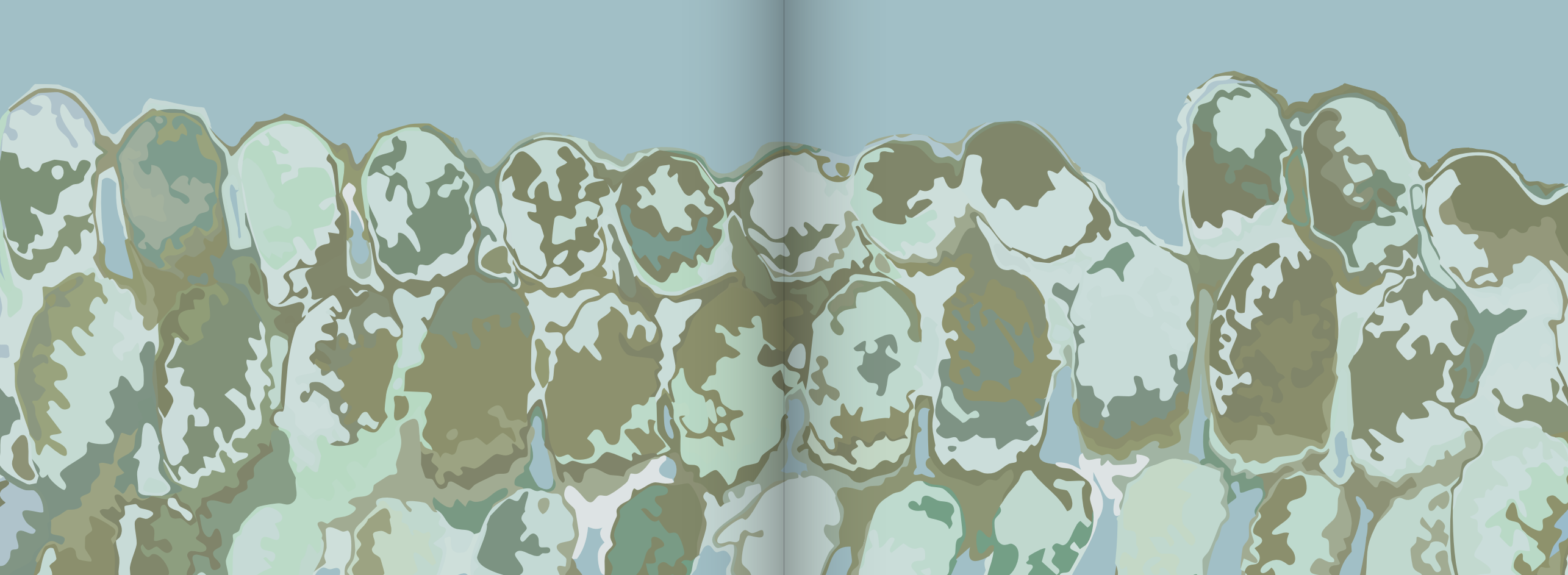

ABSTRACT

Background and Objective

Platinum therapy may cause sensorineural hearing loss. The aim was to evaluate cis- or carboplatin-induced hearing loss (CIHL) in testicular cancer patients. Potential candidates for preventive strategies against CIHL were assessed.

Methods

Forty-one patients were treated for primary (*n* = 33) or recurrent (*n* = 8) testicular cancer. Audiometry was performed at baseline and after treatment. Threshold shifts at pure tone averages vital for speech perception (PTA 1-2-4 kHz) and ultra-high sounds (PTA 8-10-12.5 kHz) were measured. CIHL was defined as a threshold shift ≥10 dB after therapy at these PTAs. CIHL was also measured via grading scales.

Key Findings and Limitations

In the primary treated group 3/33 (9.1%) developed CIHL at PTA 1-2-4 kHz and 18/32 (56%) at PTA 8-10-12.5 kHz. In the salvage treated group, this was 6/8 (75%) and 4/5 (80%), respectively. Mean threshold shift at PTA 1-2-4 kHz was 11.2 dB greater in the salvage group than the primary group ($p < 0.001$). At PTA 8-10-12.5 kHz this was 5.7 dB ($p = 0.118$). Overall, 3/41 (7%) of patients qualified for hearing aids due to treatment, of whom 2 in the salvage group. Scores on grading scales were higher in the salvage treated group. The biggest limitation was the small study population.

Conclusions

Overall, 22% and 59% of testicular cancer patients developed CIHL at frequencies vital for perception of speech perception and ultra-high sounds, respectively. Salvage treatment caused most severe CIHL.

Clinical Implications

Our findings demonstrate the need of preventive strategies against CIHL testicular cancer patients.

INTRODUCTION

Testicular cancer (TC) has the highest incidence of all neoplasms occurring in young men and about 990 new TC cases were diagnosed in the Netherlands in 2022. (1) Since the 1970's, platinum-derivates play an essential role in the chemotherapeutic treatment of TC (2, 3) and led to excellent clinical outcome, i.e. 95% 5-year relative survival in the United States. (4) However, cure goes hand in hand with platinum-related toxicities, including amongst others nephrotoxicity, neurotoxicity and ototoxicity. (5)

Primary treatment of TC limited to the testicle consists of a radical inguinal orchidectomy, often followed by surveillance or radiotherapy. Patients with disseminated disease (stage II/III) are generally treated with chemotherapy using three bleomycin-etoposide-cisplatin ((B)EP) cycles or four etoposide-cisplatin (EP) cycles. In case of recurrent disease after (B)EP, salvage treatment is given using additional high-dose carboplatin or cisplatin concomitant to other chemotherapeutic agents. (6)

Cisplatin or carboplatin-induced hearing loss (CIHL) is characterized by sensorineural hearing loss (SNHL) and is a frequent adverse event of platinum-based drugs. About 36% of adults treated with cisplatin develops CIHL (7), although it is difficult to give an exact incidence due to the heterogeneity between studies with respect to the administered cisplatin dose and the definition of ototoxicity. (5, 8-10) Since the 1970's cisplatin has been known to cause hair cell damage in the organ of Corti. Also, awareness is growing of its damaging effect to other cochlear cells, which occurs as a consequence of the release of toxic reactive oxygen species and the depletion of normally protective antioxidants. (5, 11-13) CIHLis irreversible, dose-dependent, symmetric, and starts at ultrahigh frequencies. (5, 11, 14) Thereafter and with continued treatment, hearing loss will progress to lower frequencies involved in the perception of speech (1 to 4 kHz). Hearing loss of averaged ≥ 35 dB at these frequencies results in a 50% reduction of speech intelligibility at conversation levels. Also, in the Netherlands, hearing aids are prescribed and reimbursed from this threshold. (15) Several studies emphasize the impact of CIHL on quality of life, including depression, cognitive impairment, and social isolation. (7, 16)

Patients with TC are young (17) and consequently present with relatively favorable pre-treatment hearing capacity when compared to elderly patients in need for cisplatin chemotherapy. As favorable baseline hearing capacity is a risk factor for the development of CIHL (18, 19), TC patients are particularly at risk for the development of CIHL. We hypothesize that TC patients with recurrent disease

treated with another round of platinum-therapy are particularly at risk for developing CIHL due to the further increased cumulative platinum dose.

As the prognosis of TC is excellent nowadays, there is growing focus on quality of life of cancer survivors. To this respect, our research group focusses on the search for a prophylactic intervention against CIHL (phase I trial (20) and phase III trial (*CTIS 2023-503313-30-01*)). The objective of the current study was to assess CIHL in a cohort of TC patients treated with cisplatin or carboplatin for primary or recurrent disease, as these young men may particularly benefit from such a preventive strategy against CIHL in the future.

METHODS

Patient characteristics

This study was a prospective cohort study analyzing the occurrence of and the extent of hearing loss in patients who were treated with high dose cisplatin (cumulative dose ≥ 300 mg/m^2) or carboplatin (median cumulative dose of 4471 mg/m^2) for both primary and recurrent TC between October 2019 and August 2021. All patients were treated at the department of medical oncology in the Netherlands Cancer Institute. Audiometry was performed at the department of head and neck surgery. Consent for use of data was obtained from all patients. The Institutional Internal Review Board provided approval to conduct this study.

Treatments

Patients were divided into two different groups. The first group consisted of patients with primary TC who were treated with one of the following: EP (etoposide, cisplatin), BEP (bleomycin, etoposide, cisplatin), or - in case of a contraindication for bleomycin - VIP (cisplatin, etoposide, ifosfamide). These patients received a cumulative cisplatin dose of 300 mg/m^2 or 400 mg/m^2. The second group consisted of patients with relapsed disease treated with salvage therapy, treated with one of the three following schedules: conventional dose TIP (paclitaxel, ifosfamide, cisplatin, mesna), high dose TI-CE (carboplatin, etoposide), or CTC (cyclosfamide, carboplatin, thiothepa, mesna). Five subjects in the salvage group participated in the TIGER study (phase III trial that compared TIP with TI-CE). (21)

Evaluation of hearing loss

Audiometry was performed prior to treatment and after treatment. Audiometry consisted of pure tone audiometry (in hearing level (HL)) from 0.125 kHz to 8 kHz, including air conduction (AC) and bone conduction (BC), and ultrahigh frequency audiometry (in sound pressure level (SPL)) from 8 kHz to 20 kHz. Audiometry was performed by a trained speech therapist in a soundproof booth using the Decos Audiology Workstation. Sometimes, hearing thresholds at 8 kHz SPL were missing (not measured). In these cases the value was converted from the measured 8 kHz threshold in HL to SPL following ISO 389-1 (+13 dB) (22). Occasionally, hearing loss extended the maximum output of the audiometer and was therefore not measurable. If this was the case at follow-up audiometry (de novo), these thresholds were computed by adding 5 dB to the maximum output of the audiometer so that threshold shifts could be analyzed. The patients were asked whether they experienced tinnitus and other subjective hearing problems and this was registered in the patient-file.

CIHL was determined per ear. We calculated Pure Tone Average (PTA) 1-2-4 kHz AC and BC HL as a proxy for speech perception per ear. We calculated PTA 8-10-12.5 kHz AC SPL to assess (ultra)high frequencies needed for e.g. high-pitched ringtones or the perception of high tones in music. To compare the pre- and post-treatment PTA threshold Exact Wilcoxon test for paired data was used. Next, mean threshold shifts (post-treatment minus pre-treatment) at PTA 1-2-4 kHz and PTA 8-10-1.5 kHz were calculated (in dB). Clinically relevant CIHL was defined as a threshold shift of ≥ 10 dB at one of these PTA's in one or both ears. Mann Whitney U test was used to compare the change in PTA's between the two treatment groups (primary versus salvage treatment).

Next, it was determined which patients developed a post-treatment hearing capacity of ≥ 35 dB at PTA 1-2-4 kHz BC de novo, as for hearing loss beyond this threshold hearing aids are prescribed and reimbursed in the Netherlands. Also, CIHL was assessed via the ASHA grading scale for hearing loss (23), the ASHA grading scale for hearing loss due to ototoxic drugs (for frequencies up to 20 kHz) (24), the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (based on the threshold shifts up to 8 kHz HL) (25), and the TUNE criteria (15), see Table 2.

Grading scale	Definition of SNHL
ASHA for hearing capacity (23)	For at least one tested frequency for either ear: Mild: 21 to 40 dB Moderate: 41 to 55 dB Moderately severe: 56 to 70 dB Severe: 71 to 90 dB Profound: >90 dB
ASHA for treatment-related hearing loss (24)	a) 20 dB decrease at any one tested frequency b) 10 dB decrease at any two adjacent test frequencies c) loss of response at three consecutive test frequencies where responses were previously obtained
CTCAE v5.0 (on a 1, 2, 4, 3, 6, and 8 kHz audiogram) (25)	Grade 1: Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear OR Subjective change in hearing in the absence of documented hearing loss; Grade 2: Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear; Grade 3: Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear OR hearing aid or intervention indicated Grade 4: Decrease in hearing to profound bilateral loss (absolute threshold >80 dB HL at 2 kHz and above); non-servicable hearing
TUNE (15)	Grade 0: No hearing loss Grade 1a: Threshold shift ≥ 10 dB at [8-10-12.5] OR subjective complaints in the absence of a threshold shift Grade 1b: Threshold shift ≥ 10 dB at [1-2-4] Grade 2a: Threshold shift ≥ 20 dB at [8-10-12.5] Grade 2b: Threshold shift ≥ 20 dB at [1-2-4] Grade 3: Hearing level ≥ 35 dB HL at [1-2-4] de novo Grade 4: Hearing level ≥ 70 dB HL at [1-2-4] de novo

Table 1: Grading scales used in this study for the assessment of post-treatment platinum-related hearing loss. Abbreviations: ASHA: American Speech-Language-Hearing Association; CTCAE: American Speech-Language-Hearing Association.

RESULTS

Patient characteristics are summarized in Table 2. In total 41 patients were included (82 ears). Thirty-three patients were treated for primary TC (EP, BEP and VIP protocol) and 8 patients received salvage therapy for recurrent disease. Salvage patients were previously treated with (B)EP for primary disease. Audiometry was performed pre-treatment (baseline) and median 3.8 months after treatment (range 0–11.0 months). In one patient treated with salvage therapy, follow-up audiometry was performed at a very short period, i.e. the last day of treatment. This patient was considered evaluable, as he had already developed significant hearing loss at this point (threshold shift of 14 dB at PTA 1-2-4 HL). Four patients (1 in the primary group and 3 in the salvage group) were not evaluable for analysis at PTA 8-10-12.5 kHz SPL, as the thresholds exceeded the maximum output of the audiometer for both baseline and follow-up audiometry. This resulted in 37 evaluable patients at PTA 8-10-12.5 kHz SPL, of which in 4 patients CIHL was measurable in only one ear (3 in primary group and 1 in salvage group).

Patient, tumor and treatment characteristics	
Age, median years (range)	31 (range 18 – 57)
Histology	
Seminoma	9
Non-seminoma	32
Treatment	
Number of patients receiving primary therapy (EP/BEP/VIP)	
cisplatin 300 mg/m^2	10
cisplatin 400 mg/m^2	23
Number of patients receiving salvage treatment	
TIP (cisplatin 400 mg/m^2)	3
TI-CE (carboplatin 4553 (3963 – 5125) mg/m^2)*	4
CTC (carboplatin 3527 mg/m^2)	1
Follow-up duration audiometry after therapy, median months (range)	3.8 (0.7 – 11)

Table 2: Baseline patient, tumor and treatment characteristics of the cohort treated with platinum-based treatment for testicular cancer in our institute between October 2019 and August 2021. ***median and range of the cumulative carboplatin dose per m^2, which is calculated based on renal function.**

Figure 1 illustrates the mean pure tone audiometry and mean (ultra)high frequency audiometry at baseline and follow-up. Table 3 shows the threshold shifts and *p*-values at the PTAs. When assessing threshold shifts at individual frequencies, significant *p*-values ($p < 0.05$) were seen at frequencies from 4 kHz HL up to 20 kHz SPL in the primary group and from 2 kHz HL up to 20 kHz SPL in the salvage group. When comparing the primary and the salvage treated group, the difference in mean threshold shift was 11.2 dB at PTA 1-2-4 kHz ($p < 0.001$) and 5.7 dB at PTA 8-10-12.5 kHz ($p = 0.1$).

Table 4 shows the incidences of CIHL at the two PTA's, the number of patients qualified for hearing aids after therapy, the scores at the different grading scales, and subjective patient-reported hearing symptoms as derived from the patient-files. The scores on all grading scales were higher in the salvage treated group compared to the primary treated group. Patients treated with salvage therapy

were more often qualified for hearing aids after therapy (25% versus 3%). Overall, patients treated with salvage therapy more often reported subjective hearing symptoms than patients treated with primary therapy did. Interestingly, tinnitus was more frequently reported in the primary treated group.

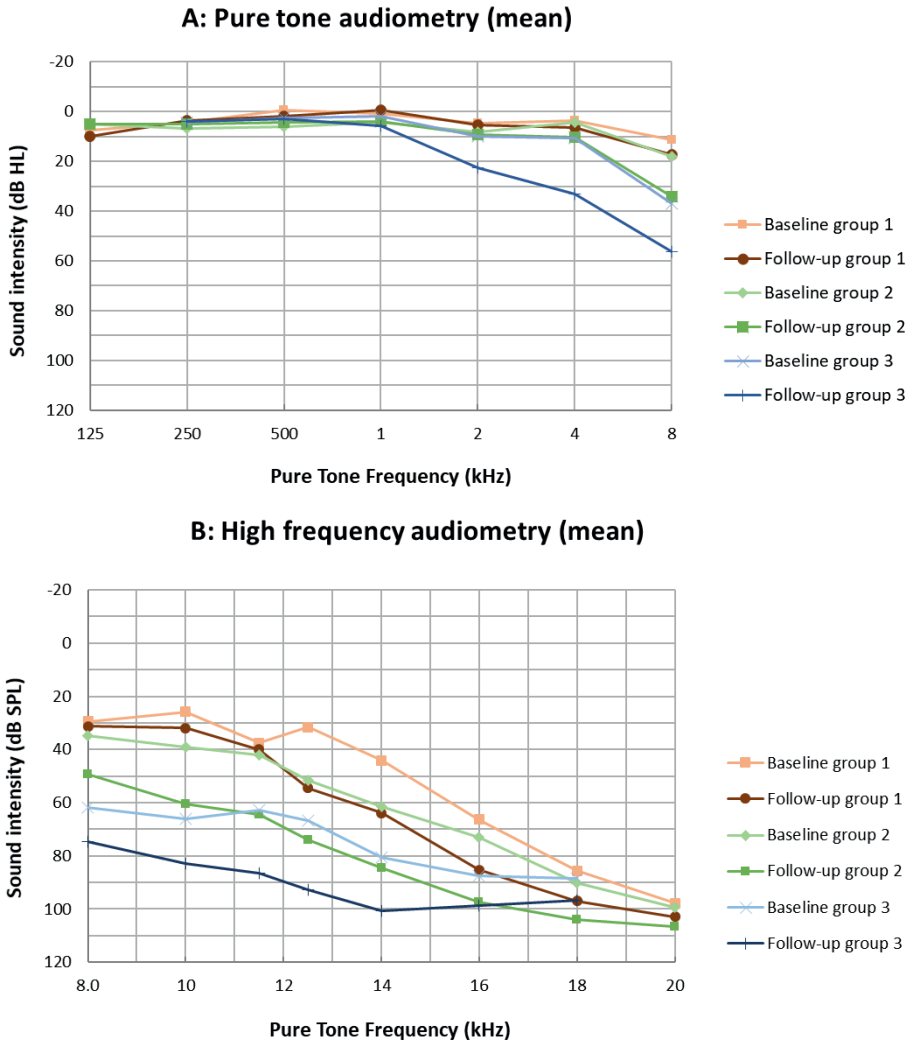

Figure 1: Audiometric results, mean thresholds.
1A) Pure tone audiometry at baseline and follow-up of the 33 testis cancer patients (66 ears) treated with primary treatment and the 8 patients (16 ears) treated with salvage therapy. 1B) High frequency audiometry of the same groups.

Pure tone average	Whole group	*p*-value	Primary	*p*-value	Salvage	*p*-value
PTA 1-2-4 kHz (in dB)	3.8 (5.3 to 9.1)	< .001	1.6 (4.9 to 6.5)	.04	12.8 (7.0 to 19.8)	< .001
PTA 8-10-12.5 kHz (in dB)	17.7 dB (38.4 to 56.1)	< .001	17.0 (36.4 to 53.4)	< .001	22.7 (51.9 to 74.6)	.002

Table 3: Threshold shifts after therapy at pure tone average (PTA) 1-2-4 kHz hearing level and 8-10-12.5 kHz sound pressure level (in dB). In the evaluation of PTA 8-10-12.5 kHz not all patients could be evaluated (n = 37) due to missing values (3 in primary group and 1 in the salvage group): hearing thresholds at one of the frequencies of the high frequency audiometry exceeded the maximum output of the audiometer (at both pre- and post-treatment) and were therefore not measurable.

	Incidence CIHL at	Incidence CIHL	Hearing aids indication de novo	Grading scales PTA 8-10-12.5 kHz SPL						Subjective treatment-related hearing symptoms		
				1. ASHA for hearing capacity		**2.** ASHA for treatment-related hearing loss		**3.** CTCAE V5.0		**4.** TUNE		Tinnitus	Impaired speech perception in noise	Profound hearing loss
Primary therapy	9% (3/33[±])	56%* (18/32)	3% (1/33)	**mild**	0 (0%)	**no hearing loss**	3 (9%)	**grade 0**	24 (73%)	**grade 0**	10 (29%)	16/33 (48%)	7/33 (21%)	6/33 (18%)
				moderate	10 (30%)	**A**	0 (0%)	**grade 1**	3 (9%)	**grade 1a**	11 (33%)			
				moderately severe	4 (12%)	**B**	12 (36%)	**grade 2**	2 (6%)	**grade 1b**	1 (3%)			
				severe profound	6 (18%)	**C**	18 (55%)	**grade 3**	4 (12%)	**grade 2a**	10 (30%)			
					13 (39%)			**grade 4**	0 (0%)	**grade 2b**	0 (0%)			
										grade 3	1 (3%)			
										grade 4	0 (0%)			
Salvage therapy	75% (6/8[±])	80%* (4/5)	25% (2/8)	**mild**	0 (0%)	**no hearing loss**	0 (0%)	**grade 0**	2 (25%)	**grade 0**	0 (0%)	1/8 (13%)	3/8 (38%)	3/8 (38%)
				moderate	0 (0%)	**A**	1 (13%)	**grade 1**	1 (13%)	**grade 1a**	1 (13%)			
				moderately severe	1 (13%)	**B**	2 (25%)	**grade 2**	2 (25%)	**grade 1b**	1 (13%)			
				severe profound	1 (13%)	**C**	5 (62.5%)	**grade 3**	3 (38%)	**grade 2a**	4 (50%)			
					6 (75%)			**grade 4**	0 (0%)	**grade 2b**	0 (0%)			
										grade 3	2 (25%)			
										grade 4	0 (0%)			

Table 4: Overview of hearing loss, the indication for hearing aids de novo, scores at different grading scales, and subjective hearing symptoms after both primary and salvage therapy. *missing values: hearing thresholds at one of the frequencies of the high frequency audiometry (PTA 8-10-12.5 kHz SPL) exceeded the maximum output of the audiometer (at both pre- and post-treatment) and were therefore not measurable. In the primary group this was the case for 3 patients and in the salvage group for one patient. [±] Two patients are included in both groups

DISCUSSION

In this study, we evaluated CIHL in a consecutive series of patients treated for primary or recurrent TC. A substantial percentage of patients developed clinically relevant CIHL at frequencies relevant for the perception of speech (59%, PTA 1-2-4 kHz) and the perception of ultrahigh sounds (22%, PTA 8-10-12.5 kHz). The number of patients developing CIHL was larger in the group of patients treated with salvage therapy than in the group treated with primary therapy. Also, CIHL at frequencies relevant for the perception of speech was significantly more severe in terms of decibels hearing loss in the salvage group than in the primary treated group. The most likely explanation for this dissimilarity is the difference in cumulative platinum-dose, as patients with recurrent disease receive a higher cumulative platinum-dose over the complete course of their disease. Accordingly, patients treated with salvage therapy developed higher scores on all grading scales for hearing loss and were more often qualified for a hearing aid after therapy.

Several previous studies described CIHL in TC patients, but they generally lacked baseline audiometry (14, 26-30), except for Osanto et al. (N = 32) (31) and Haughnes et al. (N = 46) (32), who showed significant threshold shifts at 4 and 8 kHz HL and a significantly higher prevalence of SNHL at 2 and 4 kHz in platinum-treated patients in the first decade after therapy when compared to aged-matched controls, respectively. Also, Zhang et al. tested a large cohort of TC patients after platinum therapy and found 45% of patients with SNHL according to the ASHA criteria (i.e. a threshold of ≥ 20 dB at any frequency (33) (here 0.25 to 12 kHz)), without reporting baseline audiometry and/or changes after therapy. (30) Furthermore, Frisina et al. (N = 488) - who also did not measure baseline hearing - reported that 18% of primary treated TC patients had severe to profound SNHL upon platinum-based chemotherapy according to the ASHA criteria. In our study, this percentage was a lot higher in both the primary (58%) and the salvage (88%) treated group. This can be explained by the way audiometry was performed, as we measured up to 20 kHz instead of 12 kHz and the ASHA scoring system involves CIHL per individual frequency. We agree with Chattaraj et al. that measuring baseline audiometry is essential for proper evaluation of CIHL, as pre-treatment hearing can vary widely between individuals. (7) Also, in our opinion, there are limitations to the use of the ASHA criteria, as pre-treatment hearing is not taken into account and the incidence of CIHL depends on the frequencies measured.

