Long-term Hearing Loss Assessment and PEACH Scores of Children Exposed to Platinum Chemotherapy

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List of Abbreviations & Symbols

ACA – Air Conduction Audiometry

BCA – Bone Conduction Audiometry

BM- Basilar Membrane

Ca^{2+} - Calcium Ion

Cis - Substitute Groups on the Same Side

Cl -Chlorine

CN - Cochlear Nucleus

COMPT - Catechol- O -Methyltransferase

CP - Cisplatin

CTCAE - National Cancer Institute Common Terminology Criteria for Adverse Events

dB - Decibel

DNA - Deoxyribonucleic Acid

DPOAE - Distortion Products Otoacoustic Emissions

EOAE - Evoked Otoacoustic Emissions

ERCC - Excision Repair Cross-complementing Rodent Repair Deficiency

FU - Follow-up

g/cm^3 - Gram Per Cubic Centimeter

g/mol - Molar Mass

GST - Glutathione S-transferase

Gy – Gray Units

Hz - Hertz

IHC- Inner Hair Cells

kHz - Kilohertz

kg - Kilogram

K^+ - Potassium Ion

LRP2 - Low-density Lipoprotein Receptor-related Protein 2

mg - Miligram

Na^+ - Sodium Ion

NH_3 - Ammonia

NIHL - Noise Induced Hearing Loss

OAE - Otoacoustic Emissions

OHC - Outer Hair Cells

Pt – Platinum

SLC22A2 - Solute Carrier Family 22 Member 2

SLC31A1 - Solute Carrier Family 31 Member 1

SNHL – Sensorineural Hearing Loss

SOAE - Spontaneous Otoacoustic Emissions

SPL - Sound Pressure Level

$t_{1/2}$ - Half-life

TPMT - Thiopurine S -Methyltransferase

TM – Tempanic Membrane

XPC - Xeroderma Pigmentosum Complementation Group C

° - Degree

I Dedicate This Graduate Thesis to My

Omi and Opa

For Their Support and Love

Abstract

Introduction: Platinum drugs (cisplatin and carboplatin) used in chemotherapy is responsible for permanent hearing loss with higher incidence rates in pediatric populations. This ototoxicity can be immediate and/or progressive. To date, there are no implemented treatments to prevent this issue resulting in a lower quality of life of cancer survivors.

Objectives: To determine the length of time for platinum-induced ototoxicity to occur and assess the ability of using Evaluation of Aural/Oral Performance of Children (PEACH) questionnaire to identify this type of hearing loss.

Methods: A cohort of 98 children treated with cisplatin and/or carboplatin from the CHU Sainte-Justine and the Montreal Children's Hospital. Hearing was assessed audiograms using the ASHA criteria and the Chang grading system at the following time points: pre-treatment, end of treatment, first follow-up (3-9 months), second follow-up (15-24 months), third follow-up (24-60 months), fourth follow-up (60-96 months) and final follow-up (96 or more months) following platinum-based chemotherapy. The parents of 56 children in this cohort completed the PEACH questionnaire either before or after platinum treatment.

Results: 58% of children demonstrated hearing loss following treatment and in 14% of these cases, hearing loss progressed up to 2 years following treatment. Out of the participants who completed the PEACH questionnaires, individuals with hearing loss demonstrated lower survey scores and the more significant the hearing loss, the worse the lower PEACH scores.

Conclusion: Due to the presence of progressive nature of hearing loss, it is important for medical professionals to follow pediatric cancer and identify the presence of progressive hearing loss. Implementing the PEACH questionnaire in the follow-up of patients undergoing platinum treatments may facilitate this inquiry.

Résumé

Introduction: Les médicaments à base de platinum (cisplatine et carboplatine) utilisés en chimiothérapie provoquent une perte auditive permanente avec des taux d'incidence plus élevés dans les populations pédiatriques. Cette ototoxicité peut être immédiate et/ou progressive. À ce jour, il n'y a pas de traitements mis en œuvre pour éviter ce problème, ce qui réduit la qualité de vie chez les survivants du cancer.