Patient-reported outcomes of our study are in line with results described in literature. Haugnes et al. showed that platinum-treated patients more often reported tinnitus when compared to age-matched controls (42% versus 24%, $p = 0.06$). (32) In the study of Bokemeyer et al., 20% of the primary treated patients reported symptoms of hearing loss (59% tinnitus, 18% hearing loss, 23% both). (27)

In the study of Frisina et al. 30% and 40% of patients had symptoms of hearing loss and tinnitus, respectively. (33)

Although cisplatin is considered to be more ototoxic than carboplatin (34), our results confirmed that high-dose carboplatin as part of a salvage treatment regimen for TC can also result in severe CIHL up to the lower frequencies involved in the perception of speech. This is in line with the audiometric results of a study of Shea et al. and a case report of three TC patients treated with high-dose carboplatin. (35, 36)

TC survivors will ultimately develop age-related hearing loss (presbycusis). Skalleberg et al. analyzed 82 previously treated TC patients (37) and found that hearing capacity remained worse in patients compared to healthy subjects at median 12 years after treatment. However, after three decades, hearing capacity of TC survivors approached the hearing capacity of the general population. The pathophysiology of presbycusis and CIHL are both characterized by hair cell loss in the basal cochlear windings and damage to the stria vascularis, resulting in high-frequency SNHL. (37) Cisplatin may accelerate the onset of SNHL in younger patients with excellent hearing capacity, however ultimately, it seems that presbycusis and CIHL are non-cumulative. Accordingly, Zuur et al. showed that patients with excellent baseline hearing generally develop greater platinum-related threshold shifts (in dB) than patients with preexistent presbycusis. (19)

Preventive strategies against CIHL may become available in the future. A recent phase I trial in our center showed that the injection of transtympanic sodium thiosulfate (STS), which is an antioxidant and platinum-binder, is safe and potentially effective as a prophylactic agent against CIHL. (20) Next, a multicenter phase III trial, is conducted to assess its efficacy. Young TC patients treated with salvage therapy are most prone to suffer severe CIHL and may benefit from this strategy. However, as transtympanic STS needs injection prior to each platinum infusion, we believe this intervention is only feasible in patients treated with a limited number of platinum infusions. Because TC patients receive daily platinum infusions, a pilot study is needed to investigate both the benefit and feasibility of multiple transtympanic STS injections to prevent from developing CIHL.

A limitation of this study is the small number of patients. Also, ideally, subjective hearing loss and tinnitus would have been evaluated by validated questionnaires. Nevertheless, to our knowledge, this small cohort contains the largest number of TC patients treated with salvage therapy that received both pre- and post-treatment audiometric evaluation.

CONCLUSION

In conclusion, 22% and 59% of TC patients suffered CIHL at speech frequencies and ultrahigh frequencies, respectively. TC patients treated with salvage therapy developed most severe CIHL. A pilot study is needed to investigate the feasibility and potential benefit of preventive strategies against CIHL.

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4

THE ROLE OF GENETIC VARIANTS IN THE PREDICTION OF HEARING LOSS DUE TO CISPLATIN CHEMORADIOTHERAPY

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ABSTRACT

Concomitant high-dose cisplatin with radiotherapy is commonly used for treating head and neck squamous cell carcinoma. Cisplatin, often used with radiotherapy, is known for causing irreversible sensorineural hearing loss, with individual variability suggesting a genetic component. This study aims to enhance the predictive ability of the clinical prediction model for cisplatin-induced hearing loss in head and neck squamous cell carcinoma patients, as outlined in Theunissen et al. (2015), by incorporating significant genetic variants. Conducted at the Netherlands Cancer Institute, this retrospective study included 74 patients treated between 1997 and 2011. 31 SNPs that were previously associated with cisplatin-induced hearing loss or other cisplatin-induced toxicities were identified and incorporated into the model. The primary outcome measured was the change in decibels at post-treatment 1-2-4 kHz hearing levels per additional minor allele of these SNPs, evaluated using linear mixed-effects regression models. The model's predictive accuracy was determined by the area under the curve using 10-fold cross-validation. The rs2289669 SNP in the *SLC47A1/MATE1* gene was linked to a significant 2.67 dB increase in hearing loss per allele (95% CI 0.49 to 4.86, P=0.017). Incorporating rs2289669 improved the model's area under the curve from 0.78 to 0.83, a borderline significant improvement (P=0.073). This study underscores the importance of the rs2289669 SNP in cisplatin-induced hearing loss and demonstrates the potential of combining genetic and clinical data for enhanced predictive models in personalized treatment strategies.

INTRODUCTION

Cisplatin is a widely used chemotherapeutic agent for patients with advanced head and neck squamous cell carcinoma (HNSCC), who are treated with concurrent chemoradiotherapy. (1) Dose-limiting side effects of cisplatin include nephrotoxicity, neurotoxicity, and ototoxicity. (2-4) There is no standardized protective or curative agent available for neuro- and ototoxicity. (2) However, there is an increasing interest in the search for otoprotective agents, including amifostine, dexamethasone, and vitamin E, with varying success. (5-9) Recently, trans-tympanic sodium thiosulfate has also been studied as an otoprotector in a phase I study at the Netherlands Cancer Institute (NKI) and in a meta-analysis. (5, 10)

Cisplatin has been known to instigate cochlear dysfunction, leading to sensorineural hearing loss (SNHL). According to a recent meta-analysis, the prevalence of cisplatin-induced hearing loss (CIHL) is 49% in adults with HNSCC, (8) although the incidence of CIHL varies across studies due to differing definitions and cisplatin doses. (2, 3, 11-13) CIHL is characterized by symmetric and irreversible SNHL starting at high frequencies, but after continued cisplatin courses it may progress to lower frequencies involved in speech perception. (2, 14, 15) Other risk factors for the development of CIHL include favorable pre-treatment hearing capacity, the use of other ototoxic drugs, and radiation exposure of the cochlea. (8, 9, 12, 16, 17) Also, genetic variants have been associated with CIHL, such as single-nucleotide polymorphisms (SNPs) including *ACYP2* (18-22), *WFS1 (18, 22)*, *ABCC3* (23, 24), and *MATE1* (25).

Theunissen and colleagues developed a prediction model for post-treatment hearing loss in patients with HNSCC who are treated with combined chemoradiation, see eFigure 1. (26) This model uses clinical input variables, such as baseline hearing thresholds, cisplatin dose, and cochlear radiation dose, to predict post-treatment hearing thresholds averaged at frequencies 1, 2, and 4 kHz. The model shows a 97% specificity and 29% sensitivity for the indication of hearing aids in the Netherlands (35 dB threshold). (26) Despite the model being a significant step toward improving individual recommendations for HNSCC patients at risk for CIHL, the authors called for future research concerning additional risk factors. In particular, they hypothesized the role of genetic variants in hearing loss severity, based on observations of substantial individual vulnerability for the prevalence and severity of CIHL. (26, 27) Identifying SNPs linked to CIHL is crucial for personalized care, enabling better pre-treatment guidance and interventions against chemoradiation side effects, including the use of trans-tympanic sodium thiosulfate for prevention.

The main aim of the present study is to describe the contribution of genetics to individualized susceptibility to CIHL in an extension cohort of Theunissen et al. (26) and investigate whether using SNPs that are significantly associated with CIHL increases the predictive ability of our previously designed clinical prediction model.

METHODS

Data Sources and Patient Selection

A retrospective cohort study was performed at the NKI from patients treated with intravenous (IV) cisplatin (100 mg/m^2, for 3 courses) or with intra-arterial (IA) cisplatin (150 mg/m^2; for 4 courses, within the "RADPLAT trial") (28) during 7 weeks of radiotherapy (total dose of 70 Gy in 35 fractions on tumour-bearing areas) for advanced-stage HNSCC. All patients were treated between January 1, 1997, and December 31, 2011. Patients were selected based on available pre- and post-treatment audiometry and formalin-fixed paraffin-embedded tissue blocks. The patients treated with IA cisplatin have also been treated with concurrent IV sodium thiosulphate in order to prevent from platinum-related toxicity. We chose to also include these patients in the current cohort, as in these IA treated patients sodium thiosulphate did not protect against CIHL. (28) The cochlear radiotherapy dose was also measured (methods described by Zuur et al. (17)). All patients gave informed consent for further use of their data.

Audiometry

Pure-tone audiometry was conducted pre-treatment, 15-20 days after the first cisplatin infusion, and 3-31 weeks post-treatment in a sound-proof booth using the Decos Audiology Workstation. Audiometry consisted of pure tone audiometry (in hearing level (HL)) from 0.125 kHz to 8 kHz, including both air conduction (AC) and bone conduction (BC), and ultrahigh frequency audiometry (in sound pressure level (SPL)) from 8 kHz to 20 kHz. BC thresholds were used for the frequencies 0.5, 1, 2 and 4 kHz to correct for potentially fluctuating conductive components. Three Pure Tone Averages (PTAs) were calculated: for the relatively low-frequency range relevant to quiet speech perception, we calculated PTA 0.5-1-2 kHz HL BC (PTAL). For the relatively high-frequency range relevant to speech perception in noise, we used PTA 1-2-4 kHz HL BC (PTAH). For the perception of ultrahigh sounds (e.g., music or nature), we investigated PTA 8-10-12.5 kHz SPL AC (PTAU). Missing audiometric data at the PTAs at baseline and after the first cisplatin cycle were imputed following the method described previously by Theunissen et al. (26), while patients with missing post-treatment audiometry were excluded.

Isolation of DNA and Sequencing

DNA was isolated from FFPE tissue blocks at NKI. Next, the Princess Margaret Cancer Centre in Toronto performed sequencing of 31 SNPs by MassArray SNP genotype method (Sequenom) using Assay Design Suite software.

Gene Selection

The genetic variants were selected based on published literature, had a minor allele frequency in individuals of European descent of at least 1%, and had the ability to incorporate the polymorphism or a surrogate in high (D' > 0.95) linkage disequilibrium in the multiplex reaction. Included SNPs and associated references from literature review can be found in eTable1.

In our analysis, certain SNPs identified as significant in previous literature were not available in our genotyping panel; therefore, we selected proxy variants based on linkage disequilibrium data and prior association studies. Specifically, rs1051740 was used as a proxy for rs1142345, rs2273697 for rs1800462, and rs4646316 for rs11568591.

Statistical Analysis

Descriptive statistics were used to summarize baseline patient characteristics and allele and genotype frequencies of the 31 SNPs of interest.

Multivariate imputation was performed using the panImpute function from the mitml package in R to account for missing data. Missing audiometry data at baseline (4 ears [3.5%] for PTAL and 4 ears [3.5%] for PTAH), and after the first cisplatin infusion (57 ears [49.6%] for PTAL, 57 ears [49.6%] for PTAH, and 31 ears [27.0%] for PTAU), were imputed. Imputations were functions of the outcome (i.e., post-treatment PTAs) as well as PTAs at baseline and first chemotherapy, chemotherapy dosage and cochlear radiation dose. PTAs at baseline, the first cisplatin infusion, and post-treatment were log-transformed to ensure normally distributed residuals where necessary and back-transformed for use in the main model of interest.

Quality control analysis was performed on the genetic data. This included the exclusion of SNPs without variation in allele frequency. The rs77382849 SNP *(EIF3A)* was excluded from the analysis, as all patients were observed to have the homozygous major genotype, rendering it uninformative for association analysis. Additionally, the Hardy-Weinberg equilibrium was assessed, and no SNPs violated the assumption (p-value > 0.05).

CIHL may differ in both ears, as the ear ipsilateral to the tumour may receive a higher dose of radiation compared with the contralateral ear. Thus, the outcome was expressed per ear on 2 occasions: left and right ears after the first cisplatin infusion, and the left and right ears after the end of treatment. To determine if any of the candidate SNPs were independently associated with post-treatment hearing capability, linear mixed-effects models on post-treatment PTA with patient-specific intercepts were run for one SNP at a time.

We created two sets of models: 'Unadjusted models' evaluated the association of a SNP with post-treatment hearing capability adjusted for pre-treatment high-frequency PTA (PTAH) only. 'Adjusted models' evaluated the association of a SNP on the same post-treatment hearing capability adjusted for all pre-treatment (low, high, and ultra-high) PTAs (PTAL, PTAH, PTAU), cumulative chemotherapy and radiation dose to the cochlear. We adjusted for the same clinical factors as those used in the original model by Theunissen et al. To account for additive gene effects, the genotype with the major allele homozygous was coded as '0', the heterozygous genotype as '1', and the genotype with the minor allele homozygous as '2'. Pooling of results of 100 imputed datasets was performed using the summary function (testEstimates) from the mitml package. Unadjusted and adjusted p-values were computed without correction for multiple testing; rather than dismissing associated SNPs to control family-wise error rate, we included significant SNPs with a liberal p-value threshold to preserve any potentially clinically important SNPs in the final model.

To ensure consistency in findings, a sensitivity analysis was performed using co-dominant, adjusted linear mixed-effects regression models. For this model, patients with two major alleles in a given gene were coded as zero, heterozygous patients were coded as 1, and patients with two minor alleles were coded as 2. We utilized the co-dominant genetic model due to its flexibility and detailed categorization of genotypes.

We performed a likelihood ratio test (LRT) to determine the goodness of fit of the two models, with and without the SNP. Model comparisons were calculated and summarized over the 100 imputed datasets.

To evaluate and compare the performance of the new prediction model to predict observed PTAH of at least 35 dB de novo (the Dutch threshold for hearing aid qualification) to the original prediction model, we computed the 10-fold cross-validated sensitivity and specificity of both models at all possible thresholds of predicted hearing levels. Cross-validation was performed at the patient level. Sensitivity and specificity for each cut-off were imposed on the model predictions to classify patients as "hearing loss" (predicted hearing at/above cut-off in either ear) versus "no hearing loss" (predicted hearing below cut-off in both ears). Patients with missing data for any identified statistically significant SNP associated with the outcome, or those who had baseline PTAH ≥ 35dB (therefore classified as "hearing loss" before treatment) were removed from cross-validation. We then constructed the receiver operating characteristics (ROC) curve by plotting 10-fold cross-validated sensitivity vs 1-specificity and calculated the area under the curve (AUC) with 95% confidence intervals. To assess the discriminatory performance of the models, we performed DeLong's test to compare the ROC curves of the new and the original prediction model to statistically evaluate and compare the predictive accuracy of the two models.

Data were analyzed using R software (v.2022.12.0). All tests were two-sided and α=0.05 was used to denote statistical significance.

RESULTS

Patient Selection

115 HNSCC patients received high dose chemoradiation as a primary treatment and had genetic SNP and audiometry data available. If a patient had complete data for only one ear, that ear was retained for analysis. We excluded 35 ears with missing radiation dose to the cochlea (both ears from 17 patients, one ear from 1 patient), and 54 ears without post-treatment PTAH (both ears from 24 patients, one ear from 6 patients). In total, 74 patients (64.3%) and 141 ears were included (eFigure 2). Due to the unavailability of tissue samples for some subjects from Theunissen et al.'s cohort, our current cohort differs slightly. Additionally, it includes 18 patients who underwent treatment with IA cisplatin.

Descriptive Analysis

Table 1 summarizes the patient sample. The mean age of participants was 53.9 (SD=8.5) years and 77% (n=57) of participants were male. The cumulative cisplatin dose among patients ranged from 315 to 1200 (median= 579.5) mg. The radiation dose to the cochlea ranged from 1.1 to 70.5 Gy (median= 11.4 Gy) because the patients were, in general, treated with intensity-modulated radiotherapy.

eTable 2 details allele and genotype frequency across the patient sample. Homozygous minor allele genotypes were not present for 6 SNPs (rs11085735 *(KEAP1)*, rs12201199 *(TPMT)*, rs316019 *(SLC22A2)*, rs596881 *(SLC22A2/OCT2)*, and rs2291767 and rs77124181 *(OTOS)*) and homozygous major allele genotype only was observed for one SNP (rs77382849 *(EIF3A)*), and therefore it was removed from analysis. Hence, 30 SNPs in total were included in the model building.

For the majority of patients, as time progressed from the first cisplatin infusion to post-treatment, PTAH (kHz) increased over time (i.e., hearing loss occurred) (Figure 1).

Variable	Value
No. of Patients	74
Sex (%)	
Male	57 (77.0%)
Female	17 (23.0%)
Age, mean (SD), years	53.9 (8.5)
Tumor Site (%)	
Oropharynx	45 (60.8%)
Hypopharynx	17 (23.0%)
Oral Cavity	5 (6.8%)
Larynx	3 (4.1%)
Other head and neck sites	4 (5.5%)
Cisplatin Dose per Cycle, median (min, max), mg	195.0 (145.0, 300.0)
Cumulative Cisplatin Dose, median (min, max), mg	579.5 (315.0, 1200.0)
Cochlear Radiation Dose, median (min, max), Gy	11.4 (1.1, 70.5)

Table 1: Descriptive statistics of patient population (n=74) demographics, chemotherapy, and radiation dose to the cochlea.
SD: standard deviation; Min: minimum; Max: maximum.

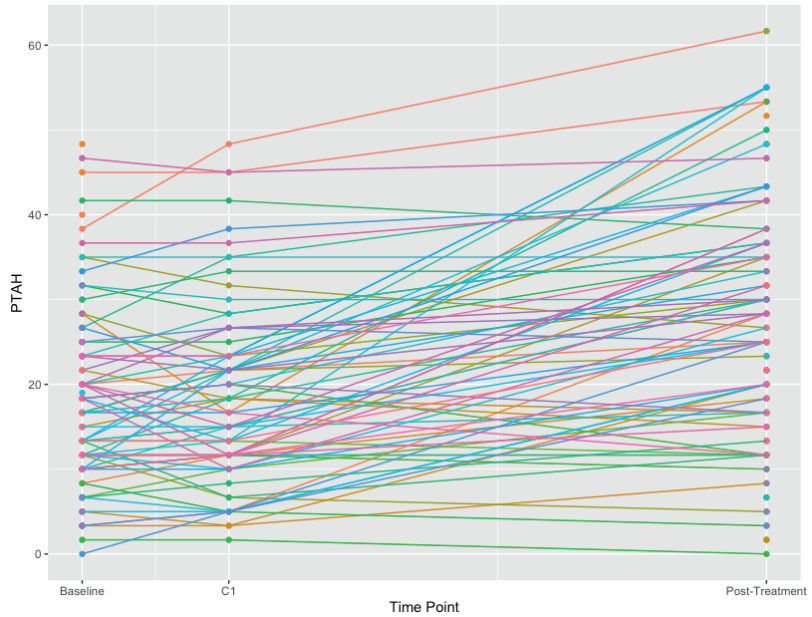

Figure 1. Spaghetti plot of Pure Tone Audiometry at High frequencies (PTAH) in kHz, a measure of hearing loss, over time, from baseline to post-treatment. C1: first cisplatin dose. Time between baseline and C1 was 3 weeks, time between C1 and post-treatment was median 14 weeks.

SNP	Unadjusted Model[b]			Adjusted Model[c]		
	Coefficient[a]	95% CI	*p*-value	Coefficient	95% CI	*p*-value
rs1048290	-0.99	-3.17, 1.20	0.376	-0.6	-3.05, 1.84	0.629
rs1051640	-1.1	-3.71, 1.52	0.412	-1.27	-4.04, 1.5	0.37
rs1051740	-0.25	-2.86, 2.35	0.851	-0.01	-2.75, 2.73	0.995
rs10981694	-1.98	-4.47, 0.51	0.118	-2.14	-4.77, 0.5	0.112
rs11085735	-1.35	-5.91, 3.21	0.561	-1.92	-6.81, 2.97	0.442
rs11615	0.14	-1.99, 2.26	0.9	0.16	-2.08, 2.4	0.888
rs12201199	1.84	-2.51, 6.19	0.407	2.6	-2.05, 7.26	0.273
rs13181	-0.63	-2.83, 1.57	0.572	-0.31	-2.65, 2.02	0.791
rs1695	-1.31	-3.54, 0.91	0.247	-0.85	-3.22, 1.52	0.483
rs1801133	0.73	-1.42, 2.89	0.506	-0.37	-2.68, 1.94	0.752
rs1806649	-0.87	-3.32, 1.57	0.483	-1.11	-3.68, 1.47	0.4
rs1872328	1.43	-2.49, 5.35	0.474	-0.16	-4.42, 4.1	0.942
rs2075252	-0.71	-3.09, 1.67	0.559	-0.48	-3.05, 2.08	0.711
rs2228001	-0.3	-2.47, 1.87	0.787	0.2	-2.16, 2.55	0.87
rs2228171	0.06	-2.13, 2.24	0.959	0.4	-1.92, 2.72	0.734
rs2273697	-1.84	-4.85, 1.16	0.229	-2.12	-5.31, 1.07	0.193
rs2289669*	2.96	0.92, 4.99	0.004	2.67	0.49, 4.86	0.017
rs2291767	-3.99	-11.48, 3.51	0.297	-1.19	-9.32, 6.93	0.774
rs316019	0.79	-3.02, 4.60	0.686	3.12	-0.92, 7.16	0.13
rs3212986	-0.01	-2.46, 2.44	0.993	0.28	-2.33, 2.88	0.835
rs3740066*	2.14	-0.36, 4.63	0.093	2.4	-0.27, 5.06	0.078
rs4480	1.28	-0.87, 3.44	0.244	0.93	-1.38, 3.24	0.431
rs4646316	-0.16	-2.82, 2.50	0.904	-1.38	-4.29, 1.52	0.351
rs4788863	-1.05	-3.44, 1.34	0.388	-0.81	-3.37, 1.75	0.535
rs596881	1.31	-2.66, 5.28	0.519	3.27	-0.92, 7.46	0.126
rs62283056	0.75	-1.99, 3.49	0.589	2.11	-0.79, 5.02	0.154
rs717620	0.29	-2.52, 3.09	0.842	0.43	-2.53, 3.38	0.777
rs77124181	-2.54	-8.13, 3.05	0.373	-2.22	-8.12, 3.67	0.46
rs7851395	0.36	-1.82, 2.54	0.748	0.72	-1.59, 3.03	0.541
rs9332377	0.62	-2.45, 3.69	0.692	0.07	-3.19, 3.32	0.968

Table 2: Unadjusted and adjusted additive, linear mixed-effects regression model results for each of the 30 SNPs of interest.
*Significant or borderline significant SNPs.
[a] **Model coefficients** are interpreted as the change in follow-up PTAH for each additional minor allele. For example, each additional minor allele of the rs2289669 SNP was associated with a 2.67 dB increase in follow-up PTAH Lower values of PTAH indicate better hearing capability.
[b] **Unadjusted models** include adjustment for pre-treatment PTAH only.
[c] **Adjusted models** include adjustment for all pre-treatment (low, high, and ultra-high) PTAs (PTAL, PTAH, PTAU), chemotherapy dosage, and radiation dose to the cochlea.

Gene Analysis

Of the 30 candidate SNPs, only one was a statistically significant predictor of post-treatment hearing capability. The rs2289669 SNP (within gene *SLC47A1/MATE1*) showed a 2.67 dB (95% CI 0.49 to 4.86, *p*=0.017) greater hearing loss for each additional minor allele (Table 2).

Sensitivity Analysis

In a sensitivity analysis, regression models were re-analyzed using a co-dominant model. The rs2289669 SNP (particularly the homozygous minor AA genotype) was again the only significant predictor of post-treatment hearing capability, consistent with our additive gene model findings *(eTable 3)*.

Model Evaluation

The inclusion of the rs2289669 SNP to our pre-existing clinical prediction model including baseline PTA, chemotherapy dose and radiation dose, significantly improved its goodness-of-fit (LRT *P*=0.017). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at different hearing cut-offs for both models ("SNP model" with the SNP and "Clinical-only model" with clinical factors only) are displayed in Table 3. The addition of rs2289669 improved sensitivity and specificity at a threshold of 30dB and 35dB (sensitivity 0.44 vs 0.38; specificity 0.96 vs. 0.94) but not at 25dB and 40dB.

We performed a supplementary analysis to observe changes in predictive ability with the addition of the top ten SNPs, chosen based on significance (i.e., p-values). We found that adding ten more SNPs to the model did not improve the predictive ability, and, due to substantial overfitting, resulted in a decrease in the AUC.

		Cut-off for determining predicted hearing loss (dB)			
		25	**30**	**35**	**40**
Clinical-only model[a]	**Sensitivity**	0.833	**0.611**	**0.389**	0.278
	Specificity	0.596	**0.766**	**0.936**	0.979
	Positive Predictive Value (PPV)	0.441	**0.500**	**0.700**	0.833
	Negative Predictive Value (NPV)	0.903	**0.837**	**0.800**	0.780
SNP model[b]	**Sensitivity**	0.778	**0.722**	**0.444**	0.278
	Specificity	0.553	**0.787**	**0.957**	0.979
	Positive Predictive Value (PPV)	0.400	**0.565**	**0.800**	0.833
	Negative Predictive Value (NPV)	0.867	**0.881**	**0.818**	0.780

Table 3: Sensitivity and specificity at different hearing cut-offs (25dB – 40dB). Patients (N=65), Ears (N=127).
[a] **Clinical-only model** predicts hearing loss from baseline PTA, chemotherapy dose, and cochlear radiation dose. [b] **SNP model** predicts hearing loss from these factors plus rs2289669 genotype. Bolded rows are where the SNP model has improved performance over the clinical-only model.

The SNP model had a moderately, borderline significant (p=0.073), higher AUC (AUC=0.83, 95% CI 0.71 to 0.94) compared to the clinical-only model (AUC=0.78, 95% CI 0.66 to 0.91). The observed predictive gains were not consistent across all hearing thresholds, with the SNP model demonstrating the greatest advantage at the 30dB hearing level, as evident from the ROC curve plots (Figure 2).

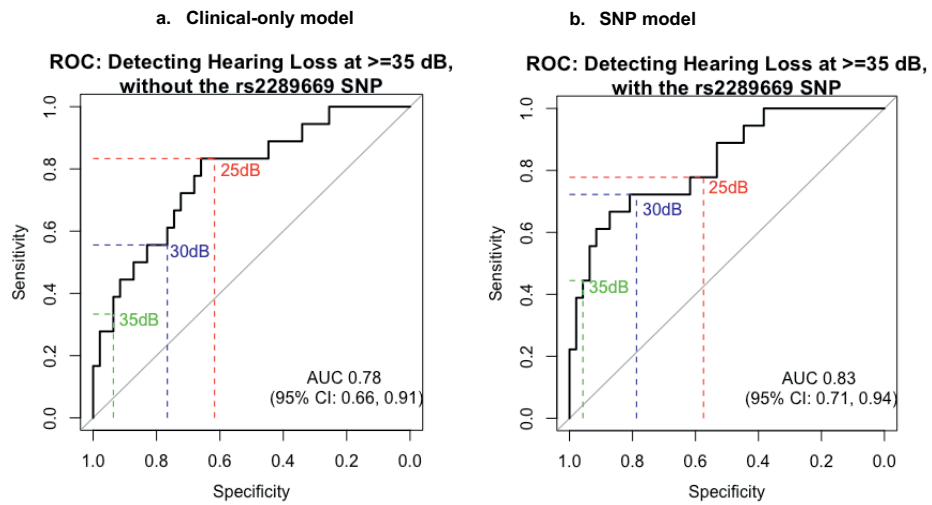

Figure 2. Receiver Operating Characteristics Curve
for models without (left, clinical-only model) and with (right, SNP model) incorporation of the rs2289669 SNP.

DISCUSSION

This study aimed to enhance the predictive ability of the clinical prediction model for cisplatin-induced hearing loss designed by Theunissen et al. by adding genetic information. We examined the association between 30 SNPs and post-treatment hearing capacity in HNSCC patients who received combined chemoradiation. Our gene analysis indicated that one SNP, rs2289669 within the *SLC47A1/Multidrug and toxin extrusion 1* (*MATE1*) gene, significantly predicted post-treatment hearing capability. Our findings suggests that individuals with less frequent variants of this SNP (i.e., homozygous minor) are more likely to develop CIHL, compared to those with more common variants. For context, in our cohort, 17.8% of individuals carried the homozygous minor allele for rs2289669, highlighting the potential impact of these rarer variants. When the rs2289669 SNP was incorporated into the previously designed clinical prediction model, it showed enhanced predictive power. Although predictive gains were not consistent across all hearing thresholds, adding the SNP improved the clinical predictive model at key hearing levels and thresholds, namely 35dB at PTA 1-2-4 kHz, relevant for the perception of speech and reimbursement of hearing aids in the Netherlands.

Genetic variations in cellular transporters may explain individual vulnerabilities to cisplatin-related toxicity. Recently, the role of *MATE1* in the development of cisplatin toxicity was studied. (29-32) *MATE1* is an H+-coupled organic cation bidirectional antiporter, expressed on the apical membrane of the tubular epithelium of the kidneys. (25, 32) The human tissue distribution of *MATE1* is comparable to that in mice, where it has been shown to play an important role in the pharmacokinetics of several drugs. (25) Nakamura et al. found that the plasma and renal levels of cisplatin were significantly higher in *MATE1* knock-out mice than in wild-type mice. (31) *MATE1* likely interacts with the *organic cation transporter 2 (OCT2)* in the systemic distribution of cisplatin. (29, 32) *OCT2* was already reported to be expressed in the cochlea and to be involved in the cochlear uptake of platinum. Interestingly, very recently, Waissbluth et al. showed that *MATE1* is expressed in the inner ear, and that cisplatin decreases both *OCT2* and *MATE1* expression in the cochlea. (30)

What is known about the *MATE1* rs2289669 variant from a study with patients using metformin—of which the uptake is also regulated by *MATE1*—is that it is likely associated with reduced transporter function. (33) A mice study demonstrated that *MATE1* deficiency leads to increased cisplatin-related nephrotoxicity and hematological toxicity (OR=1.92, 95% CI 1.13 to 3.25, *P*=0.016). (32) Hence, if *MATE1* rs2289669 is indeed associated with reduced transporter function, this variant may also be associated with a higher risk of CIHL. This is supported by our results, as we found that the A-allele is associated with more hearing loss. However, this contradicts Teft et al.'s findings that the rs2289669 *MATE1* A/A homozygous variant significantly reduces CIHL risk in 206 HNSCC patients (HR=0.46, 95% CI 0.26-0.84). (25) The exact role of *MATE1* in the cochlea needs to be further studied, although this may be challenging due to its bidirectional properties. (25)

Our analysis did not show significant associations between CIHL and several SNPs (*TPMT* (23), *ABCC3*(23, 24), *COMT* (24, 25, 34), *WFS1* (18, 22), *ACYP2* (18-22, 34-36), *ERCC2* (34), X*PC* (34), and *GSTP1* (34)) as previously reported. The lack of consistency and reproducibility in study results, along with heterogeneity in study populations (in terms of ethnicity/ancestry, cisplatin treatment protocols, and definition of CIHL), contribute to the challenges in drawing definitive conclusions. (13, 20) Additionally, many studies did not assess ultrahigh frequencies (8.0-20.0 kHz SPL), which generally are more sensitive frequencies for identifying CIHL, considering its initial manifestation at these frequencies.

The present study benefits from a well-defined cohort of patients who received combined chemoradiation as a primary treatment for HNSCC. The inclusion of SNPs and audiometry data allowed for a comprehensive analysis of the relationship between genetic variants and CIHL. Our study adopted a targeted approach by including 30 candidate SNPs in the model-building process, strategically focusing on specific genetic markers of interest, based on published literature.

A limitation of the current study is that it relied on a retrospective analysis. Information on chemo- and radiotherapy dosage, audiometry, and other clinical factors was missing in a small number of patients. To address potential data gaps, we employed a mechanism of multiple imputation based on chained equations. Second, the selection of the 31 SNPs in this study was informed by a review of literature available up until 2017. Therefore, any genetic markers linked to ototoxicity discovered post-2017 were not included in our analysis. This could mean that potentially relevant SNPs identified after this cutoff were not included in the analysis. Finally, the predictive ability of the model, despite the inclusion of genetic variants, still demonstrated limitations in sensitivity and specificity. This suggests that factors beyond the current set of variables, such as environmental factors or additional genetic markers, may contribute to the variability in CIHL susceptibility.

CIHL is a common and debilitating side effect of cisplatin treatment, with important consequences on patients' quality of life. (8) Understanding the genetic factors contributing to CIHL may enhance clinical prediction models, allowing clinicians to provide tailored pre-treatment counselling, inform patients about the potential risk of CIHL, and enable the identification of individuals who may benefit from otoprotectants. Moreover, the identification of SNPs related to CIHL may facilitate

the development of novel preventive strategies and interventions. Additional research is needed to assess the validity and generalizability of the prediction model in various genetic backgrounds and clinical settings in other HNSCC patient populations. Furthermore, the specific mechanisms through which these genetic variants contribute to the development of CIHL need to be elucidated.

CONCLUSION

Our findings demonstrate a significant association between the rs2289669 SNP within the *SLC47A1 (MATE1)* gene and post-treatment hearing capability in patients undergoing combined chemoradiation. These results contribute to a deeper understanding of the genetic factors influencing hearing outcomes in this population and provide insights into potential strategies for personalized treatment approaches in the future.

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5

THE ASSOCIATION BETWEEN SKELETAL MUSCLE MASS AND SENSORINEURAL HEARING LOSS UPON CISPLATIN-BASED CHEMORADIOTHERAPY IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

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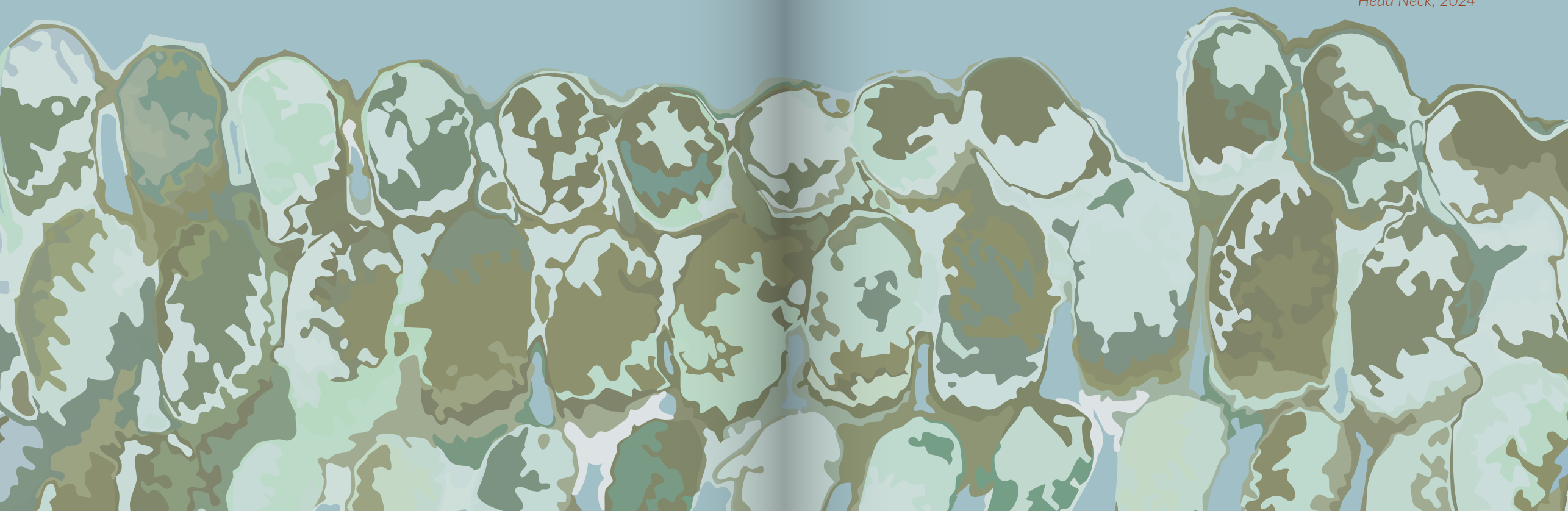

ABSTRACT

Introduction

Patients with head and neck squamous cell carcinoma (HNSCC) treated with cisplatin-based chemoradiotherapy (CRT) frequently experience irreversible sensorineural hearing loss (SNHL). Patients with low lumbar skeletal muscle index (LSMI) may experience higher serum peak dosages of cisplatin. This study investigated whether pre-treatment low LSMI is associated with increased SNHL upon cisplatin-based CRT.

Material and methods

LSMI was assessed using routine pre-treatment CT scans. Pure tone audiometry was performed at baseline and at follow-up to assess treatment-related SNHL. Linear mixed models were used to reveal a potential association between the continuous variable LSMI and SNHL.

Results

This retrospective cohort study included 81 patients and found a significant association between low LSMI and increased treatment-related SNHL at pure-tone frequencies vital for the perception of speech (averaged of 1, 2, and 4 kHz) (p = 0.048).

Conclusions

HNSCC patients with low LSMI suffer increased treatment-related SNHL upon cisplatin-based CRT.

INTRODUCTION

Therapy for locally advanced head and neck squamous cell carcinoma (HNSCC) often consists of concomitant high-dose cisplatin chemo-radiotherapy (CRT). As standard of care, triweekly cisplatin, dosed at 100 mg/m^2, or weekly cisplatin, dosed at 40 mg/m^2, is given intravenously. (1, 2) Cisplatin has proven efficacy in the improvement of locoregional disease control compared to RT alone. However, cisplatin treatment is often accompanied by toxicities such as nausea, stomatitis, myelosuppression, nephrotoxicity, and ototoxicity. (3-5)

Sensorineural hearing loss

Cisplatin may cause symmetrical and irreversible ototoxicity. This may consist of tinnitus and/or sensorineural hearing loss (SNHL). SNHL due to cisplatin-based CRT starts at the extended high-frequencies and gradually progresses to lower frequencies with ongoing treatment. The severity depends on the cumulative cisplatin dose. (5-7) Cisplatin can potentially damage various cochlear structures, such as the inner and outer hair cells, spiral ganglion, and stria vascularis. The development of SNHL involves several biological processes, including the release of toxic reactive oxygen species and the depletion of the cochlea's protective antioxidants. (5, 6, 8-10) Varying incidences of cisplatin-induced SNHL have been reported by previous studies, which however, were based on different treatment schedules and definitions of ototoxicity. A recently published meta-analysis concerning 1,021,700 patients treated with platinum for various cancer types, showed that 43% of the subjects developed SNHL. (11) In contrast, earlier studies reported even higher incidence rates, reaching up to 80%. (5, 12-14) Risk factors for cisplatin-induced SNHL are, amongst others, favorable pre-treatment hearing capacity, as is often seen in younger patients, and a concomitant cochlear radiation dose ≥ 30 Gray. (13, 15, 16) Also, earlier research showed that patients of younger age and with better pre-treatment hearing capacity suffer increased treatment-related hearing loss (threshold shifts). (17)

Ototoxicity can be classified as dose limiting toxicity (DLT) during cisplatin-based CRT and consequently compromise reaching a cumulative cisplatin dose of ≥ 200 mg/m^2, generally accepted to be the minimum dose needed for therapeutic benefit. (18-20) Recently, it was found that patients treated with triweekly cisplatin-based CRT, DLT was caused by ototoxicity in 42% of the cases, followed by nephrotoxicity (27%). (21) It was shown that patients with low skeletal muscle mass (SMM) had significantly more DLT compared to patients without low SMM (66% versus 34%; p < 0.01). (21) Also, other studies showed that patients with low SMM are at higher risk of developing cisplatin DLT. (22-24) Furthermore, DLT caused by ototoxicity was more frequently, although not significantly, observed

in patients with low SMM compared to patients without low SMM (67.2% versus 32.8%; p = 0.12). (21) However, only ototoxicity that was believed to be the cause of DLT was evaluated, thereby leading to the possible exclusion of patients with clinically relevant ototoxicity that was not severe enough to change the dose. (21)

Skeletal muscle mass

SMM can easily be assessed prior to treatment using diagnostic computed tomography (CT) or magnetic resonance imaging (MRI) scans. Cross-sectional muscle area delineation at the third cervical vertebra (C3) as a proxy for the lumbar skeletal muscle index (LSMI) (25-30) can be used as a predictor of DLT for patients with HNSCC treated with CRT. (21-23) An LSMI below or equal to 43.2 cm^2/m^2 was defined as low SMM. (22) Since cisplatin mainly distributes to the fat-free mass, it is plausible that patients with low SMM, hence less fat-free mass, might experience higher peak dosages of cisplatin. (31) A higher peak dosage can hypothetically increase the risk of toxicity, especially in a vulnerable organ, such as the ear, that is highly affected by cumulative cisplatin dose. (32, 33)

To our knowledge, no data is available regarding the association between pre-treatment the continuous variable LSMI and binary variable SMM and the severity of SNHL in patients with HNSCC. Therefore, the primary aim of this study is to assess whether pre-treatment LSMI is associated with increased treatment-related SNHL in HNSCC patients treated with cisplatin-based CRT, using either a weekly or triweekly cisplatin regimen. Secondary aims are evaluations of whether pre-treatment low SMM is associated with clinically relevant treatment-related SNHL, with SNHL graded according to several ototoxicity grading systems (American Speech-Language-Hearing Association (ASHA) (34); Common Terminology Criteria for Adverse Events CTCAE (35); and TUNE (36), and if patients with low SMM have a higher incidence of indications for hearing aid after treatment.

METHODS

Patients and study design

This article presents a retrospective cohort study conducted at the Netherlands Cancer Institute between January 2020 and May 2023. This study was approved by the Institutional Review Board of the Netherlands Cancer Institute (ID IRBd22-261) and executed according to the Declaration of Helsinki.

We included patients with HNSCC with an indication for curative primary and adjuvant high-dose cisplatin-based CRT. This consisted of RT given five times a week for seven consecutive weeks with a cumulative dose of 70 Gray (primary setting) or 66 Gray (adjuvant setting) with concomitant intravenous cisplatin. All patients received cisplatin administered in a triweekly (days 1, 22 and 43, 100 mg/m^2) or weekly (days 1, 8, 15, 22, 29, 36 and 43, 40 mg/m^2) regimen. Patients needed to have a scan prior to CRT assessable for SMM measurement, which means that the scan was free of artifacts and bilateral lymph node metastases involvement of the sternocleidomastoid muscle at the level of the C3. For all eligible patients, both baseline and follow-up audiometry records were available. Patients with a cochlear radiation dose of ≥ 30 Gray were excluded due to the risk of clinically relevant radiation-induced SNHL. (37, 38)

Information about age, sex, human papillomavirus (HPV) status of the tumor, tumor localization, Tumor Node Metastasis (TNM) stage from the Union for International Cancer Control (UICC, 8th edition) (39), weight (in kg) and height (in cm), cisplatin treatment regimen (triweekly versus weekly), cumulative cisplatin dose given (in mg/m^2), DLT and cause of DLT was collected. DLT was defined as any toxicity leading to a cisplatin dose reduction of ≥ 50%, a treatment delay of ≥ 4 days, or early termination of the chemotherapy. (23, 40)

Audiometry assessment

Audiometry was performed at baseline and on average five weeks (range 0 – 14 weeks) post-CRT. Air conduction (AC) thresholds were measured for standard frequency pure tone audiometry at 0.125 to 8.0 kHz and expressed in dB Hearing Level [dB (HL)] and extended high-frequency pure tone audiometry at 8.0 to 16.0 kHz expressed in dB Sound Pressure Level [dB (SPL)]. Bone conduction (BC) thresholds were measured for standard frequency pure tone audiometry at 0.5, 1.0, 2.0 and 4.0 kHz [dB (HL)]. If the difference between AC and BC was ≥ 10 dB at 0.5, 1, 2, or 4 kHz, BC thresholds were used to ensure that sensorineural hearing levels were used for analysis. The measurements were obtained in a sound-proof booth using Decos Audiology Workstation. The Telephonics TDH-39P headphone was used for standard frequency AC, the Radioear B71 bone conductor for BC, and the Sennheiser HDA 200 headphone was used to measure extended high-frequency audiometry.

A Pure Tone Average (PTA) was then computed at frequencies 1-2-4 kHz HL (PTA 1-2-4 kHz), relevant for the perception of speech. A PTA at frequencies 8-10-12.5 kHz SPL (PTA 8-10-12.5 kHz) was also calculated, pertinent to the perception of (ultra-)high sounds, as in music, but also for speech perception in noise. (41, 42) If the threshold at extended high-frequency audiometry was not available for 8.0 kHz SPL, this threshold was calculated by taking the dB (HL) value of the standard frequency pure tone threshold at 8 kHz plus adding 13

dB, following the guidelines of ISO 389-1. (43) If a patient's hearing threshold exceeded the maximum output capacity of the audiometer during the follow-up measurement, rendering it untestable, this threshold was computed by adding 10 dB to the maximum measurable threshold of the audiometer (e.g., 100 + 10 = 110 dB, depending on the audiometer's settings).

Grading scale	Definition of hearing loss
ASHA	a) 20 dB decrease at any one tested frequency
	b) 10 dB decrease at any two adjacent test frequencies
	c) loss of response at three consecutive test frequencies where responses were previously obtained
CTCAE v5.0	Based on the threshold shifts up to 8 kHz HL
	Grade 1: Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear OR Subjective change in hearing in the absence of documented hearing loss;
	Grade 2: Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear;
	Grade 3: Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear OR hearing aid or intervention indicated
	Grade 4: Decrease in hearing to profound bilateral loss (absolute threshold >80 dB HL at 2 kHz and above); non-serviceable hearing
TUNE	Grade 0: No hearing loss
	Grade 1a: Threshold shift ≥ 10 dB at [8-10-12.5] OR subjective complaints in the absence of a threshold shift
	Grade 1b: Threshold shift ≥ 10 dB at [1-2-4]
	Grade 2a: Threshold shift ≥ 20 dB at [8-10-12.5]
	Grade 2b: Threshold shift ≥ 20 dB at [1-2-4]
	Grade 3: Hearing level ≥ 35 dB HL at [1-2-4] de novo
	Grade 4: Hearing level ≥ 70 dB HL at [1-2-4] de novo

Table 1: Grading scales that were used in this study for the assessment of post-treatment platinum-related hearing loss.
Abbreviations: ASHA: American Speech-Language-Hearing Association; CTCAE: Common Terminology Criteria for Adverse Events.

Threshold shifts and PTA threshold shifts, were calculated as post-treatment hearing thresholds minus pre-treatment hearing thresholds at single frequencies and as post-treatment PTA minus pre-treatment PTA, for both PTA 1-2-4 kHz and PTA 8-10-12.5 kHz. Clinically relevant treatment-related SNHL was defined as a threshold shifts of ≥ 10 dB at one of these PTAs in one or both ears. In addition, the severity of treatment-related SNHL was assessed using three distinct ototoxicity grading scales, as outlined in Table 1. Namely, the ASHA grading scale for hearing loss due to ototoxic drugs (44), the CTCAE version 5.0 (35), and the TUNE criteria (36) were applied. It may be appreciated that all three grading scales use a combination of threshold shift and post-treatment hearing level to grade ototoxicity. Therefore we also defined another outcome measure to reflect the post-treatment hearing level, namely the lower threshold for audiological rehabilitation in the Netherlands; A new indication for hearing aids due to therapy was defined as a PTA 1-2-4 kHz of < 35 dB before treatment and ≥ 35 dB after treatment. (45, 46) The incidence of patients with a clinically relevant treatment-related SNHL at PTA 1-2-4 kHz, and PTA 8-10-12.5 kHz, and an indication for hearing aids de novo was subsequently calculated, for patients with and without low SMM.