Objectifs: Déterminer le délai d'apperition de l'ototoxicité induite par du platinum et évaluer la capacité d'utiliser d'évaluation des performances sonores/Oral des enfants (PEACH) afin d'identifier ce type de perte auditive.

Méthodes: Une cohorte de 98 enfants a été traitée avec cisplatine et/ou carboplatine du CHU Sainte-Justine et de l'Hôpital de Montréal pour enfants. L'audition a été évaluée à l'aide d'audiogrammes en utilisant les critères de l'ASHA et le système de classement Chang au temps suivants : pré-traitement, fin de traitement, premier suivi (3-9 mois), deuxième suivi de (de 15 à 24 mois), troisième suivi (24-60 mois), quatrième suivi (60-96 mois) et suivi final (96 mois ou plus) après une chimiothérapie à base de platine. Les parents de 56 enfants de cette cohorte ont complété le questionnaire PEACH avant ou après le traitement de platinum.

Résultats: 58 % des enfants ont démontré une perte auditive après le traitement et dans 14 % de ces cas, la déficience auditive a progressé jusqu'à 2 ans après le traitement. Parmi les participants qui ont rempli les questionnaires PEACH, les individus avec une perte auditive ont montré des scores inférieurs et plus la perte auditive étais significative, plus la diminution du score de PEACH était importante.

Conclusion: En raison de la nature progressive de la perte de l'audition, il est important pour les professionnels de la santé d'assurer un suivi des survivants du cancer pédiatrique, et pour identifier la présence de perte progressive de l'audition. L'utilisation du questionnaire de PEACH peut faciliter ce suivi.

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Contribution of Authors

Study 1

Stephanie Fay Lenhart, Dr. Anne-Sophie Carret and Dr. Sam Daniel contributed to the designing of the experiment. Stephanie Fay Lenhart performed the patient recruitment, literature review, data collection and analysis. Melissa Perrault provided any documentation and information of the participant's pharmaceutical records. Both Aren Bezdjian and Stephanie Fay Lenhart participated in the writing of the paper.

Study 2

Stephanie Fay Lenhart, Dr. Anne-Sophie Carret and Dr. Sam Daniel worked together to design the experiment. Stephanie Fay Lenhart conducted patient recruitment, literature review and with the help of Justine Ratelle collected the PEACH questionnaires. Dr. Lawrence Joseph conducted the data analyses. Stephanie Fay Lenhart and Dr. Yehuda Schwarz wrote the manuscript.

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CHAPTER 1: Introduction

1.1 Thesis Rational

Platinum drugs, cisplatin and carboplatin, are used in the treatment of many soft tissue neoplasms, including cancers to the following areas: bones, connective tissues, muscles, brain, nerve tissues, head, neck, lungs, eyes, kidneys, adrenal glands, lymph nodes tissues, liver, and reproductive organs (1,2). Over the past 40 years, following their introduction into clinical practice, the 5-year survival rates of childhood cancer has increased from 50% to 90% (3). This effectiveness made the use of platinum compounds an important aspect of cancer treatment, even if their side effects constitute: ototoxicity, nephrotoxicity and neurotoxicity (4). As ototoxicity remains the focus of this thesis, other secondary effects will not be discussed. The word "ototoxicity" refers to damage either to human hearing mechanisms or to the vestibular apparatus. These damages may result in hearing loss which can increase significantly the burden of cure in survivors of childhood cancers (5).

Hearing loss caused by platinum compounds is usually bilateral and common in children (6). Studies have recorded degrees of pediatric hearing loss up to 33% following carboplatin treatment and higher rates of 13% to 96% after cisplatin treatment (7-10). These auditory decreases may be immediate and/or progressive. A retrospective study by Peleva et al. (2014) conducted with the McGill Auditory Laboratory attempted to determine the incidence rate of platinum-induced ototoxicity. Post-chemotherapy ototoxicity was detected in 48% of children and significant ototoxicity was present in 30%. In addition 48% of patients with long-term follow-up had further hearing loss following 60 months post-treatment (10). Over the

past few years, platinum-induced hearing loss has become a recognized problem and several more long-term studies have surfaced assessing children's hearing using different follow-up time intervals (11,12).

While not life threatening, early hearing loss causes drastic deterioration to children's quality of life by impairing their ability to learn, perform academically and develop social interactions. These issues increase stress and affect the child's family unit (13,14). In addition to these problems, hearing loss creates a financial burden on society. In 2003, it was estimated that the additional lifetime cost per one individual with hearing loss will exceed 383 000$ US (15). Today, there is no recognized and implemented measures to prevent platinum-induced ototoxicity. Taking into account these negative factors, it is important to find better ways of identifying early symptoms of platinum ototoxicity. This would allow clinical professionals the opportunity to alter the dosage and/or the use of these medications in patients demonstrating signs of early hearing loss thereby improves the patient's quality of life after cancer.

1.2 Objectives & Thesis Structure

The first of two studies focuses on assessing the long-term progression of hearing loss in a group of children tested during the same time intervals following treatment. The objective of the second study is to determine if the "parental evaluation of aural/oral performance of children" (PEACH) questionnaire (for assessment of children's quality of life) could be used as an early identification tool for discovering pediatric ototoxicity during chemotherapy.

To better understand the impact of platinum-induced ototoxicity, this thesis provides background information through a literature review in Chapter 2. Section 2.1 contains a basic overview of the principles of sound (phase, frequency and intensity) followed by a general overview of the anatomy and physiology involved in the human processing of sound. Section 2.2 contains a description of the three types of hearing disorders: conductive hearing loss, sensorineural hearing loss and mixed hearing loss. Emphasis is placed on sensorineural hearing loss that is caused by platinum compounds and its impact on survivor's quality of life. Section 2.3 discusses the techniques used to uncover sensorineural hearing loss. Section 2.4 focuses on the molecular configuration, mechanisms and clinical implications of using platinum drugs (cisplatin and carboplatin). Finally, section 2.5 focuses on the combined effect of platinum compounds and other ototoxic agents used during chemotherapy.

This study looked at the long-term hearing loss caused by cisplatin and carboplatin (study 1, Chapter 3). This first manuscript allows for a better understanding of the progression of ototoxicity in a pediatric population. Chapter 4 looked at how the PEACH questionnaire can be used to see hearing depreciation in children exposed to platinum drugs (study 2). Chapter 5 concludes the thesis with a summary of the manuscript's findings. It also provides a general overview of future directions for research and clinical practice.

CHAPTER 2: Background & Literature Review

2.1 Basics of Sound, Anatomy & Physiology of the Human Ear

In physics, sound is a result of vibration caused by pressure from an object or person. This vibration will displace itself in a sinusoidal wave through air or water. The shape of this wave is characterized by: frequency, phase and intensity. Sound waves are received and processed by the human ear's three parts: external, middle and inner ear (16, 17). A simple understanding of the anatomy and physiology of the ear is needed, since platinum compounds affect the integrity of the auditory pathway.

2.1.1 Properties of Sound

Frequency depicts the speed of vibrations. It represents the number of cycles occurring in one second expressed in Hertz (Hz) units (17). A normal person has a frequency range of 125 Hz to 20, 000 Hz, (18) with better hearing between 500 Hz and 8 000 Hz (19) in order to determine speech (250 Hz to 8, 000 Hz) (17).

Phase refers to the location where a vibration would be situated if it was on a circle and it is expressed in degrees (0° to 360°). For example, a vibration starts at 0° and ends its cycle at the end of wave at 360°. When two pure tones are situated 180° opposite each other in the same frequency, then the waves cancel each other out. However, when two waves originate from different frequencies, the vibrations are combined creating a complex waveform (20).

Intensity is the amplitude or loudness of a sound described in decibels (dB). It is used to depict human hearing range. Normal conversation occurs between 40 dB to 50 dB (17) but humans can hear up to the threshold of pain at 140 dB (21).