Assessment of lumbar skeletal muscle index

LSMI, a continuous variable, was assessed using the pre-treatment CT or MRI scan, using SliceOmatic software v 5.0 (Tomovision, Canada), using verified methods. (25, 27, 28, 30) First, the slice at the level of C3 was selected by scrolling through the vertebra from cranial to caudal direction until the entire vertebral arc was visualized. If it was not possible to select a slice showing the entire vertebral arc due to slice thickness, the most caudal slice with a nearly closed vertebral arch was selected. Secondly, the cross-sectional muscle area at C3 was measured by delineating the sternocleidomastoid muscles and paravertebral muscles semi-automatically, subsequently excluding the fatty tissue. If unilateral lymph node metastasis compromised a sternocleidomastoid muscle, the unaffected sternocleidomastoid muscle was delineated twice. (25) Thirdly, the cross-sectional muscle area at the level of the L3 was calculated (22):

$$cross-sectional\ muscle\ area\ at\ L3\ (cm^2) = 27.304 + 1.363 * cross-sectional\ muscle\ area\ at\ C3\ (cm^2) - 0.671 * Age\ (years) + 0.640 * Weigth\ (kg) + 26.442 * Sex\ (Sex = 1\ for\ female, 2\ for\ male)$$

The cross-sectional muscle area at L3 was then normalized for height, which resulted in the LSMI:

$$LSMI\ (cm^2/m^2) = \frac{\text{cross} - \text{sectional muscle area}\ at\ L3\ (cm^2)}{Height\ (m)^2}$$

A patient with LSMI ≤ 43.2 cm^2/m^2 was considered as having low SMM, which is a binary variable. (22, 47)

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) if they were normally distributed or as median with interquartile range (range between first (Q1) and third (Q3) quartile) when their distribution was skewed. The normality of the distributions was tested using the Kolmogorov-Smirnov test. Categorical variables are presented as absolute and relative frequencies. Differences in baseline characteristics between patients with a low SMM and those without a low SMM were tested using independent sample *t*-tests for normally distributed continuous variables or, Mann-Whitney U tests for non-normally distributed continuous variables, and Pearson's Chi-square tests, Fisher exact tests or Fisher-Freeman-Halton exact tests for categorical data. Differences in baseline characteristics and occurrence of DLT between patients treated with weekly versus triweekly cisplatin were assessed using the same statistical methods.

Linear mixed models (LMM) for nested data were applied to assess the association between the continuous variable LSMI and other covariates with the two primary outcomes for treatment-related SNHL, i.e., the threshold shift at PTA 1-2-4 kHz in dB (HL) and the threshold shift at PTA 8-10-12.5 kHz in dB (SPL). The outcomes were expressed per ear (left or right) and measurements from individual patients were clustered. In all models, only the intercept was estimated as a random parameter. Univariable models to test associations with SNHL on both PTAs included the following variables: LSMI, age, cumulative cisplatin dose, baseline hearing at PTA 1-2-4 kHz HL, sex, cochlear RT dose, and cisplatin schedule. The multivariable LMM incorporated variables with a p-value < 0.1 in the univariable analysis.

Mean threshold shifts at PTA 1-2-4 kHz in dB (HL), and PTA 8-10-12.5 kHz in dB (SPL) between patients with low SMM and patients without low SMM were compared using LMM for nested data. The occurrence of new indications for hearing aids de novo (threshold of ≥ 35 dB after treatment) was compared between both groups using a Chi-square test. Using a linear-by-linear test, grading scores for ototoxicity were compared between patients with low SMM and those without low SMM.

Data analysis was performed using IBM SPSS Statistics 27. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Subjects and general characteristics

A cohort of 136 patients treated in the Netherlands Cancer Institute between 2018 and 2023 was identified. Fifty-five patients were excluded for further analysis, because they received a cochlear radiation dose ≥ 30 gray on one or both ears (n = 10) or baseline and/or follow-up audiometry was not available (n = 45). This resulted in 81 patients (162 ears) being eligible for analysis. The median age was 62 [Q1-Q3: 54-67] years, and the mean LSMI was 42.39 ± 6.58 cm^2/m^2. General characteristics stratified by SMM category are presented in Table 2. The proportion of patients with low versus without low SMM (a binary variable) was similar (48% versus 52%), which is in agreement with previous literature (21, 23). In total, 56 male patients were included, of whom 16 (29%) had low SMM, and 25 female patients were included, of whom 23 (92%) had low SMM. The proportion of female patients was statistically significantly different between both groups (p < 0.01), as reported in previous literature.(21) Patients with low SMM had significantly less often HPV-positive tumors (28% versus 57%; p = 0.01), which is also observed in previous literature.(48) DLTs were evenly distributed between patients with and without low SMM (41% versus 43%; p = 0.99), as was cumulative cisplatin dose (median 280 mg/m^2 versus 280 mg/m^2; p = 0.86), and cisplatin schedule (triweekly 28% versus 26% and weekly 72% versus 74%; p = 0.99).

Additionally, no differences between characteristics and DLT in patients receiving weekly versus triweekly cisplatin were found: sex (p = 0.59), HPV-status (p = 0.09), tumor location (p = 0.35), LSMI (42.77 cm^2/m^2 versus 41.35 cm^2/m^2; p = 0.39), age at diagnosis (median 60.00 versus 63.00 years; p = 0.98), cumulative cisplatin dose (median 280 mg/m^2 versus 200 mg/m^2; p = 0.82) and DLT (41% versus 46%; p = 0.80). Only TNM-stage differed significantly between cisplatin regimens: patients receiving the weekly regimen more often had a lower TNM stage (p = 0.03).

Multivariable analysis of the association between lumbar skeletal muscle mass and treatment-related sensorineural hearing loss

Results of the univariable and multivariable LMM are presented in Table 3 and Table 4. In the univariable LMM, a significant negative association between treatment-related SNHL at PTA 1-2-4 kHz HL and LSMI was found (p = 0.03). Also, significant positive associations between treatment-related SNHL at PTA 1-2-4 kHz HL and cisplatin schedule (p = 0.03) and cochlear radiotherapy dose (p < 0.01) were found. In the multivariable LMM, threshold shifts at PTA 1-2-4 kHz HL were significantly smaller in patients with higher LSMI (p < 0.05) and significantly larger with increased cochlear radiotherapy dose (p < 0.01). The LMM for the

threshold shift at PTA 8-10-12.5 kHz SPL did not show a significant association with LSMI (p = 0.77). However, a larger threshold shift at PTA 8-10-12.5 kHz SPL was associated with a higher cochlear radiotherapy dose (p = 0.02).

Variable	Total	Low SMM	No low SMM	p-Value
	81 (100%)	39 (48%)	42 (52%)	
Age at diagnosis (median [Q1-Q3])	62 [54-67]	63.00 [56-69]	61.50 [53-66]	0.184[b]
Sex				**< 0.001[c]**
Female	25 (31)	23 (59)	2 (5)	
Male	56 (69)	16 (41)	40 (95)	
HPV-status				**0.013[d]**
Negative/unknown	46 (57)	28 (72)	18 (43)	
Positive	35 (43)	11 (28)	24 (57)	
Tumor site				0.506[e]
Larynx	9 (11)	5 (13)	4 (10)	
Hypopharynx	4 (5)	2 (5)	2 (5)	
Oropharynx	48 (59)	20 (51)	28 (66)	
Oral cavity	12 (15)	7 (18)	5 (12)	
Nasopharynx	4 (5)	1 (2.5)	3 (7)	
Unknown primary	1 (1)	1 (2.5)	0 (0)	
Sinonasal	1 (1)	1 (2.5)	0 (0)	
Other	2 (3)	2 (5)	0 (0)	
UICC category				**0.023[e]**
I	6 (7)	2 (5)	4 (10)	
II	24 (30)	7 (18)	18 (43)	
III	15 (19)	9 (23)	5 (12)	
IVA/IV	22 (27)	10 (26)	12 (29)	
IVB	14 (17)	11 (28)	3 (7)	
LSMI (cm^2/m^2) (mean±SD)	42.39 ± 6.58	37.02 ± 4.49	47.37 ± 3.60	**<0.001[a]**
Treatment type				0.999[d]
Cisplatin weekly (40 mg/m^2)	59 (73)	28 (72)	31 (74)	
Cisplatin triweekly (100 mg/m^2)	22 (27)	11 (28)	11 (26)	
Cumulative cisplatin dose given (median [Q1-Q3])	280 [200-280]	280 [200-280]	280 [200-280]	0.862[b]

Table 2: Continued

Variable	Total	Low SMM	No low SMM	p-Value
	81 (100%)	39 (48%)	42 (52%)	
DLT				0.999[d]
No	47 (58)	23 (59)	24 (57)	
Yes	34 (42)	16 (41)	18 (43)	
Cause of DLT				0.340[e]
Ototoxicity	0 (0)	0 (0)	0 (0)	
Nephrotoxicity	9 (31)	7 (44)	4 (22)	
Kidney dysfunction, pre-renal	1 (4)	0	1 (5)	
Hematologic toxicity	12 (41)	7 (44)	7 (39)	
General condition of the patient	3 (10)	0	3 (17)	
Other	4 (14)	2 (12)	3 (17)	

Table 2: General characteristics of the study population according to the presence or absence of low skeletal muscle mass
[a] = tested with independent sample t-test [b] = tested with Mann-Whitney U Test, [c] = tested with Fisher's Exact Test, [d] = tested with Pearson Chi-Square test, [e] = tested with Fisher-Freeman-Halton Exact Test
DLT = dose limiting toxicity; HPV = Human Papillomavirus; Q1-Q3 = range between first and third quartile; LSMI = lumbar skeletal muscle index; SMM = skeletal muscle mass; SD = standard deviation; UICC = tumor stage based on the Union for International Cancer Control

Treatment-related sensorineural hearing loss, grading scores and new indications for hearing aids and their associations with skeletal muscle mass

Figure 1 represents mean hearing thresholds obtained with pure tone audiometry (0.125 to 8 kHz HL) and pure tone extended high-frequency audiometry (8 to 16 kHz SPL) at baseline and after treatment. The mean threshold shift at 4 kHz HL was 14.4 dB for patients with low SMM (a binary variable) compared to 6.8 dB for patients without low SMM (p = 0.03). The mean threshold shift at 8 kHz HL was 23.5 dB for patients with low SMM compared to 14.4 dB for patients without low SMM (p = 0.03).

Variable	Univariable analysis	95% Confidence Interval			Multivariable analysis	95% Confidence Interval		
	Estimate	Lower bound	Upper bound	*p*-value	Estimate	Lower bound	Upper bound	*p*-value
Lumbar skeletal muscle index (cm^2/m^2)	-0.280	-0.539	-0.021	**0.034**	-0.253	-0.504	-0.003	**0.048**
Age at diagnosis (years)	0.120	-0.079	0.319	0.232				
Sex								
Male	Reference							
Female	2.109	-1.637	5.856	0.266				
Cisplatin schedule								
Triweekly	Reference							
Weekly	-4.128	-7.939	-0.317	**0.034**	-3.620	-7.370	0.130	0.058
Cumulative cisplatin dose	-0.015	-0.047	0.018	0.369				
Baseline hearing level at PTA 1-2-4 kHz HL	-0.062	-0.182	0.058	0.308				
Cochlear radiotherapy dose	0.240	0.071	0.403	**0.005**	0.228	0.066	0.389	**0.006**

Table 3: Linear mixed models for treatment-related sensorineural hearing loss at pure tone average 1-2-4 kHz (in HL)

Variable	Univariable analysis	95% Confidence Interval			Multivariable analysis	95% Confidence Interval		
	Estimate	Lower bound	Upper bound	*p*-value	Estimate	Lower bound	Upper bound	*p*-value
Lumbar skeletal muscle index (cm^2/m^2)	-0.063	-0.507	0.377	0.774				
Age at diagnosis (years)	-0.358	-0.679	-0.038	**0.029**	-0.233	-0.583	0.117	0.189
Sex								
Male	Reference							
Female	1.646	-4.559	7.851	0.599				
Cisplatin schedule								
Weekly	Reference							
Triweekly	-2.995	-9.642	3.653	0.372				
Cumulative cisplatin dose	0.016	-0.042	0.074	0.575				
Baseline hearing level at PTA 1-2-4 kHz	-0.224	-0.421	-0.027	**0.026**	-0.115	-0.331	0.100	0.290
Cochlear radiotherapy dose	0.355	0.074	0.747	**0.013**	0.337	0.058	0.616	**0.018**

Table 4: Linear mixed models for treatment-related sensorineural hearing loss at pure tone average 8-10-12.5 kHz (in SPL)

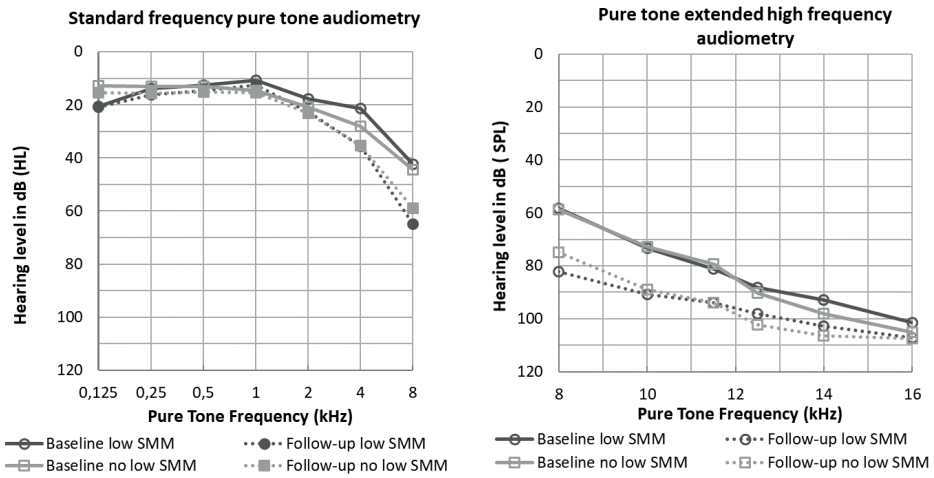

Figure 1: Baseline and follow-up mean audiometry values
for both skeletal muscle mass groups
Abbreviations: SMM = skeletal muscle mass, HL = Hearing Level, SPL = Sound Pressure Level.

The incidence of clinically relevant treatment-related SNHL (threshold shift of ≥ 10 dB) at PTA 1-2-4 kHz HL was 36% in patients with low SMM compared to 17% in patients without low SMM ($p < 0.05$), Table 5. Mean threshold shifts at PTA 1-2-4 kHz were 7.1 dB in patients with low SMM and 3.4 dB in patients without low SMM ($p = 0.04$). The incidence of clinically relevant treatment-related SNHL at PTA 8-10-12.5 kHz SPL was 77% in patients with low SMM compared to 62% in patients without low SMM ($p = 0.14$). Mean threshold shift at PTA 8-10-12.5 kHz SPL was 19.0 dB in patients with low SMM and 14.7 dB in patients without low SMM ($p = 0.13$). The incidence of a novel indication for hearing aids for one or both ears after treatment was higher (but statistically not significant) in patients with low SMM (26%) compared to patients without low SMM (12%; $p = 0.11$). There were no statistically significant differences in scores at all grading scales for ototoxicity between patients with low SMM and patients without low SMM (ASHA: $p = 0.80$; CTCAE: $p = 0.37$; TUNE: $p = 0.10$).

Variables	Low SMM	No low SMM	*p*-value
	39 patients or 78 ears	42 patients or 84 ears	
Hearing loss at PTA 1-2-4 kHz HL			
Incidence of treatment-related SNHL (%)	14 (36)	7 (17)	0.048[a]
Hearing loss in dB per ear (mean ± SD)	7.1 dB (9.6)	3.4 dB (7.0)	**0.037[c]**
Hearing loss at PTA 8-10-12.5 kHz SPL			
Incidence of treatment-related SNHL (%)	30 (77)	26 (62)	0.144[a]
Hearing loss in dB per ear (mean ± SD)	19.0 (16.0)	14.7 (11.5)	0.129[c]
Hearing aids indicated			
Incidence (%)	10 (26)	5 (12)	0.112[b]
ASHA			0.802[d]
no hearing loss (%)	6 (15)	8 (19)	
grade A (%)	3 (8)	4 (10)	
grade B (%)	28 (72)	26 (62)	
grade C (%)	2 (5)	4 (10)	
CTCAE v5.0			0.366[d]
grade 0 (%)	23 (59)	32 (76)	
grade 1 (%)	3 (8)	3 (7)	
grade 2 (%)	2 (5)	1 (2)	
grade 3 (%)	11 (28)	6 (14)	
grade 4 (%)	0 (0)	0 (0)	
≥ grade 3 (%)	11 (28)	6 (14)	0.124[a]
TUNE			0.100[d]
grade 0 (%)	6 (15)	10 (24)	
grade 1a (%)	7 (18)	12 (29)	
grade 1b (%)	1 (3)	1 (2)	
grade 2a (%)	14 (37)	12 (32)	
grade 2b (%)	1 (3)	1 (2)	
grade 3 (%)	10 (26)	6 (14)	
grade 4 (%)	0 (0)	0 (0)	
≥ grade 2a (%)	25 (64)	19 (45)	0.089[a]

Table 5: The incidence of clinically relevant sensorineural hearing loss (≥ 10 dB threshold shift) at PTA 1-2-4 kHz (in dB [HL]) and PTA 8-10-12.5 kHz (in dB [SPL]) in low and no low SMM groups, the incidence of an indication for hearing aids de novo (PTA ≥ 35 dB after CRT and < 35 dB at baseline), and scores on various grading scales.
[a]) Chi-Square test; [b]) Fisher's exact test, [c]) Linear mixed model; [d]) Linear-by-linear test. A p-value of < 0.05 is considered statistically significant
Abbreviations: ASHA = American Speech-Language-Hearing Association criteria; CTCAE = Common Terminology Criteria for Adverse Events; HL = hearing level; kHz = kilohertz; PTA = Pure Tone Average; SMM = skeletal muscle mass; SNHL = Sensorineural hearing loss; SPL = Sound pressure level; TUNE = TUNE criteria.

DISCUSSION

The primary aim of this study was to assess a potential association between SNHL and LSMI in HNSCC patients treated with high-dose cisplatin-based CRT. Patients with a low SMM, as binary variable, experience higher serum peak dosages than their no low SMM counterparts. (31) Due to its hydrophilic characteristics, cisplatin mainly distributes to the fat-free mass, of which the muscles are the largest component. (49) Patients with low SMM, hence low fat-free mass, might be relatively overdosed since cisplatin dosage is calculated normalized to body surface area. Hence, we hypothesized that patients with lower LSMI and low SMM may suffer increased treatment-related SNHL. This retrospective cohort study indeed showed that treatment-related SNHL at pure-tone frequencies essential for the perception of speech, as expressed by PTA 1-2-4 kHz HL, is associated with pre-treatment LSMI (the continuous variable) and low SMM (the binary variable). Moreover, the incidence of clinically relevant hearing loss showed a statistically significant 19%-point difference between patients with low SMM and patients without low SMM. However, LSMI and low SMM were not associated with SNHL at the extended high-frequencies, as expressed by PTA 8-10-12.5 kHz SPL. This can be explained by the fact that cisplatin-related SNHL starts at extended high-frequencies, and at these frequencies SNHL may have reached its maximum at an earlier stage during treatment in all patients, despite their individual sensitivity to develop ototoxicity. (15)

The number of studies that reported on the association between SMM and cisplatin-induced SNHL is limited. To our knowledge, one previous study investigated the predictive impact of low SMM on cisplatin DLT and analyzed the frequencies of ototoxicity (21), which reported a higher incidence of dose limiting ototoxicity in patients with low SMM compared to patients without low SMM (67% versus 33%; $p = 0.12$). (21) The investigators did not define a specific degree of hearing deterioration from which ototoxicity was classified as DLT. Surprisingly, none of the DLTs in our cohort was due to ototoxicity. (21)

No associations between SNHL and cumulative cisplatin dosage could be found. This is noteworthy since it is generally accepted that cisplatin-induced hearing loss is dose dependent. (32, 45) Also, there was no association between SNHL and cisplatin regimen. Weekly cisplatin is non-inferior in terms of oncologic outcomes to the triweekly regimen in the postoperative setting for patients with HNSCC. (50, 51) Due to the lower toxic peak dosages, weekly cisplatin is thought to be less toxic when compared to a triweekly cisplatin schedule. (50, 51) A recent study showed that SMM is also a predictor for DLT (specifically for hematologic toxicities) in the weekly regimen, but this study did not report on ototoxicity specifically. (52) In our cohort, the distributions of weekly versus triweekly regimens were equal between patients with and without low SMM, but no differences in DLT and SNHL between these groups could be observed. It should be emphasized that we defined low SMM as LSMI ≤ 43.2 cm^2/m^2 in accordance to previous studies towards DLT in patients with HNSCC (21-23), but this cutoff remains under debate since others propose different criteria for low SMM (53), for example, subgroups for male and female patients. (54) Studies that use as LSMI ≤ 43.2 cm^2/m^2 as cutoff for low SMM despite sex, have high rates of low SMM in the female population (up to 95%) (21, 22) compared to studies that used sex specific cutoffs (25 to 50%). (23, 48, 54)

Our study showed a significant association between cochlear RT dose and SNHL, which aligns with previous studies (12, 38, 55), as a cochlear RT dose of ≥ 30 Gy may lead to clinically relevant hearing loss. (37, 38, 45) Although patients who received a cochlear RT dose ≥ 30 Gy RT were excluded from this study, we still observed a significant association between the RT dose and SNHL. This partly contradicts another review, which states that clinically relevant treatment-related SNHL (≥ 10 dB) is rarely seen with a mean cochlear radiation dose under 45 Gray. (55)

Limitations of this study are the retrospective design and, most of all, the relatively small study group and single-center set-up. The range in timing of post-treatment audiometry was 0 – 14 weeks, however we do not expect that this influenced our results as cisplatin induced SNHL develops semi-instantaneous after treatment. Additionally, earlier research did not find a difference in hearing loss between directly post treatment, months and years after treatment. (33, 56) We did not exclude patients with missing 8 kHz SPL values (n = 22) nor those that exceeded the maximum output of the audiometer upon CRT (n = 36). If we were to exclude every patient who exceeded the maximum output level of the audiometer during CRT, we would have excluded 36 patients with treatment-related SNHL, thereby biasing our results. It's important to note that excluding these patients would mean excluding those who actually performed poorly, as their values were not measurable. One of the strengths is the availability of elaborate and complete audiometry data, including extended high-frequency audiometry. Finally, our research can easily be compared to future studies since various ototoxicity grading systems have been applied.

In conclusion, this is the first study showing a significant association between lower LSMI, as a continuous variable, and low SMM, as a binary variable, and increased treatment-related SNHL at frequencies essential for the perception of speech in HNSCC patients treated with cisplatin CRT.

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6

TREATMENT-RELATED HEARING LOSS IN WEEKLY VERSUS TRIWEEKLY CISPLATIN CHEMORADIATION FOR HEAD AND NECK CANCER

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ABSTRACT

Purpose

Cisplatin-induced hearing loss is a common side effect in patients treated with cisplatin-based chemoradiation (CRT) for head and neck squamous cell carcinoma. The extent of hearing loss after concurrent CRT was compared between triweekly (3x100 mg/m^2) and weekly (7x40 mg/m^2) cisplatin CRT.

Method

This retrospective cohort study was conducted in the Antoni van Leeuwenhoek Hospital and included 129 patients with cisplatin-based CRT for head and neck cancer (72 treated in the triweekly and 57 in the weekly regimen). Baseline and follow-up pure-tone audiometry was conducted to assess hearing loss. Clinically relevant hearing loss was defined as a decline upon treatment of ≥ 10 decibel at a pure tone average 1-2-4 kilohertz and/or 8-10-12.5 kilohertz.

Results

The incidence of clinically relevant cisplatin CRT induced hearing loss was 42% in the triweekly versus 19% in the weekly group ($p < 0.01$). The mean threshold shift at a pure tone average (PTA) 1-2-4 kilohertz was 9.0 decibel in the triweekly compared to 4.3 decibel in the weekly CRT group ($p < 0.01$). At PTA 8-10-12.5 kilohertz, the incidence of clinically relevant hearing loss was 75% in the triweekly compared to 74% in the weekly CRT group ($p = 0.87$). The mean threshold shift at PTA 8-10-12.5 kHz was 20.2 decibel versus 15.6 decibel, respectively ($p = 0.07$).