2.1.2 External Ear

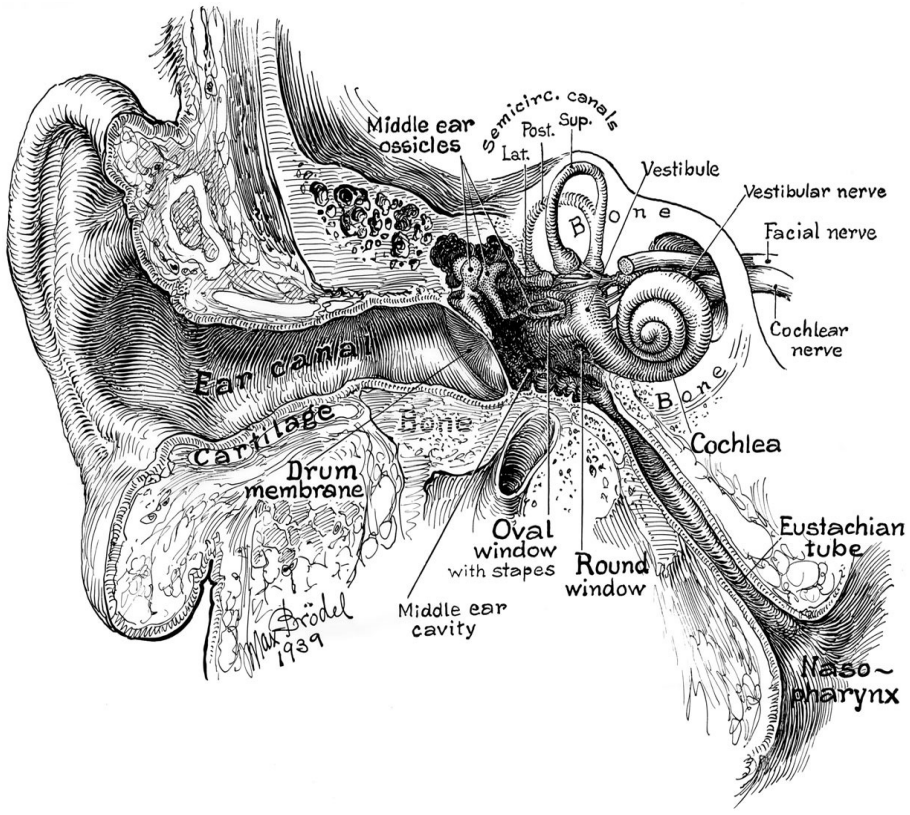


Figure 1. Drawing of the human ear depicting the anatomy of the outer, middle and inner ear drawn by Max Brödel in 1939. Image retrieved and given permission to use from Van De Water (2012) for the purpose of this thesis (22).

The goal of the external ear is to transmit sound to the tympanic membrane using the auricle (pinna) and external acoustic meatus (auditory canal) (see Figure 1). The pinna is the first part of ear. It is slightly angled with an irregularly shaped concave cartilage covered by skin. Connecting anterior and posterior ligaments allow the pinna to remain outside of the temporal bone. This structure permits the collection of sound waves to pass into the adjoining auditory canal. The auditory canal also called the external auditory meatus is approximately 4 centimeters long and connects the external and the middle ears.

Encompassing this length are hair follicles which containing oily sebaceous and sweat glands. These glands produce dry and wet wax (earwax), which prevents the entrance of foreign bodies and serve as a disinfectant. Because little sound is absorbed in the auditory canal, the passage allows for sounds to travel directly onto the tympanic membrane (TM, eardrum or drum membrane). The TM is a semi-transparent one-centimeter divider between the external and middle ear. With this round membrane less than 1/10th millimeter thick, sound waves are capable of creating vibrations when they reach it (23-25).

2.1.3 Middle Ear

The middle ear consists of a small lateral air-filled chamber known as the tympanic cavity. The tympanic cavity is surrounded by conductive tissue, the eardrum and the Eustachian tube (pharyngotympanic tube) (see Figure 2). The Eustachian tube connects to the nasopharynx. During swallowing and yawing this tube is opened to acclimatize pressure build-up in the inner ear (23,24).

Inside the tympanic cavity are the ossicles. These are the three smallest bones in the body: hammer (malleus), anvil (incus) and the stirrup (staples). They create a chain from the tympanic membrane (TM) to the round window of the cochlea. The malleus has a form of a hammer with the club-shape part attached to the tympanic membrane. The incus has an elongated shape linking the hammer to the staples. The bottom of the sideways U-shaped staples is connected to the incus and the oval window. This bone chain is able to amplify any vibration. When an acoustic sound vibrates on the eardrum, a chain reaction

begins, whereby the connecting ossicles move. This results in the stapes striking the oval window of the cochlea (23,24).