Conclusion

Cisplatin-dose reduction to a weekly cisplatin CRT regimen for head and neck cancer may reduce the incidence of clinically relevant hearing loss at frequencies vital for speech perception.

INTRODUCTION

Cisplatin is a widely used anti-cancer drug in treating numerous types of cancers, including head and neck squamous cell carcinoma (HNSCC). Advanced HNSCC is often treated with (adjuvant) high-dose cisplatin chemoradiation (CRT), i.e., 3x100 mg/m^2, as adding cisplatin to RT leads to improved survival rates in these patients compared to radiotherapy alone. (1-3) However, high-dose cisplatin may cause considerable side effects, including acute toxicities such as nausea, stomatitis, myelosuppression (1, 2), nephrotoxicity, neurotoxicity, i.e., peripheral nerve toxicity, and hearing loss. (2, 4)

Hearing loss may occur when cisplatin damages various cochlear structures, including the outer and inner hair cells, stria vascularis, and spiral ganglion cells. Several biological processes are involved in developing cisplatin-induced hearing loss (CIHL), amongst others, releasing toxic reactive oxygen species and depleting the cochlea's protective antioxidants (4-9). Furthermore, the development of CIHL is influenced by several co-occurring risk factors, including a cochlear radiation dose of more than 30 Gray (Gy) (10, 11), and favorable pre-treatment hearing capacity, as often seen in younger patients. (9, 12-15)

The clinical presentation of CIHL is characterized by symmetric and irreversible sensorineural hearing loss (SNHL) starting at extended high-frequencies and progressing to lower frequencies with continued treatment. (4, 5, 16) However, due to the heterogeneity in treatment schedules and used definitions of ototoxicity in studies conducted so far, it is hard to report the incidence of CIHL precisely. (4, 11, 13, 17) It is widely accepted that a cumulative concurrent cisplatin dose of ≥ 200 mg/m^2 is a prerequisite for its anticancer efficacy in advanced HNSCC patients. (18, 19) However, approximately 30% of the patients suffer from cisplatin-related dose-limiting toxicities. (20-22) Therefore, an alternative CRT schedule for HNSCC has been designed to reduce toxicity and increase compliance to this intensive treatment regimen. The standard of care triweekly CRT schedule (100 mg/m^2 cisplatin, days 1, 22, and 43; further referred to as "triweekly CRT schedule") was adapted to a weekly CRT schedule (40 mg/m^2 cisplatin, weekly during seven consecutive weeks; further referred to as "weekly CRT schedule"). Earlier research showed that the weekly schedule gives less toxicities such as nephrotoxicity and neutropenia (2, 23), however these studies did not elaborate on the difference in hearing loss between both schedules. Therefore, the aim of our study was to compare hearing loss in HNSCC patients treated with weekly and triweekly high-dose cisplatin CRT.

METHODS

Study design and subjects

This is a retrospective cohort study with HNSCC patients treated with radiotherapy (five times a week for seven weeks, with a cumulative radiotherapy dose of 70 Gray) and concomitant intravenous cisplatin in a cumulative dose of at least 200 mg/m^2. All patients were treated at the Netherlands Cancer Institute. The triweekly CRT group received 100 mg/m^2 cisplatin every three weeks (on days 1, 22 and 43). Most of these patients were treated between 1999 and 2004 (24) and 2018 and 2020. The weekly CRT group received a weekly cisplatin dose of 40 mg/m^2 (on days 1, 8, 15, 22, 29, 36 and 43) between 2020 and 2023. Due to dose limiting toxicities, some patients did not complete the full planned cisplatin schedule and continued treatment with RT only. We included patients that received a cumulative dose of 200 mg/m^2 or more, as this is the minimum cumulative dose of cisplatin needed for increased CRT-related anticancer efficacy. In view of future informed consent for patients we wished to assess treatment-related hearing loss in patients receiving ≥ 200 mg/m^2 cisplatin CRT. Only patients with both baseline and follow-up audiometry were included in the current study.

RESULTS

Subjects

One hundred ten patients were treated in the triweekly CRT cohort. Thirty-eight patients were excluded because of baseline audiometry missing (n = 5), follow-up audiometry missing (n = 16), dose-limiting toxicity leading to a cumulative cisplatin dose < 200 mg/m^2 (n = 2), and cochlear radiation dose ≥ 30 Gy (n = 15), resulting in 72 evaluable patients. Sixty-nine patients were treated with the weekly CRT schedule. Twelve of them were excluded from the analysis because of baseline audiometry missing (n = 3), follow-up audiometry missing (n = 1), dose-limiting toxicity leading to a cumulative cisplatin dose < 200 mg/m^2 (n = 5), and radiotherapy dose on cochlea ≥ 30 Gy (n = 3), resulting in 57 evaluable patients.

The baseline characteristics of both treatment groups are shown in Table 5. Patients in the triweekly CRT group were relatively younger (56.2 versus 60.7 years old, p < 0.01). In the weekly CRT group, 65% of all patients were treated for oropharyngeal cancer compared to 36% in the triweekly CRT group. The mean cochlear radiotherapy dose was higher in the triweekly CRT group, namely 14.2 Gy versus 8.1 Gy (p < 0.01).

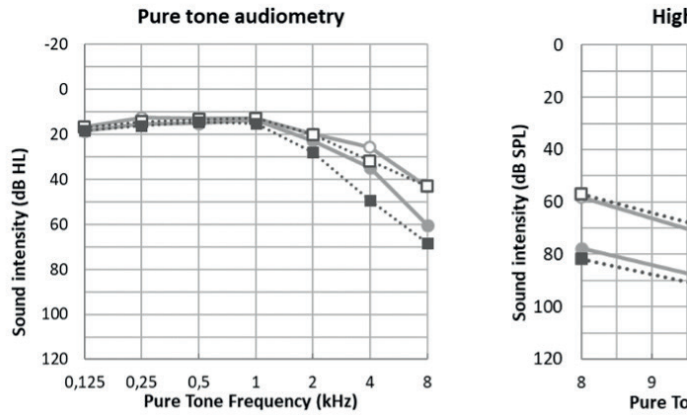
	Triweekly CRT group (n = 72)	Weekly CRT group (n = 57)	p-value
Gender (%)			0.33[a]
Male	56 (78)	40 (71)	
Female	16 (22)	17 (29)	
Age (years) (mean, SD)	56.2 (± 9.9)	60.9 (± 8.4)	< 0.01[b]
Tumor localization (%)			< 0.01[c]
Oropharyngeal	26 (36)	37 (65)	
Oral cavity	9 (13)	8 (14)	
Laryngeal	21 (29)	3 (5)	
Hypopharyngeal	13 (18)	5 (9)	
Nasopharyngeal	2 (3)	3 (5)	
Unknown primary	0 (0)	1 (2)	
Other	1 (1)	0 (0)	
Cumulative cisplatin dose (%)			n.a.
300 mg/m^2	67 (93)	0 (0)	
280 mg/m^2	0 (0)	38 (67)	
240 mg/m^2	0 (0)	13 (23)	
200 mg/m^2	5 (7)	6 (10)	
Cochlear radiation dose (gray) (mean, SD)	14.2 (7.8)	8.1 (7.6)	< 0.01[b]

Table 1: Baseline characteristics. a) Chi-Square test; b) independent samples T-test; c) Fisher's exact test, A p-value of < 0.05 is considered statistically significant. Abbreviation: CRT: chemoradiation

Audiometry results

The audiometric data of all patients in both cohorts is presented in Figure 1 and Table 2. The mean threshold shift at PTA 1-2-4 kHz was 9.0 (± 9.9) dB in the triweekly CRT group and 4.3 (± 8.2) dB in the weekly CRT group (p < 0.01). The mean threshold shift at PTA 8-10-12.5 kHz was 20.2 (± 16.4) dB in the triweekly CRT group and 15.6 (± 14.0) dB in the weekly CRT group (p = 0.07). At the frequencies of 1-2-4 kHz, we observed clinically relevant CIHL, defined as a threshold shift of 10 dB or more, in 31 out of 72 patients (42%) from the triweekly CRT group and 11 out of 57 patients (19%) from the weekly CRT group (p < 0.01). At frequencies of 8-10-12.5 kHz, clinically relevant CIHL was observed in 54 out of 72 patients (75%) from the triweekly CRT group and 42 out of 57 patients (74%) from the weekly CRT group (p = 0.87). Significantly higher grading scale scores were observed in the triweekly

CRT schedule compared to the weekly CRT schedule on both the CTCAE and TUNE (both $p < 0.01$). However, hearing loss, as defined by the ASHA criteria, was not significantly different between both groups ($p = 0.81$). Furthermore, more patients in the triweekly CRT group had an indication for hearing aids de novo after treatment compared to the weekly CRT schedule (36% versus 14%, $p < 0.01$).



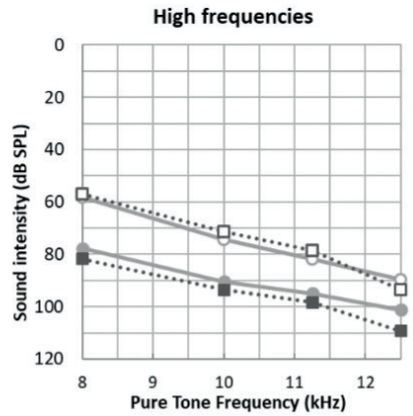


Figure 2. Baseline and follow-up average audiometry for both treatment groups.
A. Pure tone audiometry. B. High frequency audiometry. Abbreviations dB: decibel, HL: hearing level; SPL: sound pressure level; kHz: kiloHertz

Linear mixed model

Results of univariable and multivariable linear mixed model analyses are presented in Table 3 and Table 4. The threshold shift in hearing after therapy at PTA 1-2-4 kHz HL was significantly higher by 3.5 dB in the triweekly CRT group compared to the weekly CRT group (estimate 3.55, 95% CI 0.15 – 6.95, $p = 0.04$) after adjustment for radiotherapy dose to the cochlea, age and baseline hearing level at PTA 1-2-4 kHz. The threshold shift was significantly higher with higher cochlear radiation dose (estimate 0.28, 95% CI 0.12 – 0.44, $p < 0.01$). However, the difference in threshold shift in hearing after therapy at PTA 8-10-12.5 kHz HL was smaller and not significant between the two cisplatin CRT schedules (estimate – 0.50, 95% CI -4.71 – 5.71, $p = 0.85$) after adjustment for baseline age, radiotherapy dose to the cochlea and baseline hearing level at PTA 1-2-4 kHz. The threshold shift at PTA 8-10-12.5 kHz was significantly smaller in older patients (estimate – -0.36., 95% CI -0.64 – -0.09, $p = 0.01$) and in patients with worse baseline hearing level at PTA 1-2-4 kHz (estimate – -0.21, 95% CI -0.39 – -0.05, $p = 0.01$). The threshold shift at PTA 8-10-12,5 kHz was significantly higher with higher cochlear radiation dose (estimate 0.33, 95% CI 0.08 – 0.58, $p = 0.01$). No significant interactions were found between the variables used in the multivariable model.

Variables		Triweekly CRT n = 72 patients	Weekly CRT n = 57 patients	p-value
Hearing loss at PTA 1-2-4 kHz HL				
	Incidence (%)	31 (43)	13 (23)	0.02[a]
	Hearing loss in dB per ear (mean, range)	9.1 (-10.0 – 50.0)	4.3 (-5.0 – 38.3)	< 0.01[c]
Hearing loss at PTA 8-10-12.5 kHz SPL				
	Incidence (%)	54 (75)	42 (74)	0.87[a]
	Hearing loss in dB per ear (mean, range)	20.7 (-15.2 – 60.0)	16.0 (-20.0 – 61.7)	0.06[c]
Indication for hearing aids (number of patients (%))		26 (36)	8 (14)	0.01[b]
Grading scales				
ASHA				0.84[d]
	no hearing loss (%)	8 (11)	9 (16)	
	grade A (%)	3 (4)	4 (7)	
	grade B (%)	59 (82)	37 (65)	
	grade C (%)	2 (3)	7 (12)	
CTCAE v5.0				< 0.01[d]
	grade 0 (%)	31 (43)	41 (72)	
	grade 1 (%)	7 (10)	5 (9)	
	grade 2 (%)	8 (11)	1 (2)	
	grade 3 (%)	26 (36)	10 (17)	
	grade 4 (%)	0 (0)	0 (0)	
TUNE				< 0.01[d]
	grade 0 (%)	9 (12)	9 (16)	
	grade 1a (%)	5 (7)	17 (30)	
	grade 1b (%)	4 (6)	2 (3)	
	grade 2a (%)	24 (33)	19 (33)	
	grade 2b (%)	4 (6)	1 (2)	
	grade 3 (%)	26 (36)	9 (16)	
	grade 4 (%)	0 (0)	0 (0)	

Table 2: Incidence of ≥10 dB hearing loss at PTA 1-2-4 kHz HL and PTA 8-10-12.5 kHz SPL, threshold shift at both PTAs, incidence of an indication for hearing aids de novo (PTA ≥ 35 dB after CRT and < 35 dB at baseline), and scores on grading scales for both CRT groups. a) Chi-Square test; b) Fisher's exact test; c) Linear mixed model; d) Linear-by-linear association. A p-value of < 0.05 is considered statistically significant.Abbreviations: kHz: kiloHertz; SPL: sound pressure level; HL: hearing level; dB: decibel, ASHA: American Speech-Language-Hearing Association criteria; CRT: chemoradiation; CTCAE: Common Terminology Criteria for Adverse Events

Variable	Univariable analysis			Multivariable analysis		
	Estimate	*95% CI*	*p-value*	*Estimate*	*95% CI*	*p-value*
Cisplatin schedule						
Weekly CRT	Ref.			Ref.		
Triweekly CRT	4.79	1.86 – 7.72	< 0.01	3.50	0.27 – 6.74	0.03*
Baseline age in years	-0.12	-0.28 – 0.03	0.12			
Gender						
Female	Ref.					
Male	2.27	-1.18 – 5.71	0.20			
Cumulative cisplatin dose/m²	0.03	-0.02 – 0.08	0.32			
Cochlear radiation dose in Gray	0.36	0.20 – 0.52	< 0.01	0.29	0.13 – 0.46	< 0.01*
Baseline hearing level at PTA 1-2-4 kHz, in dB HL	-0.10	-0.20 – 0.00	0.05	-0.11	-0.21 – -0.00	0.04*

Table 3: Linear mixed model for PTA 1-2-4 kHz HL. A p-value < 0.05 is considered statistically significant. Abbreviations: CRT: chemoradiation; PTA: pure tone average.

Variable	Univariable analysis			Multivariable analysis		
	Estimate	*95% CI*	*p*-value	Estimate	*95% CI*	*p*-value
Cisplatin schedule						
Weekly CRT	Ref.			Ref.		
Triweekly CRT	4.85	-0.10 – 9.80	0.06	0.88	-4.37 – 6.12	0.72
Baseline age in years	-0.54	-0.79 – -0.29	< 0.01*	-0.32	-0.60 – -0.04	0.03
Gender						
Female	Ref.					
Male	-0.60	-6.30 – 5.09	0.83			
Cumulative cis-splatin dose/m²	0.03	-0.06 – 0.11	0.54			
Cochlear radiation dose in Gray	0.38	0.13 – 0.63	< 0.01*	0.31	0.05 – 0.56	0.02
Baseline hearing level at PTA 1-2-4 kHz, dB HL	-0.34	-0.50 – -0.19	< 0.01*	-0.23	-0.41 – -0.06	0.01

Table 4: Linear mixed model for PTA 8-10-12.5 kHz HL. P-value < 0.05 is considered statistically significant. Abbreviations: CRT: chemoradiation; PTA: pure tone average.

DISCUSSION

Although the main goal of anticancer therapy remains to achieve better survival and loco-regional control, improving post-treatment quality of life by reducing treatment-related toxicity has become increasingly important. (12, 25) In HNSCC, weekly cisplatin CRT (7 cycles of 40 mg/m^2 cisplatin during seven consecutive weeks) achieves similar survival rates when compared to triweekly CRT (3 cycles of 100 mg/m^2 cisplatin every three weeks). (2, 23) Also, it is accompanied by less cisplatin toxicities such as nephrotoxicity, neutropenia, and electrolyte disturbances. (2, 23) The objective of this study was to assess whether adopting a weekly CRT schedule reduces CIHL.

The implementation of weekly CRT may contribute to preserving hearing capacity and improving quality of life. (12, 25) In our research, the incidence of clinically relevant hearing loss of ≥ 10 dB at PTA 1-2-4 kHz, representing the perception of speech in noise, was found significantly higher in the triweekly CRT group compared to the weekly CRT group (42% versus 19%, $p < 0.01$), in agreement with previous studies. (2, 26) The 23%-point difference in the incidence, the marked difference in hearing-aid candidacy (36% versus 14%), CTCAE criteria ($p < 0.01$) and TUNE criteria ($p < 0.01$) indicate benefit of a weekly cisplatin regimen over a triweekly cisplatin regimen with respect to CRT-induced hearing loss in HNSCC patients. Consequently, a weekly cisplatin regimen might reduce adverse effects commonly observed in HNSCC patients' health-related quality of life, including social isolation, anxiety, and depression. (25, 27) Careful interpretation of our data is warranted in view of the retrospective nature of our research. A limitation of this retrospective design was that the lack of speech audiometry in most patients, which would have provided valuable extra information about speech processing capacity prior and after CRT. However, detailed description of data was available for all patients, including audiometric hearing thresholds up to 12.5 kHz SPL, the cochlear radiation dose per ear, and the gradation of hearing loss as defined by different grading scales.

We found a significant association between cochlear radiation dose and CIHL, in agreement with previous studies that found a cochlear radiation dose ≥ 30 Gy to cause clinically relevant sensorineural hearing loss of ≥ 10 dB. (10, 28) Other literature advises to limit the radiation dose to the cochlea to ≤ 35 Gy (29, 30), however we chose to use the most strict cut-off value. The triweekly CRT group, mainly treated between 1999 and 2004, received a higher mean cochlear radiation dose, attributed to a difference in radiation techniques and planning in the years 1999 – 2004 when compared to more recently treated patients in both the weekly and triweekly CRT schedule (16.1 Gy versus 8.4 Gy). Despite the limitation of this time difference and difference in radiation technique, after correcting for the mean

cochlear radiation dose in our multivariable analysis, significantly more hearing loss at PTA 1-2-4 was found in the triweekly compared to the weekly CRT group. Also, we found no significant difference in CIHL between patient in the triweekly groups treated between 1999 – 2004 and 2018 – 2020 on PTAs 1-2-4 ($p = 0.08$) and 8-10-12,5 ($p = 0.36$). Therefore, we believe that it is justified to evaluate all triweekly patients as one cohort, regardless of the difference in treatment period.

Even though weekly CRT may decrease the incidence of cisplatin-CRT induced hearing loss, there is still a need for an otoprotectant in both treatment regimens. Recently, both systemic and topical (transtympanic) approaches have been studied to reduce CIHL with varying successes. (12, 14, 31, 32) Antioxidants are probably the most encouraging otoprotective agents, as they can neutralize the toxic formation of reactive oxygen species by cisplatin. Interestingly, the antioxidant sodium-thiosulphate (STS) can also inactivate cisplatin. When STS is injected into the middle ear (transtympanically), it may locally inactivate cisplatin without interfering with its systemic anticancer effect. In a recent phase I trial, this method was safe and feasible. (33) Its efficacy is currently studied further in a multicenter phase 3 randomized controlled setting (*CTIS 2023-503313-30-01).* The current study shows that patients treated in both schedules are still prone to develop clinically relevant CIHL. Therefore, HNSCC patients treated in both the triweekly and the weekly CRT schedule are eligible to participate in our phase 3 trial regarding the efficacy of transtympanic STS against CIHL.

In conclusion, hearing capacity seems to be relatively preserved after treatment with a weekly cisplatin CRT regimen (7 cycles of 40 mg/m^2 cisplatin during seven consecutive weeks) when compared to triweekly cisplatin CRT regimen (3 cycles of 100 mg/m^2 cisplatin every three weeks) for HNSCC. However, both treatment schedules induce clinically relevant CIHL at extended high-frequencies, which impairs the quality of higher sounds (e.g., for music) and speech perception in noise (34, 35). Currently a multicenter phase 3 study to evaluate the efficacy of transtympanic STS against CIHL is underway. Ultimately, these efforts should reduce CIHL and thereby increase the quality of life in HNSCC patients and survivors.

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APPENDIX A

Grading scale	Definition of hearing loss
ASHA (36)	A) 20 dB decrease at any one tested frequency B) 10 dB decrease at any two adjacent test frequencies C) loss of response at three consecutive test frequencies where responses were previously obtained
CTCAE v5.0 (on a 1, 2, 4, 3, 6, and 8 kHz audiogram) (37)	Grade 1: Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear OR Subjective change in hearing in the absence of documented hearing loss; Grade 2: Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear; Grade 3: Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear OR hearing aid or intervention indicated Grade 4: Decrease in hearing to profound bilateral loss (absolute threshold >80 dB HL at 2 kHz and above); non-serviceable hearing
TUNE (38)	Grade 0: No hearing loss Grade 1a: Threshold shift ≥ 10 dB at [8-10-12.5] OR subjective changes in the absence of a threshold shift Grade 1b: Threshold shift ≥ 10 dB at [1-2-4] Grade 2a: Threshold shift ≥ 20 dB at [8-10-12.5] Grade 2b: Threshold shift ≥ 20 dB at [1-2-4] Grade 3: Hearing level ≥ 35 dB HL at [1-2-4] de novo Grade 4: Hearing level ≥ 70 dB HL at [1-2-4] de novo

Table 5: **Grading scales that were used for the assessment of post-treatment platinum-related hearing loss.** Abbreviations: ASHA: American Speech-Language-Hearing Association; CTCAE: Common Terminology Criteria for Adverse Events.

7

SENSORINEURAL HEARING LOSS AFTER ADOPTIVE CELL IMMUNOTHERAPY FOR MELANOMA USING MART-1 SPECIFIC T CELLS: A CASE REPORT AND ITS PATHOPHYSIOLOGY

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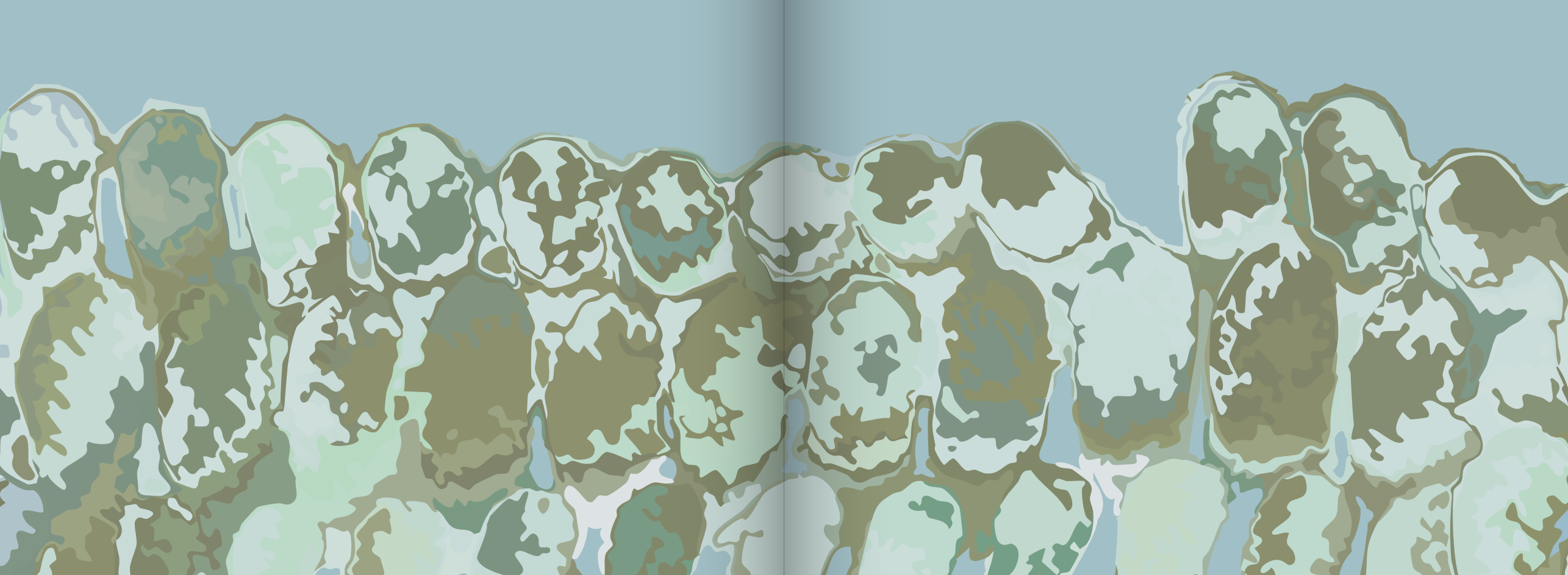
Otology & Neurotology, 2019



ABSTRACT

Objective

To illustrate a case of sensorineural hearing loss (SNHL) after immunotherapy based on T cell receptor (TCR) gene therapy using modified T cells recognizing melanoma antigen recognized by T cells 1 (MART-1) for disseminated melanoma.

Patient

We present a 59-year-old woman with profound subacute bilateral SNHL including unilateral deafness after immunotherapy based on TCR gene therapy using modified T cells recognizing MART-1 for disseminated melanoma. Ten days after treatment, the patient developed hearing loss of 57 dB hearing loss (HL) air conduction (AC) at pure tone average (PTA) 0.5-1-2-4 kHz in the right ear, and >100 dB HL AC at PTA 0.5-1-2-4 in the left ear. The right ear recovered partially, while the left ear remained deaf, despite oral prednisolone (1.0 mg/kg) and salvage treatment with three transtympanic injections of 0.5 mL dexamethasone (4.0 mg/mL).

Conclusion

Based on our presented case and a vast amount of literature there is circumstantial evidence that TCR gene therapy for melanoma targets the perivascular macrophage-like melanocytes in the stria vascularis, resulting in SNHL. We suggest that SNHL after TCR gene therapy may be caused by a disruption of the blood-labyrinth-barrier and the endolymphatic potential and/or a sterile inflammation of the stria vascularis. In severe cases like our subject, we posit that endolymphatic hydrops or hair cell loss may cause irreversible and asymmetrical deafness. Steroid prophylaxis via transtympanic application is debatable.

INTRODUCTION

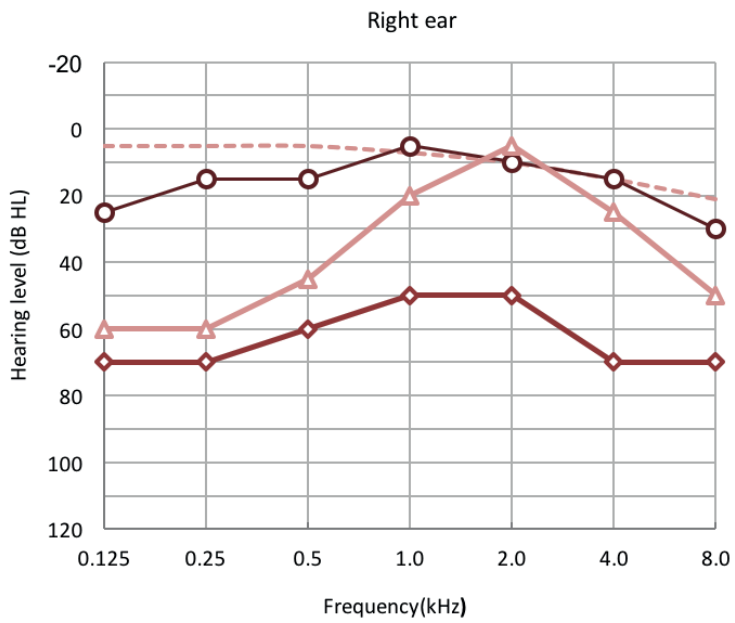
T cell receptor (TCR) gene therapy is an internationally appreciated novel treatment option for metastatic melanoma, which is currently tested for its efficacy and toxicity. (1) In melanoma antigen recognized by T cells 1 (MART-1) specific TCR gene therapy, peripheral blood T cells are adapted by engraftment of a MART-1 specific TCR that recognizes melanoma cancer cells. (1-3) Small phase I/II studies have reported partial tumor response rates up to 13-30% for patients with melanoma progressing upon prior therapies. (1) Interestingly, this specific form of TCR gene therapy introduced a novel likely cause of treatment-related sensorineural hearing loss (SNHL) into our clinical practice. (4) We aim to present a case of asymmetric SNHL including deafness of one side after MART-1 specific TCR gene therapy and to discuss its potential pathophysiology. The unilateral deafness demonstrated here may interfere with the design of possible future treatment strategies for these patients with regard to the indication for TCR gene therapy itself or possible combinations with other forms of immunotherapy.

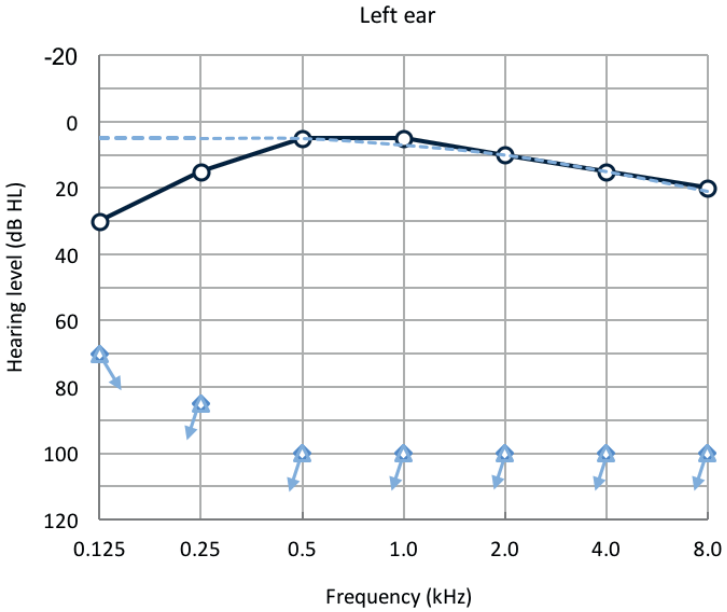
CASE PRESENTATION

A 59-year-old woman with metastatic cutaneous melanoma was included in a phase I/IIa clinical trial (EudraCT no. 2011-002941-36) using MART-1 specific TCR transduced T cells. Audiometry revealed normal baseline hearing capacity (Figure 1). Firstly, the patient underwent apheresis. Next, she was treated with cyclophosphamide and fludarabine for the depletion of lymphocytes. One week later, 2.5 x 10^8 TCR-modified T cells were reinfused into the patient.

In the evening of day nine after infusion of T cells, she complained of subacute bilateral hearing loss. The next morning, she reported bilateral “deafness”. Audiometric testing showed asymmetric SNHL with a pure tone average (PTA) 0.5-1-2-4 kHz of 57dB hearing level (HL) air conduction (AC) in the right ear and a deaf left ear. Immediately, systemic prednisolone (1mg/kg/day) was administered. After two weeks, the right ear significantly improved. In an attempt to rescue the left ear, 0.5mL topical dexamethasone (4.0mg/mL) was given transtympanically at day 25, 27 and 33 after T cell infusion. Nevertheless, the left ear remained deaf. The right ear recovered to 24dB HL AC at PTA 0.5-1-2-4 kHz with speech discrimination of 97% at 60dB sound pressure level (SPL) (Figure 1C). Repeated audiometry after one month showed similar results (not shown). The patient did not complain of vestibular symptoms or tinnitus. Unfortunately, the patient died from progressive disease four months after treatment, therefore further follow-up was impossible.

A


Right ear
Hearing level (dB HL)
-20
0
20
40
60
80
100
120
0.125
0.25
0.5
1.0
2.0
4.0
8.0
Frequency(kHz)


Left ear
Hearing level (dB HL)
-20
0
20
40
60
80
100
120
0.125
0.25
0.5
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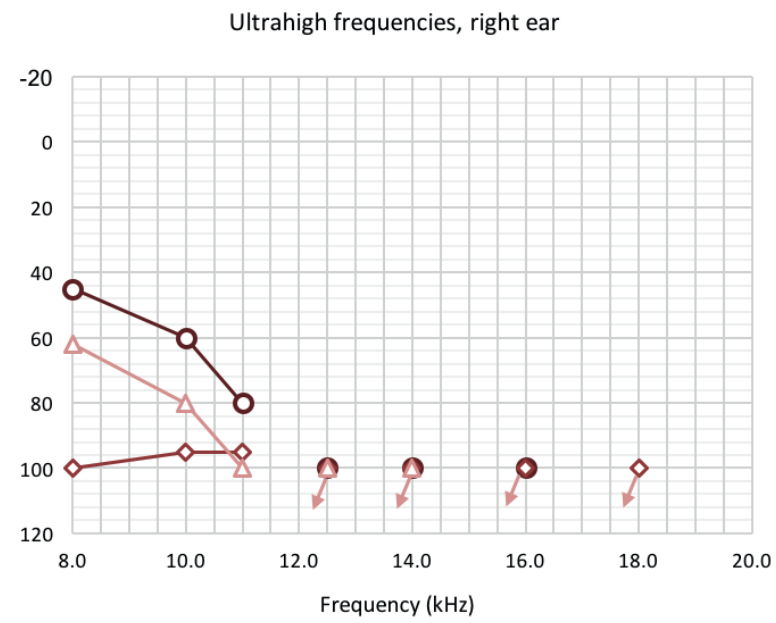
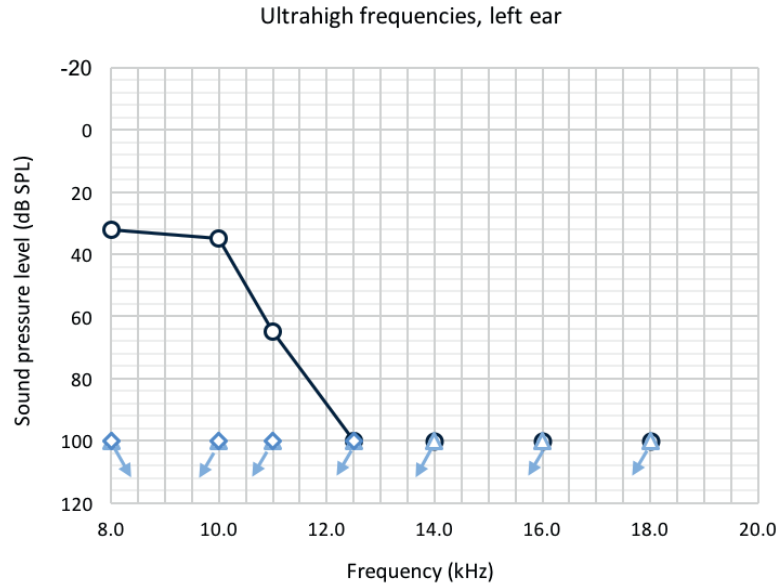
2.0
4.0
8.0
Frequency (kHz)

B


Ultrahigh frequencies, right ear
-20
0
20
40
60
80
100
120
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Frequency (kHz)


Ultrahigh frequencies, left ear
Sound pressure level (dB SPL)
-20
0
20
40
60
80
100
120
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Frequency (kHz)

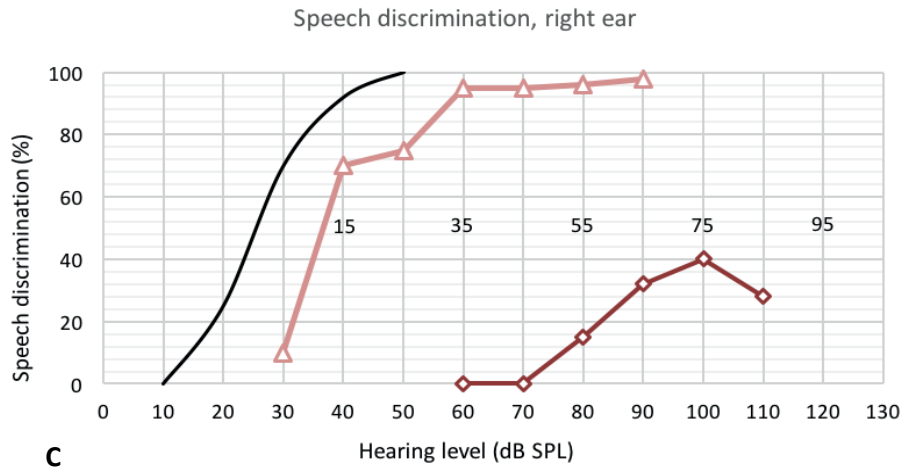


Figure 1. Audiometric testing results for baseline hearing level (); hearing level at onset of clinically overt hearing loss, which is ten days after T cell receptor modified T cell infusion (); and the hearing level at end of steroid treatment (). **Figure 1A**. Pure tone audiometry, only air conduction (AC) thresholds are shown. Ten days after infusion of TCR transduced T cells, hearing level was asymmetrically reduced to 57 dB HL AC at pure tone average (PTA) 0.5-1-2-4 kHz in the right ear and >100 dB HL AC at PTA 0.5-1-2-4 in the left ear - the left ear could not be measured with adequate masking. After systemic steroid treatment, the right ear recovered to 24dB HL AC at PTA 0.5-1-2-4 kHz. The left ear remained deaf. **Figure 1B.** High frequency audiometry. The high frequencies in the right ear partially recovered. However, high frequencies could not be heard in the left ear, which did not recover after steroid treatment. *the SPL thresholds shown at 8 kHz have been converted from HL according to ISO 389-1. **Figure 1C.** Speech perception for the right ear. Baseline discrimination scores are missing. At onset of symptoms, the maximum discrimination score was reduced to 40% at 100dB SPL, which improved to a maximum of nearly 100% at 60 dB SPL. In the left ear, there was no measurable speech perception.

DISCUSSION

To our knowledge, this is the first patient described in literature that suffered asymmetric SNHL including deafness on one side after TCR gene therapy for melanoma.

TCR gene therapy is a relatively novel type of immunotherapy using autologous T cells obtained from the patient through apheresis. These T cells are genetically modified *in vitro* to possess specific TCRs in order to target antigens expressed on melanoma cells, i.e. gp100 or MART-1. (5) Next, the T cells are expanded and reintroduced into the patient to kill the melanoma. (3, 4, 6) Interestingly, this therapy can be accompanied by SNHL, decreased vision and skin rash. (4, 7) These are probably "*off-tumor on-target*" side-effects, because melanocytes with melanoma-identical antigens can be found in the inner ear, uvea and epithelium of the skin. (8)

The cochlear stria vascularis plays an important role in the cochlear electromechanical transmission of sound (Figure 2A). (9-12) In the endolymph of the ductus cochlearis a potential of +80mV is preserved, the so-called endolymphatic potential (EP). The EP is needed for the transmission of mechanical sound to electrical propagation of sound by the hair cells (HCs). (11) Various ion-channels facilitate potassium (K^+)-recycling into the endolymph to maintain the EP. (9, 11, 13, 14) Both the basal and marginal cellular layer of the stria vascularis are linked by impermeable tight-junctions that prevent electrochemical communication with adjacent structures, (Figure 2A). Hence, the stria vascularis serves as a blood-labyrinth barrier (BLB) that protects the cochlea from toxic substances and prevents ionic exchange. (9, 11, 15) BLB impairment, amongst others, causes several forms of SNHL, including noise-induced hearing loss and autoimmune SNHL. (9, 10, 12, 14, 16-18) The intermediate cells, also known as perivascular macrophage-like melanocytes (PVM/Ms), are essential for BLB function and K^+-homeostasis. (9-16, 18, 19) We hypothesize that the TCR-modified T cells target these PVM/Ms, because these melanocytes express melanoma-identical antigens. (8) In two trials of Seaman et al. and Johnson et al. half of the 68 patients developed SNHL after TCR gene therapy. (4, 7) Generally, SNHL recovered completely after topical steroid application, but the role and timing of administration remain unclear. We aim to discuss the potential pathophysiology of SNHL after TCR gene therapy.

Firstly, we believe that TCR gene therapy can disrupt the BLB and cochlear K^+-recycling. Once the reinfused T cells have invaded the stria vascularis, they may target the PVM/Ms. PVM/Ms produce the pigment epithelium-derived factor (PEDF), which upregulates tight-junction proteins. (12, 16, 18) If TCR gene therapy targets the PVM/Ms, these tight-junctions may be disrupted (Figure 2B). Subsequent leakage of electrolytes may cause an EP drop and diminished mechano-electrical transduction of sound. Additionally, TCR gene therapy possibly affects cochlear K^+-transportation. Different ion-transporters regulate K^+-recycling, including *KCNJ10* expressed by the PVM/Ms (Figure 2A). (11, 13) If the PVM/Ms are being targeted, K^+-recycling may be hampered and subsequently, insufficient K^+ will be available for the marginal cells to maintain the EP. Severe EP changes may induce HC loss, resulting in permanent SNHL. (20) The ability to recover may depend on the duration and/or degree of EP deterioration. Therefore, it is plausible that SNHL after TCR gene therapy may be the result of an interruption of the BLB and K^+-homeostasis.

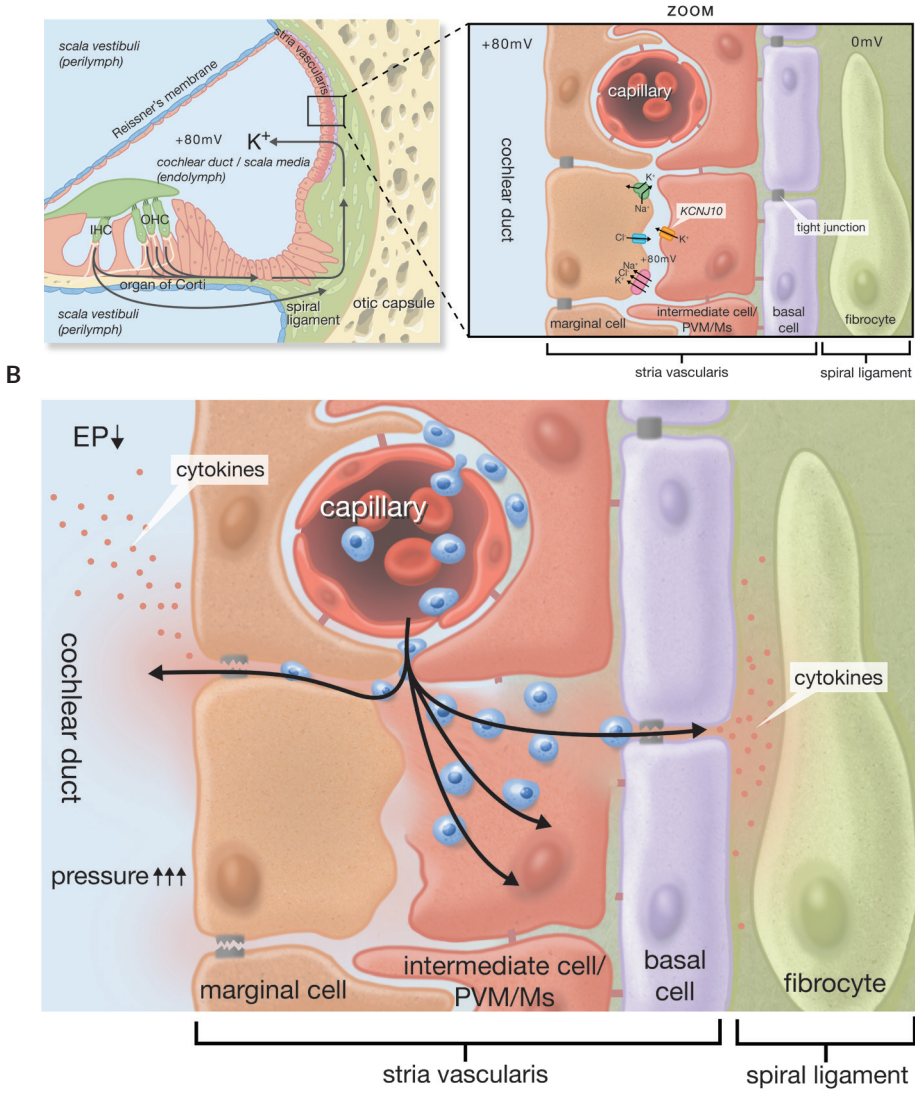


Figure 2.
Figure 2A. *Left:* a schematic cross-section through the human cochlea, depicting the potassium (K^+) recycling. K^+ is recycled from the organ of Corti's inner and outer hair cells (IHC and OHC) into the spiral ligament and subsequently via the stria vascularis back into the endolymph of the cochlear duct to become available again for the hair cells for the electrochemical transmission of sound. *Right (zoom):* a schematic model of the capillaries and the three types of cells situated in the stria vascularis: the basal cells, the intermediate cells (or PVM/Ms) and the marginal cells. Furthermore, the most important ion-transporters for K^+-recycling are shown, like the *KCNJ10* transporter located at the PVM/Ms. The tight-junctions play an important role in the function of the blood labyrinth barrier because they prevent electrochemical communication between the stria vascularis and adjacent structures.

Figure 2B. An overview of the proposed hypothetical mechanisms behind the development of hearing loss after T cell receptor gene therapy for melanoma. The T cells may infiltrate the stria vascularis and then recognize and damage the PVM/Ms, potentially resulting in ion- and fluid-leakage of the blood labyrinth barrier. The tight-junctions may break, resulting in an increased permeability of the stria vascularis. The leakage of ions and fluid may lead to a drop of the endolymphatic potential (EP), edema and/or an elevated pressure in the stria vascularis and cochlear duct. Ultimately, it could be that this results in the formation of endolymphatic hydrops and/or a laceration of the Reissner's membrane. The infiltration of T cells may also be accompanied by an inflammatory response with the involvement of cytokines, which may damage the cochlear structures.

Secondly, it could be that TCR gene therapy causes a (sterile) inflammation of the stria vascularis with cytokine involvement and reactive edema (Figure 2B). Cytokines may cause HC degeneration, as is seen in neomycin ototoxicity (21), noise-induced SNHL (22), and cytomegalovirus-related SNHL (23). Furthermore, toxic levels of reactive oxygen species (ROS) are known to induce cochlear cell apoptosis in cisplatin ototoxicity (24, 25) and cytomegalovirus-related SNHL (23). Potentially, TCR gene therapy might similarly initiate cochlear cell degeneration by cytokines and ROS.

As a result of the above suggested changes after TCR gene therapy, it may be that endolymphatic hydrop formation takes place, as is for example seen after BLB destruction in guinea pigs. (26, 27) Leakage of the BLB enables osmotic influx of ions and fluids into the cochlear duct, which elevates the endolymphatic volume. Perhaps, this might have caused a laceration of the Reissner's membrane with subsequent deafness. Although there is circumstantial evidence that TCR gene therapy causes SNHL by targeting the PVM/Ms, direct evidence for this causality is lacking. Therefore, it would be valuable to study our proposed pathophysiology in for example MART-1 knock-out mice.

Interestingly, the pathophysiology of SNHL after TCR gene therapy seems comparable to SNHL in the Vogt-Koyanagi-Harada disease (VKH). In this autoimmune disease, which is initiated by anti-gp100 (28, 29) and/or anti-MART-1 (30) antibodies, melanocytes in the epithelium of the skin, uvea and cochlea are damaged. Remarkably, in both VKH (28) and TCR gene therapy SNHL can progress asymmetrically, while other forms of ototoxicity generally develop symmetrically. (31) It could be that the immunopathology in VKH and TCR gene therapy is site-dependent, resulting in asymmetric hearing changes. In our opinion, the asymmetric aspect of our patient's SNHL and recovery may be another indication of the supposed underlying multifactorial pathophysiology discussed above.

The ability to recover from cochlear inflammation depends on the severity of inflammation. (27) Indeed, the occurrence of SNHL after TCR gene therapy seems to be dose-dependent (7). PVM/Ms are supposedly capable of self-renewal (10, 17, 32) and subsequent normalization of the homeostasis likely improves hearing

capacity. We suggest that our patient developed cochlear damage before the PVM/Ms got the chance to recover. However, the follow-up was relatively short compared to previous studies. (4, 7) Theoretically, hearing capacity could have improved at a later state. Unfortunately, the patient died of progressive disease after four months, leaving no opportunity for repeated audiometry.

Hearing capacity significantly improves after steroid treatment in VKH. (29) Accordingly, steroid therapy may prevent potential sterile strial inflammation proposed by us to potentially be caused by TCR gene therapy. In this respect, transtympanic application might be an ideal strategy, as systemic steroids may affect the anti-cancer effect of the T cells and higher cochlear dosing can be achieved with transtympanic application. (33) Following earlier observations that SNHL starts 7-10 days after the infusion of T cells (4, 7), it may be considered to start steroid application around the time of the infusion of T cells and continue this for ten days.

In conclusion, there is circumstantial evidence that TCR gene therapy for melanoma targets the PVM/Ms in the stria vascularis, resulting in SNHL. We suggest that SNHL may be caused by a disruption of BLB and the EP and/or a sterile inflammation of the stria vascularis. Hypothetically, endolymphatic hydrops or HC loss may cause irreversible and asymmetric deafness. It would be valuable to test our proposed theories in animal studies.

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8

SUMMARY, DISCUSSION AND IMPLICATIONS FOR FURTHER RESEARCH, CONCLUSION

SUMMARY

The main goal of this thesis was to develop an otoprotective strategy against cisplatin-initiated hearing loss (CIHL). To this respect, we successfully completed a phase I trial to assess whether preventive transtympanic sodiumthiosulphate (STS) injections were safe, feasible and effective against CIHL in humans. Also, our research aimed to identify patient groups that may particularly benefit from CIHL prevention strategies in the future. Finally, we present a novel form of cancer-therapy related hearing loss. We provide a rationale for severe sensorineural hearing loss with unilateral deafness that may occur during T-cell receptor gene therapy applied for metastatic melanoma.

In **Chapter 2** we describe the investigator-initiated phase I trial "N12MTG" (EudraCT: 2012-004653-80). This trial was designed to assess whether the use of transtympanic injections with sodium thiosulfate (STS) gel was safe, feasible and effective as prophylaxis against CIHL in humans. (1) Twelve patients treated with high dose cisplatin (≥ 75 mg/m^2) were included. Primary objective of the trial was safety and feasibility. Preliminary efficacy of transtympanic STS and pharmacokinetics of systemically available cisplatin were also assessed. One ear was treated with 0.1 M STS gel, injected transtympanically (into the middle ear), while the contralateral ear served as internal control. Results showed that transtympanic application of STS was safe. Based on our pharmacokinetic data, we hypothesize that transtympanically injected STS does not interfere with the systemically available cisplatin. Four of the 12 subjects did not develop CIHL. Four of 8 patients suffering CIHL were considered "responders" to STS: the threshold shift after cisplatin therapy at pure tone average (PTA) 8-10-12.5 kHz sound pressure level (SPL) was 18.4 dB greater (i.e. worse) in the untreated ears when compared to the STS-treated ears (6.8 dB versus 25.2 dB, respectively (p = 0.068)). Although we were not able to show statistically significant efficacy in this very small patient cohort, we believe that the results of these four patients rise above random observations. In our view, these observations may suggest the first in-human clinically relevant efficacy of transtympanic application of STS to prevent CIHL in adults, without affecting the anti-cancer effect of cisplatin. In order to further assess its efficacy, a new protocol for a multicenter randomized phase III trial was written (for summary see **Appendix II**).

In **Chapter 3** we evaluated cis- and carboplatin related hearing loss in patients with testicular cancer (TC). Usually, testicular cancer patients are young with excellent baseline hearing, as they do not suffer from age-related hearing loss (presbycusis). Because this is one of the risk factors for the development of CIHL and TC may be treated with high-dose platinum therapy, TC patients are particularly at risk to develop CIHL. (2-6) In order to assess whether they are suitable candidates for a prophylaxis against CIHL in the future, we determined the degree of CIHL in 41 TC patients treated with different protocols using multimodality treatment including high-dose cisplatin or carboplatin. (7) The first group consisted of 33 patients treated for primary disease. The second group consisted of 8 patients with relapsed disease, who were treated with salvage therapy using another round of platinum. We evaluated CIHL in terms of threshold shifts after therapy (in dB), scores on different grading scales for ototoxicity, subjective symptoms, and qualification for hearing aids de novo after therapy. In total, 22% of all subjects developed CIHL at the PTA for speech perception (1-2-4 kHz hearing level (HL)), and 59% at the ultrahigh frequencies (8-10-12.5 kHz SPL). The mean threshold shift (change in decibels after therapy) at PTA 1-2-4 kHz HL was 11.2 dB greater in the salvage group than the primary group (p < 0.001). At PTA 8-10-12.5 kHz SPL this difference was not significant (5.7 dB, p = 0.118). This can be explained by the fact that, at these ultrahigh frequencies, sensorineural hearing loss (SNHL) is already occurring after the lower platinum doses of primary therapy, as CIHL starts at these frequencies. Overall, 3/41 (7%) of patients qualified for hearing aids due to treatment and two of them were in the salvage group. Scores on all evaluated grading scales were higher in the salvage group. The biggest limitation of the current study was the relatively small study population. In conclusion, all TC patients are at risk for the development of CIHL, but patients treated for recurrent disease developed most severe CIHL. This can be addressed to the high cumulative platinum dose that is given over the course of their disease. A selection of TC patients may be suitable for a preventive strategy against CIHL in the future, also dependent on the feasibility of repetitive STS injections before each platinum infusion.

In **Chapter 4** we report on genetic features associated with CIHL. (8) Theunissen et al. developed a prediction model to predict CIHL in patients treated with concomitant chemoradiotherapy (CRT) using high dose cisplatin for advanced head and neck squamous cell carcinoma (HNSCC). (9) We evaluated whether the performance of this prediction model could be improved by incorporating single nucleotide polymorphisms (SNPs) previously reported to be associated with CIHL. Based on published literature, 31 SNPs were selected (see eTable 1 (8)). We assessed whether these SNPs were correlated with CIHL in a retrospective cohort of 74 platinum-treated HNSCC patients (providing 141 ears). Patients were selected based on the availability of pre-treatment and post-treatment audiometry and FFPE tissue blocks that could be used for the isolation of DNA. Genotyping was done by MassArray SNP genotype method (Sequenom). The threshold shift (change in decibels after therapy) at PTA 1-2-4 kHz HL was analyzed per additional minor allele. In our cohort, only the rs2289669 SNP within the *SLC47A1/MATE1* gene was significantly associated with post-treatment hearing capability. Incorporating this SNP to the prediction model by Theunissen et al. moderately

improved its predictive capacity (p = 0.073). *MATE1* is an H+-coupled organic cation bidirectional antiporter and recently, its cochlear expression has been described. (10) Genetic variations in cellular transporters may explain individual vulnerabilities to cisplatin-related toxicity. Presumably, the *SLC47A1/MATE1* variant is associated with hearing loss due to a reduced transporter function, but the exact role *MATE1* in the inner ear is currently unknown.

In **Chapter 5** we assessed the role of a low skeletal muscle mass (SMM) (sarcopenia) in the development of CIHL. (11) We hypothesized that low SMM was associated with CIHL, because patients with low SMM receive a relative overdose of cisplatin as it mainly distributes to free fat mass. (12) In a retrospective study cohort of 81 HNSCC patients treated with cisplatin-based CRT, a significant association between low SMM and CIHL on the speech related frequencies (PTA 1-2-4 kHz HL) was seen (p = 0.048). In the ultrahigh frequencies (PTA 8-10-12.5 kHz SPL) this association could not be found. Probably, sensorineural hearing loss (SNHL) already reaches its maximum at these frequencies in the early phase of treatment, regardless of individual vulnerability for the development of CIHL. (6) Regarding the incidence of clinically relevant CIHL, no significant difference could be found between patients with (n = 39) and without (n = 42) sarcopenia. This may be attributed to the relatively small population studied. In conclusion, our results show that low SMM is significantly associated with increased CIHL at frequencies vital for the perception of speech.

In **Chapter 6** we retrospectively analyzed the difference in CIHL between two different cisplatin dose-intensity CRT schedules for advanced HNSCC. (13) In order to reduce cisplatin-related toxicity, a weekly cisplatin CRT schedule (weekly 40 mg/m^2 cisplatin during seven consecutive weeks of CRT) has been introduced to our clinical practice as an alternative to the 3-weekly protocol (100 mg/m^2 cisplatin during seven consecutive weeks of CRT (cisplatin on days 1, 22, and 43). A total of 129 patients were analyzed: 72 subjects were treated with weekly cisplatin, further referred to as “weekly group”, and 57 subjects with 3-weekly cisplatin, further referred to as “3-weekly group”. CIHL was defined as a decline of mean ≥ 10 dB at speech related frequencies (PTA 1-2-4 kHz HL) or at the ultrahigh frequencies (PTA 8-10-12.5 kHz SPL). At the speech related frequencies, the incidence of CIHL was 43.1% in the 3-weekly and 22.8% in the weekly treated group (p = 0.02), with a mean threshold shift of 9.1 (± 9.9) dB and 4.3 (± 8.3) dB, respectively (p = 0.03). At PTA 8-10-12.5 kHz SPL, no significant differences were seen between both treatment groups in both the incidence of CIHL and the threshold shift after therapy. Using multivariable linear mixed model, significant associations were seen between CIHL and treatment schedule (p = 0.03), cochlear radiotherapy dose (p < 0.01), and baseline hearing capacity (p = 0.04). Based on these results, we believe

that dose reduction to a weekly cisplatin regimen reduces CIHL at frequencies vital for speech perception when compared to the 3-weekly schedule.

In the last part of this thesis (**Chapter 7**) is described how an innovative type of immunotherapy against melanoma led to severe sensorineural hearing loss and unilateral deafness. (14) In our center, a phase I/IIa trial was conducted to study the safety and feasibility of TCR gene therapy using modified T cells recognizing MART-1 for disseminated melanoma. (15) One of the subjects developed profound subacute bilateral sensorineural hearing loss including unilateral deafness ten days after therapy. In the right ear, sensorineural hearing loss of 57 dB at PTA 0.5-1-2-4 kHz HL was seen. The left ear was functionally deaf (> 100 dB at PTA 0.5-1-2-4 kHz HL). Oral and transtympanic corticosteroids were given in an attempt to improve hearing. The right ear recovered partially, but the contralateral ear remained deaf. Based on this case and literature, we posit that the modified T cells probably did not only target melanoma cancer cells, but also targeted the pigmented perivascular macrophage-like melanocytes (PVM/Ms) in the stria vascularis, see Figure 1 (left), as these cells probably express melanoma-identical antigens. Consequently, the blood-labyrinth barrier, which is essential for normal hearing, was damaged, resulting in (irreversible) sensorineural hearing loss. An amendment to the trial protocol was made for the upcoming patients, who were consequently treated with repetitive prophylactic transtympanic dexamethasone injections before and after the transfer of T cells. (15) No permanent hearing loss was seen after this implementation (n = 4).

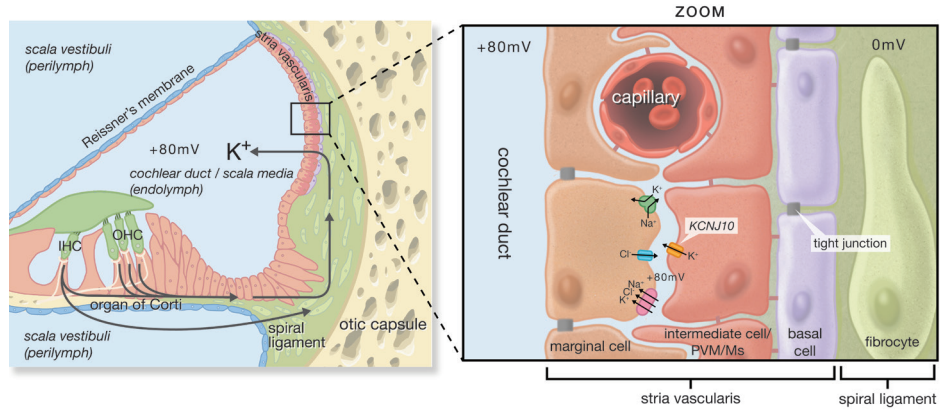

Figure 1. Anatomy of the inner ear.
Left: a schematic cross-section through the human cochlea, depicting the otic capsule (circled in red), which embraces the cochlear compartments and cochlear cells. The route of potassium (K^+) recycling is shown. *Right (zoom):* a schematic model of the capillaries and the three types of cells situated in the stria vascularis: the basal cells, the intermediate cells (or PVM/Ms, circled in blue) and the marginal cells. Furthermore, the most important ion-transporters for K^+-recycling are shown, like the *KCNJ10* transporter located at the PVM/Ms. Abbreviations: IHC: inner hair cell; OHC: outer hair cell. (14)

DISCUSSION AND IMPLICATIONS FOR FURTHER RESEARCH

While the primary approach to treatment of cancer remains curation, there is a growing focus on the reduction of cancer-therapy related toxicity in cancer patients and survivors. Cisplatin chemotherapy can lead to severe SNHL. Currently, there is great interest in the search for an otoprotective strategy against CIHL. A prophylaxis may contribute to the hearing preservation and the improvement of quality of life. (2, 16) Based on our study results, the topical use of an antioxidant by transtympanic injection may be an appropriate choice. The antioxidant STS may hold particular significance because STS can inactivate cisplatin by binding to its active form without any systemic anticancer effect of cisplatin. (17, 18) However, the observed efficacy against CIHL in advanced HNSCC needs to be proven in a larger patient population. To this purpose a KWF-funded multicenter randomized controlled phase III trial will be conducted in the next years (*CTIS 2023-503313-30-01)*. A total of 100 HNSCC patients treated with CRT using cisplatin in a cumulative dose of ≥ 200 mg/m^2 will be included. Subjects will be treated with transtympanic STS injection in one ear, which is determined by randomization. The contralateral ear will be left untreated and serves as an optimal matched pair to the STS-treated ear, as CIHL generally develops symmetrically. In the power calculation we used a cautiously chosen expected efficacy rate of 33%, which is based on a most conservative interpretation of the results of the phase I trial. (1) If, ultimately, efficacy is proven with this phase III trial, transtympanic STS can be implemented in our clinical practice.

However, there are some important continuing issues in the use of transtympanic STS as an otoprotectant**.** Most importantly: which patients should we offer such prophylaxis? In order to answer this question, pretreatment prediction of CHIL is of utmost importance. Next, it needs also to be determined if administration of STS by repetitive transtympanic STS injections (before each platinum infusion) is feasible for each selected patient. Although we were able to find some relevant risk factors for the development of CIHL (7, 8, 11, 13, 19), it remains challenging to forecast on an individual basis whether a patient will develop CIHL. Currently, the use of prediction models seems to be the most accurate approach in CIHL risk assessment. For HNSCC patients treated with cisplatin CRT, we propose to use the prediction model as presented in this thesis, including the SNP that was found to be associated with increased CIHL. From a logistic point of view, however, it is challenging to offer all patients repeated transtympanic STS injections, especially in TC patients undergoing repetitive platinum administrations in multiple consecutive days.

Proper timing of transtympanic STS administration is likely one of the most critical factors for achieving optimal efficacy. In the phase I trial, we opted to inject 3 hours before cisplatin infusion based on a preclinical study that showed impressive hair cell preservation in guinea pigs with this timing. (20) However, cisplatin can be detected in the inner ear directly after infusion and its elimination rate from the cochlea is slow. (21) One may advocate to inject STS directly before cisplatin infusion. However, it is impossible to translate these pre-clinical results directly to humans, as the human's otic capsule (see Figure 1, right) is thicker and the round window permeability is lower compared to guinea pigs. (18, 20) In healthy individuals, Tmax (time to peak drug concentration) was seen already after one hour after transtympanic STS application and this rapid absorption of STS into the systemic circulation is suggested to occur through uptake by the cochlea rather than through the Eustachian tube. (22) If the cochlear absorption of STS does indeed occur within the first hour after transtympanic application (which is also seen in transtympanic application of triamcinolone acetonide (23)), the optimal timing of STS application would be one or two hours before cisplatin infusion. Due to logistic reasons, it is impractical to inject STS such a short time prior to cisplatin application. The patient needs to lie down with the treated ear upwards for 30 minutes (60 minutes when both ears are treated). Also, it takes time to transport the patient from the oncology ward towards an otolaryngologic consultation room equipped with a microscope, which is needed for transtympanic application of the gel. Based on the preclinical studies earlier mentioned and and the preliminary efficacy observed in our phase I trial with an injection administered three hours prior to cisplatin infusion, for now we decided to continue with this timing in the phase III trial.

In the end, not all patients may be willing to undergo an additional otoprotective intervention alongside their platinum-based therapy. We believe that individuals who depend on their hearing for their profession (e.g., musicians) or relatively young patients (e.g., those with testicular cancer) are likely to be the primary groups opting for STS prophylaxis in the future. It is important to note that, three decades after cisplatin therapy, overall hearing capacity in most patients approaches that of age-matched controls. (24) However, this was not observed in individuals under the age of 40, as the hearing gap with age-matched controls increased significantly between the first and thirth decade after therapy. Some individuals are strongly dependent on their hearing in their working and/or social life during the first three decades following cisplatin therapy. These patients might particularly benefit from an otoprotector.

Preclinical drug testing may be enhanced in the near future, as research groups are currently focusing on the development of human organoids. (25) Organoids are 3D in vitro cultured versions of human organs derived from humane stem cells and mimic the function of real organs. This innovation enables a unique opportunity to investigate disease development pathways and to conduct drug testing prior

to clinical trials in humans. Cochlear organoids could be used to elucidate the mechanisms contributing to the onset of CIHL. (26) Also, prophylactic drugs against CIHL may be tested on inner ear organoids in the future. For instance, organoids would enable the search for drugs that inhibit transporter proteins involved in the onset of CIHL (27, 28), thereby helping to prevent CIHL.

Besides the emergence of preventive strategies against CIHL, there is an alternative option to preserve hearing in patients treated with cisplatin chemotherapy. In patients treated with high dose cisplatin CRT for HNSCC, we observed that dose reduction to a weekly cisplatin protocol reduces CIHL at frequencies vital for speech perception when compared to the 3-weekly schedule respectively, from 42% to 19% ($p < 0.01$), without undermining cure rates. (29, 30) Our results were confirmed by a recent study in a similar study population. (31) Possibly, similar dose adaptations can be of value for patients with other types of tumors as well. More awareness of debilitating CIHL among medical oncologists may contribute to the development of less damaging treatment schedules in other oncology clinics. To this end, the use of uniform audiometry techniques is a prerequisite. In literature, there is still an inconsistency in the method of performing audiometry for the analysis of CIHL. Multiple studies indicate the necessity for practical guidelines for the detection and follow-up of CIHL in clinical practice. (16, 32-34) Ideally, audiometric monitoring would consist of a measurement at baseline, during treatment, and within 3 months after cisplatin therapy in order to facilitate auditory rehabilitation if needed. Also, it can be debated whether audiometry should be performed at 10 or 20 years after therapy, as cisplatin may retain in the inner ear for months to years after therapy. (35) For adequate detection of CIHL, we advise to perform standard audiometry (up to 8.0 kHz HL) plus ultrahigh frequency audiometry up to at least 12.5 kHz SPL, because CIHL starts in the ultrahigh frequencies. In order to better assess the grades of CIHL between different study groups, Theunissen et al. developed a grading scale for the assessment of CIHL, the TUNE criteria. (9) The TUNE criteria showed higher sensitivity when compared to other currently available grading scales (e.g. Common Terminology Criteria for Adverse Events (CTCAE), Brock, Chang). In order to implement the TUNE criteria into our clinical practice and for research purposes, its validation is currently being carried out in a multicenter study ("M18TUN"). Ultimately, uniformity between studies in the use of audiometry and grading scales does improve the ability to compare study results.

At last, we described a novel form of cancer-therapy related hearing loss after the treatment for disseminated melanoma. (14) This case report on a dramatic SNHL in a melanoma patient receiving adoptive cell immunotherapy using MART-1 specific T cells demonstrates that awareness of the risk of therapy-induced hearing loss can also apply to innovative treatment modalities (potentially targeting cochlear melanocytes).

CONCLUSION

The main contributions of this thesis are, next to confirming safety and feasibility of transtympanic STS as an otoprotective strategy against CIHL, the identification of clinical and genetic risk factors for the development of CIHL. These findings have laid a solid foundation for continued scientific research with focus on reconfirmation of STS efficacy in a large randomized trial and of reconfirming the beneficial effect of dose reduction by weekly cisplatin administration in prospective follow-up studies in advanced HNSCC. It can be hypothesized that clinical risk factors emerging from these studies and implementation of SNP data in the prediction model might contribute to further improvement of individualized pretreatment risk profiling of CIHL.

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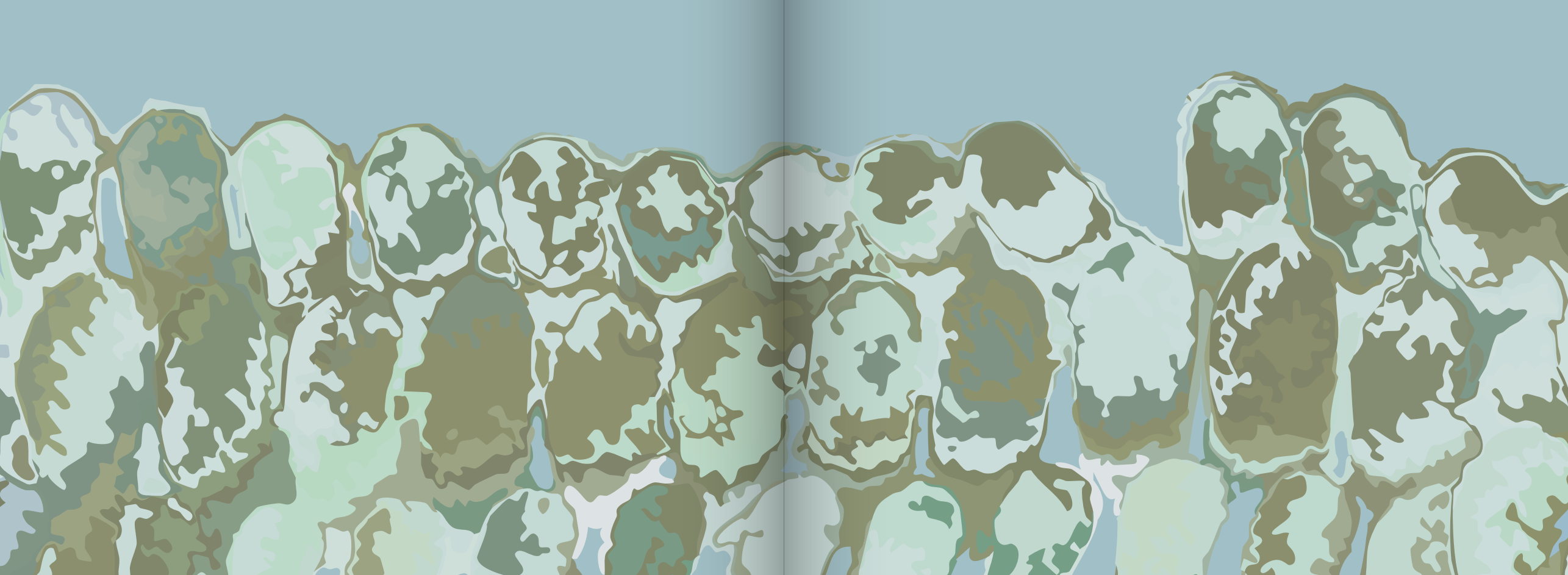
NEDERLANDSE SAMENVATTING



NEDERLANDSE SAMENVATTING

Hoewel genezing het primaire doel van oncologische behandelingen blijft, groeit de aandacht voor het verminderen van aan de behandeling gerelateerde toxiciteit en het verbeteren van kwaliteit van leven na behandeling. Cisplatin chemotherapie kent als veelvoorkomende bijwerking ototoxiciteit, die zich uit in perceptief gehoorverlies. Het voornaamste doel van dit proefschrift was het onderzoeken van de veiligheid en toepasbaarheid van natriumthiosulfaat als preventieve maatregel tegen cisplatin-geïnduceerd gehoorverlies. In dit kader hebben we met succes een fase I-studie afgerond om te beoordelen of de behandeling met preventieve intratympanale injecties met natriumthiosulfaat veilig, haalbaar en effectief was. Dit proefschrift richtte zich tevens op de identificatie van patiëntengroepen die in de toekomst zouden kunnen profiteren van preventieve strategieën tegen cisplatin-geïnduceerd gehoorverlies. Tot slot presenteren we een nieuwe vorm van gehoorverlies gerelateerd aan kankertherapie. We onderbouwen hoe ernstig perceptief gehoorverlies met unilaterale doofheid kon ontstaan tijdens de behandeling met T-cel receptor gentherapie voor gemetastaseerd melanoom.

In **hoofdstuk 2** wordt een gerandomiseerde fase I/III studie beschreven, waarin de veiligheid en toepasbaarheid van intratympanale injecties met natriumthiosulfaat ter preventie van cisplatin-geïnduceerd gehoorverlies is onderzocht. (1) De studie werd uitgevoerd bij een groep van 12 patiënten die werden behandeld met hoge dosering cisplatin (≥ 75 mg/m^2). Naast de veiligheid werd ook de voorlopige effectiviteit van deze methode en de farmacokinetiek van systemisch beschikbaar cisplatin geëvalueerd. Als interventie werd één oor met intratympanaal natriumthiosulfaat gel (0.1 M) behandeld en werd het contralaterale oor ongemoeid gelaten als interne controle. De keuze voor het te behandelen oor werd bepaald door randomisatie. De resultaten van deze studie toonden aan dat intratympanale injecties met natriumthiosulfaat veilig en toepasbaar zijn, aangezien er geen bijwerkingen gerelateerd aan de interventie werden waargenomen. Respons op natriumthiosulfaat werd gedefinieerd als een verschil van ≥ 10 dB in drempelverschuiving op PTA 8-10-12.5 kHz tussen het onbehandelde oor en het behandelde oor. In de gehele groep trad er een iets grotere drempelverschuiving op in de onbehandelde oren dan in de behandelde oren, maar dit verschil was niet statistisch significant of klinisch relevant (gemiddelde drempelverschuiving van 12.7 dB versus 8.8 dB op het gemiddelde van de frequenties 8, 10 en 12.5 kHz (pure tone average (PTA) 8-10-12.5 kHz SPL) (p = 0.402)). Vier van de 12 patiënten ontwikkelden geheel geen gehoorverlies en werden daarom uitgesloten van de analyse naar de voorlopige effectiviteit van natriumthiosulfaat. Van de acht patiënten die wel gehoorverlies ontwikkelden, waren er vier "*responders*", met een gemiddeld verschil in drempelverschuiving van 18.4 dB tussen de onbehandelde oren (25.2 dB) en de behandelde oren (6.8 dB) (p = 0.068). Hoewel

dit verschil in dit kleine cohort niet statistisch significant was, denken we dat we dat deze mate van winst wel klinisch relevant is en niet geheel op toeval berust. Op basis van de farmacokinetische resultaten lijkt het intratympanaal toegediend natriumthiosulfaat de systemische beschikbaarheid van cisplatin niet te beïnvloeden. Daarom kan deze interventie mogelijk als gehoorbeschermer fungeren zonder de chemotherapeutische werking van cisplatin tegen te gaan. Om de effectiviteit te onderzoeken, is een studieprotocol voor een multicenter fase 3 studie geschreven, die binnenkort zal starten (samenvatting te vinden in **Appendix 2**).

De mate van gehoorverlies bij patiënten die met hoge dosis platinum zijn behandeld voor testiskanker wordt beschreven in **hoofdstuk 3**. Patiënten met testiskanker zijn doorgaans jong en hebben vaak een goed uitgangsgehoor. Dit geeft een verhoogd risico op platinum-geïnduceerd gehoorverlies. (2-6) In deze studie werd het gehoorverlies in twee verschillende patiëntgroepen onderzocht. De eerste groep (n = 33) bestond uit patiënten met primaire ziekte die werden behandeld met multimodaliteitstherapie, inclusief hoge doses cisplatin. De tweede groep (n = 8) bestond uit patiënten met gemetastaseerde ziekte, die in hun behandeltraject nogmaals een cyclus cis- of carboplatin kregen als onderdeel van *salvage*-therapie. Het gehoorverlies werd beschreven aan de hand van de volgende onderdelen: als drempelverschuiving na behandeling (in dB), aan de hand van scores op verschillende gradaties systemen voor ototoxiciteit, aan de hand van subjectieve gehoorsymptomen en als het hebben van een indicatie voor een hoortoestel (de novo) na therapie. Van de 41 patiënten ontwikkelde 22% gehoorverlies in de frequenties die betrokken zijn bij het spraakverstaan (PTA 1-2-4 kHz hearing level (HL)) en 59% in de ultrahoge frequenties (PTA 8-10-12.5 kHz SPL). Patiënten die werden behandeld met *salvage* therapie vertoonden een grotere mate van gehoorverlies op alle uitkomstmaten dan patiënten die primaire chemotherapie kregen. Dit kan worden verklaard door het feit dat deze groep patiënten uiteindelijk een hogere cumulatieve dosis platinum ontving door de herhaaldelijke behandelingen van hun ziekte. Een selectie van deze patiënten zou kunnen profiteren van een preventief middel tegen cisplatin- geïnduceerd gehoorverlies in de toekomst.

We hebben onderzocht of bepaalde genetische varianten geassocieerd zijn met het optreden van cisplatin-gerelateerd gehoorverlies, wat wordt beschreven in **hoofdstuk 4**. (7) Theunissen et al. ontwikkelden eerder een predictiemodel voor het voorspellen van het optreden van gehoorverlies na hoge dosis cisplatin bij patiënten die behandeld worden voor een hoofdhals-carcinoom. (8) Het doel van onze studie was om te bepalen of het voorspellend vermogen van dit model verbeterd kon worden door de toevoeging van *single nucleotide polymorphisms* (SNPs) die in verband staan met cisplatin-geïnduceerd gehoorverlies. Op basis

van literatuuronderzoek werden 31 SNPs geselecteerd. Het studiecohort bestond uit patiënten die in het verleden waren behandeld met hoge dosis cisplatin voor een hoofdhals-carcinoom, waarvan zowel audiometrie (voorafgaand en na de behandeling) als weefsel voor de isolatie van DNA beschikbaar was. DNA werd geïsoleerd middels *MassARRAY SNP genotyping*. In totaal werden 73 patiënten (met 141 oren) geïncludeerd. De verandering in decibel (drempelverschuiving) op PTA 1-2-4 kHz werd bepaald per minor allel. In dit cohort werd slechts één SNP, rs2289669 op het *SLC47A1/MATE1*-gen, significant geassocieerd met cisplatin-gerelateerd gehoorverlies. Het toevoegen van deze SNP aan het model van Theunissen et al., leidde tot een lichte verbetering in het discriminerend vermogen ten opzichte van het model zonder genetische kenmerken (p = 0.073).

In **hoofdstuk 5** werd onderzocht wat de rol van lage skeletspiermassa (sarcopenie) was in het ontstaan van cisplatin-geïnduceerd gehoorverlies bij hoofdhals-kankerpatiënten die werden behandeld met chemoradiatie met cisplatin. Onze hypothese was dat patiënten met sarcopenie een verhoogd risico hebben op ototoxiciteit, omdat deze patiënten een relatieve overdosis cisplatin krijgen. (10) In een retrospectief cohort van 81 patiënten vonden we een significante associatie tussen lage skeletspiermassa en cisplatin-geïnduceerd gehoorverlies in de frequenties die betrokken zijn bij het spraakverstaan (PTA 1-2-4 kHz) (p = 0.048). In de ultrahoge tonen (PTA 8-10-12.5 kHz SPL) werd dit echter niet waargenomen. Dit zou kunnen worden verklaard doordat ook patiënten zonder expliciete risicofactoren voor het optreden van ototoxiciteit in deze ultrahoge frequenties gehoorverlies ontwikkelen, omdat het gehoorverlies als eerst tot uiting komt in deze frequenties. Sarcopenie zou dan dus enkel een verhoogd risico geven op het optreden van gehoorverlies in de lagere frequenties. Er werd geen significant verschil gevonden in de incidentie van gehoorverlies door cisplatin tussen de groep patiënten met sarcopenie (n = 39) en zonder sarcopenie (n = 42). Dit kan komen door de beperkte omvang van de onderzoekspopulatie.

In **hoofdstuk 6** wordt het verschil in cisplatin-geïnduceerd gehoorverlies beschreven tussen twee verschillende chemoradiatie protocollen voor de behandeling van hoofdhals-kanker. Tegenwoordig wordt er steeds vaker gekozen voor chemoradiatie in een gereduceerde dosis met wekelijks cisplatin (40 mg/m^2 cisplatin per week gedurende 7 chemoradiatie weken) in plaats van het conventionele driewekelijkse schema (100 mg/m^2 cisplatin op dag 1, 22 en 43 van de 7 chemoradiatie weken) met als doel het verminderen van toxiciteit. Eerdere studies toonden dat beide behandelschema's resulteren in een vergelijkbare overleving. (11, 12) In ons retrospectieve cohort werden 129 patiënten geïncludeerd, waarvan 57 patiënten waren behandeld met het driewekelijkse cisplatin protocol en 72 patiënten met het wekelijkse protocol. Cisplatin-geïnduceerd gehoorverlies werd gedefinieerd als een drempelverschuiving van ≥ 10 dB op PTA 1-2-4 kHz of op PTA 8-10-12.5 kHz na de behandeling. De incidentie van cisplatin-geïnduceerd gehoorverlies op PTA 1-2-4 kHz was 43.1% in de driewekelijkse en 22.8% in de wekelijkse groep (p = 0.02). De gemiddelde drempelverschuiving na therapie op PTA 1-2-4 kHz was 9.1 (± 9.9) dB in de driewekelijkse en 4.3 (± 8.3) dB in de wekelijkse groep (p = 0.03). Op PTA 8-10-12.5 kHz werden geen significante verschillen gevonden tussen beide groepen in de incidentie van gehoorverlies en in de drempelverschuiving na cisplatin. Met een *multivariable linear mixed model* werd onder andere een significante associatie gezien tussen gehoorverlies en het behandelschema (p = 0.03). We kunnen concluderen dat dosisreductie naar het wekelijks cisplatin resulteert in een vermindering van zowel de ernst als de frequentie van het optreden van gehoorverlies bij patiënten met hoofdhals-kanker.

In **hoofdstuk 7** wordt beschreven hoe de behandeling van gemetastaseerd melanoom kan leiden tot ernstig perceptief gehoorverlies met zelfs unilaterale doofheid. (13) In ons centrum werd een nieuwe vorm van immunotherapie voor gemetastaseerd melanoom onderzocht in een fase I/IIa trial. Dit betrof T-cel receptor gen therapie, waarbij gebruik werd gemaakt van gemodificeerde T-cellen die het tumorantigeen MART-1 herkennen. Eén van de geïncludeerde patiënten ontwikkelde tien dagen na behandeling ernstig bilateraal perceptief gehoorverlies met unilaterale doofheid. Het betrof een perceptief verlies van 57 dB op PTA 0.5-1-2-4 kHz in het rechter oor en > 100 dB op PTA 0.5-1-2-4 in het linker oor. In een poging het gehoor te verbeteren, werd de patiënt eerst behandeld met orale corticosteroïden en later, na uitblijven van verbetering, ook met intratympanale corticosteroïden. Het beste oor herstelde gedeeltelijk, maar het contralaterale oor bleef doof. Op basis van deze casus en aanvullend literatuuronderzoek denken we dat de T-cellen de benigne melanocyten in de stria vascularis van de cochlea hebben aangevallen. Hierdoor zou de bloed-labyrint-barrière onherstelbaar beschadigd kunnen zijn geraakt, terwijl deze barrière normaliter een belangrijke bijdrage levert aan de functie van het binnenoor. Naar aanleiding van deze casus werd het studieprotocol aangepast: de rest van de patiënten is behandeld met preventieve intratympanale dexamethason injecties voorafgaand aan en na de toediening van de T-cellen, waarna er geen gehoorverlies meer werd waargenomen. (14)

CONCLUSIE

De belangrijkste bijdragen van dit proefschrift zijn, naast de bevestiging van de veiligheid en haalbaarheid van intratympanale natriumthiosulfaat injecties als interventie tegen gehoorverlies door cisplatin, de identificatie van klinische en genetische risicofactoren voor de ontwikkeling van cisplatin-geïnduceerd gehoorverlies. Deze bevindingen vormen een solide basis voor nader onderzoek, met de nadruk op de herbevestiging van de effectiviteit van natriumthiosulfaat

in een grote gerandomiseerde studie, evenals de herbevestiging van het gunstige effect van dosisreductie door wekelijks cisplatin toediening in prospectieve studies bij gevorderd hoofdhals-carcinoom. Er kan worden verondersteld dat de klinische risicofactoren die uit deze studies naar voren komen, evenals de implementatie van SNP-gegevens in het predictiemodel, zouden kunnen bijdragen aan verdere verbetering van de geïndividualiseerde risicoprofilering van cisplatin-geïnduceerd gehoorverlies voorafgaand aan de behandeling.

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APPENDICES

I: ABBREVIATIONS

II: SUMMARY OF PROTOCOL: TRANSTYMPANIC SODIUM THIOSULPHATE TO PREVENT CISPLATIN-RELATED HEARING LOSS. A RANDOMIZED CONTROLLED MULTICENTER PHASE III TRIAL; THE SOUND TRIAL

DANKWOORD

CURRICULUM VITAE

APPENDIX I - ABBREVIATIONS

AC	air conduction
ASHA	the American Speech-Language-Hearing Association
AUC	area under the curve
BC	bone conduction
BEP	bleomycin, etoposide, cisplatin
BLB	blood-labyrinth barrier
CI	confidence interval
CIHL	cisplatin induced hearing loss
C_{max}	maximum observed concentration
CRT	chemoradiotherapy
CTC	cyclosfamide, carboplatin, thiothepa, mesna
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variatiation
dB	decibel
DLT	dose limiting toxicity
EP	etoposide, cisplatin OR endolymphatic potential
FFPE	formalin fixed paraffin embedded
Gy	gray
HL	hearing level
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
IHC	inner hair cell
IV	intravenous
kHz	kilohertz
LMM	Linear mixed models
LRT	likelihood ratio test
LSMI	lumbar skeletal muscle index
MART-1	melanoma antigen recognized by T cells 1
NPV	negative predictive value
NSCLC	non-small cell lung cancer
OHC	outer hair cell
PK	pharmacokinetic
PPV	positive predictive value
PTA	pure tone average
PTAH	pure tone average 1-2-4 kHz hearing loss (bone conduction)
PTAL	pure tone average 0.5-1-2 kHz hearing loss (bone conduction)
PTAU	pure tone average 8-10-12.5 kHz sound pressure level (air conduction)
PVM/Ms	perivascular-resident macrophage-like melanocytes
ROC	receiver operating characteristics
ROS	reactive oxygen species
RT	radiotherapy
SMM	skeletal muscle mass
SPL	sound pressure level
SAE	serious adverse event
SD	standard deviation
SNHL	sensorineural hearing loss
SNP	single-nucleotide polymorphism
STS	sodium thiosulphate
TC	testicular cancer
TCR	T cell receptor
TI-CE	carboplatin, etoposide
TIP	paclitaxel, ifosfamide, cisplatin, mesna
TNM	Tumor Node Metastasis
UICC	Union for International Cancer Control
VIP	cisplatin, etoposide, ifosfamide
VKH	Vogt-Koyanagi-Harada disease
WHO	World Health Organization

APPENDIX II – NEDERLANDSE SAMENVATTING PROTOCOL "SOUND TRIAL"

Intratympanale injecties met natriumthiosulfaat ter preventie van cisplatin-gerelateerd gehoorverlies: een randomized controlled multicenter fase III trial (*SOUND trial*)

INLEIDING

Per jaar worden in Nederland ruim 4.000 patiënten behandeld met cisplatin voor verschillende soorten maligniteiten, waaronder plaveiselcarcinomen in het hoofdhalsgebied. De gouden standaard voor de behandeling van vergevorderde hoofdhals-carcinomen is behandeling met (adjuvante) chemoradiatie met hoge dosis cisplatin. (1-3) Een bekende bijwerking van cisplatin is het optreden van irreversibel, bilateraal, perceptief gehoorverlies. (2,4) Deze vorm van ototoxiciteit is dosisafhankelijk en start in de hoge frequenties, maar kan oplopen tot in de lagere frequenties die betrokken zijn bij het spraakverstaan wanneer er een hogere cumulatieve dosis cisplatin wordt toegediend. (4-6) Cisplatin tast de cochleaire haarcellen aan en leidt tot schade in andere cochleaire cellen door afgifte van vrije radicalen en een depletie van beschermende antioxidanten. (4, 5, 7-10) Het is lastig om een exacte incidentie van cisplatin-geïnduceerd gehoorverlies te geven, omdat er in verschillende studies een variatie in behandelschema's en definities van ototoxiciteit wordt gebruikt. (4, 11-14) Gehoorverlies zorgt voor een verminderde kwaliteit van leven door sociaal isolement en eenzaamheid en verhoogt het risico op cognitieve achteruitgang, dementie, depressie en valneiging op latere leeftijd. (16)

RATIONALE

Tegenwoordig wordt er veel onderzoek gedaan naar de preventie van cisplatin gerelateerde ototoxiciteit. Een van de onderzochte middelen is het antioxidant natriumthiosulfaat. Natriumthiosulfaat inactiveert cisplatin door aan het platinum molecuul te binden. Daarnaast vermindert de anti-oxidatieve werking van natriumthiosulfaat de schade die wordt aangericht door vrije radicalen. Omdat de systemische toepassing van natriumthiosulfaat mogelijk leidt tot een lagere overleving, (5, 17) werd de mogelijkheid van topicale toediening middels intratympanale injecties (in het middenoor) onderzocht. In een fase I studie met twaalf patiënten is aangetoond dat het veilig is om natriumthiosulfaat toe te dienen in het middenoor (N12MTG trial). (18) Ook had natriumthiosulfaat ingespoten in het middenoor geen nadelig effect op systemische beschikbaarheid van cisplatin. Bij de helft van de patiënten in dit onderzoek werd gezien dat gehoorverlies door cisplatin voorkomen werd door de natriumthiosulfaat injecties in het oor, maar

dit was niet significant vanwege het kleine patiëntaantal. Om de effectiviteit van natriumthiosulfaat te kunnen bewijzen, dient deze interventie in een groter aantal patiënten te moeten worden onderzocht, namelijk de beoogde M22STS trial.

DOEL STUDIE

Het doel van dit fase III onderzoek is de evaluatie van de effectiviteit (klinisch relevant voordeel) van de intratympanale injecties met natriumthiosulfaat ter preventie van cisplatin-geïnduceerd gehoorverlies.

IN- EN EXCLUSIECRITERIA

Patiënten (18 jaar of ouder) met een hoofdhals-carcinoom die behandeld worden met een cumulatieve dosis cisplatin van ≥ 200 mg/m^2 in een wekelijks of driewekelijks schema worden geïncludeerd. Exclusiecriteria zijn een cochleaire radiotherapie dosis van ≥ 30 Gray (19), asymmetrisch perceptief gehoorverlies, gemiddeld ≥ 40 dB gehoorverlies op de frequenties 1, 2 en 4 kHz tezamen, bekende overgevoeligheid voor natriumthiosulfaat en/of otologische pathologie waardoor intratympanale injecties niet toegediend kunnen worden.

STUDIEOPZET

Maximaal drie uur voorafgaand aan elke cisplatin kuur krijgt de patiënt unilateraal een intratympanale injectie met natriumthiosulfaat gel (0.1 M) toegediend. Het te behandelde oor wordt bepaald door randomisatie. Bij elke individuele patiënt wordt het ene oor behandeld met natriumthiosulfaat en wordt het contralaterale oor onbehandeld gelaten als interne controle. De injectie vindt plaats na lokale anesthesie van het trommelvlies. Na elke injectie moet de patiënt 30 minuten met het behandelde oor naar boven blijven liggen, zodat de gel via het ronde venster naar het binnenoor kan diffunderen.

Voorafgaand aan behandeling (baseline) en drie maanden na de behandeling (follow-up) zal audiometrie worden verricht. Dit betreft zowel een normaal tonen audiogram (0.5 tot 8.0 kHz hearing level (HL)) als een hoge tonen audiogram (8.0 tot 16.0 kHz sound pressure level (SPL)). Een klinisch relevant effect wordt gedefinieerd als een verschil tussen baseline en follow-up audiometrie van gemiddeld ≥ 10 decibel op drie opeenvolgende frequenties (van 0.5 tot 16 kHz) in het voordeel van het met natriumthiosulfaat behandelde oor. Tevens wordt er

gekeken naar het verschil in behoud van gehoorfunctie tussen het behandelde en onbehandelde oor.

Het gehoorverlies wordt tevens gekwalificeerd aan de hand van een aantal eerder beschreven gradatiesystemen, namelijk zoals geclassificeerd door 'the International American Speech-Language-Hearing Association' (ASHA) (20), 'the Common Terminology Criteria for Adverse Events' (CTCAE) versie 5.0 (21), en de TUNE criteria (22). Tevens worden er voorafgaand en na de behandeling *patient-reported outcome measures* (PROMs) bepaald aan de hand van vragenlijsten betreffende het gehoor en tinnitus. Daarnaast wordt gekeken naar de mediane 1- en 2 jaar overleving van onze patiënten om aan te tonen dat natriumthiosulfaat in deze toedieningsvorm niet interfereert met het chemotherapeutisch effect van cisplatin.

Bij dit onderzoek zal een analyse gedaan worden van de skeletspiermassa, dit is de verhouding spieren die de patiënt ten opzichte van zijn lichaamsverhoudingen heeft. Patiënten met een lage skeletspiermassa (sarcopenie) krijgen namelijk een hogere concentratie cisplatin in het bloed en hebben daardoor mogelijk meer kans op gehoorschade. Vanuit de diagnostische beeldvorming zal dit geanalyseerd worden.

Gedurende 3 jaar worden patiënten geïncludeerd (2024-2026). De follow-up periode betreft een mediane duur van 2 jaar (2024-2028).

Aan het eind van de studie wordt er onderzocht of het gehoor in de oren die met natriumthiosulfaat zijn behandeld beter is dan in de onbehandelde oren. Als bij de behandelde oren gehoorbescherming wordt aangetoond, zullen in de toekomst beide oren behandeld worden.

DEELNEMENDE CENTRA

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CURRICULUM VITAE

Charlotte Duinkerken was born in Groningen, the Netherlands, on June 1st, 1990. She grew up in Groningen and at Curaçao with her parents and younger sister. In 2008, she graduated from Praedinius Gymnasium in Groningen. After high school, she spent a year abroad in which she took a language course in Cambridge (UK) and she did volunteer work on Borneo, Malaysia. In 2009, she started her medical study at the University of Groningen. She spent a year doing internships on Curaçao. She completed her medical studies with a clinical and scientific internship at the Department of Head and Neck Surgery and Oncology of the Antoni van Leeuwenhoek Hospital (NKI AvL), Amsterdam. She obtained her medical degree in 2016. Next, she started working as a clinical resident (not in specialist training) at the Surgery department at the Alrijne hospital in Leiderdorp for 7 months. Subsequently, she returned to the NKI AvL where she started as a clinical resident (not in specialist training) at the Department of Head and Neck Surgery and Oncology from 2017 to 2019. During this period, she started working on her PhD trajectory focusing on the prevention of hearing loss induced by cancer treatment. Between April 2019 and January 2025, Charlotte was a clinical resident in training to become an otolaryngologist at the department of Otolaryngology and Head and Neck Surgery at the Leiden University Medical Center (LUMC), including two internships in the Groene Hart Hospital (GHZ) in Gouda and the Alrijne Hospital in Leiderdorp. She currently works as an otolaryngologist at the Haaglanden Medical Center (HMC) in The Hague. Charlotte lives in the Hague with Auke Hingstman and their two sons Stijn (2022) and Pieter (2025).