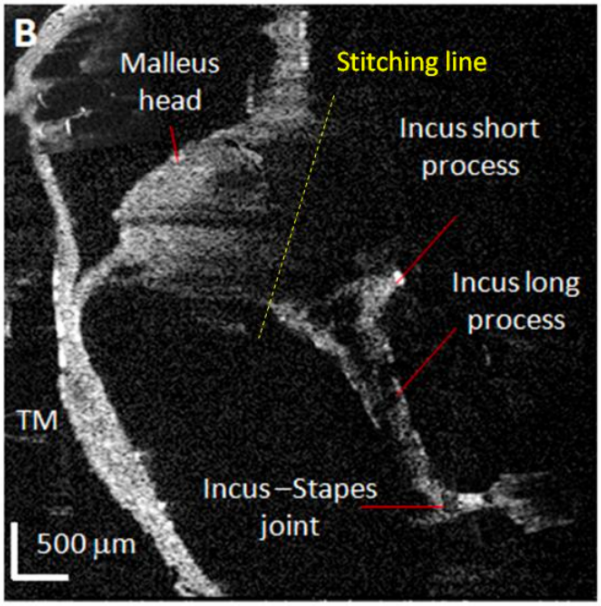


Figure 2. Images retrieved with permission from Park et al. (2016). Cross-sectional optical coherence tomography of the middle ear (26).

2.1.4 Inner Ear

Anatomy:

Deep within the temporal bone, is the inner ear (also described as the bony labyrinth) a complex system containing fluid ducts. This section is found in a bony outer osseous labyrinth inside a bony casting. The labyrinth is the basis for auditory and human balance systems. It contains: the vestibule, the semicircular canals (anterior, posterior and lateral) and the cochlea (see Figure 3) (27).

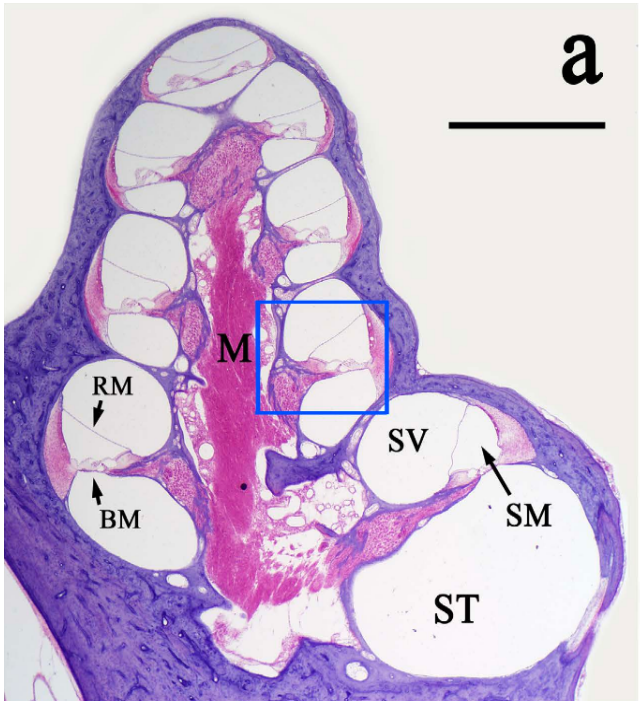


Figure 3. Guinea pig cochlea cross-section stained with hematoxylin and eosin, and cut along its longitudinal axis with Magnification = 2×; Scale = 1 mm (M: modiolus. BM: basilar membrane; RM: Reissner's membrane, ST: scala tympani; SM: scala media; SV: scala vestibuli.). The region contained in the blue box is a single half-turn of the guinea pig cochlea and the area in englobes the organ of Corti discussed further in paper using figure 4. Images used with author's permission (28).

The vestibule (roughly 5 mm horizontally and 3 mm across vertically) is in the middle of the bony labyrinth, between the semicircular canals and the cochlea. The oval window located on the vestibule lateral wall and connects to the ossicle stirrup bone. Posterior to the vestibule are the three semicircular canals: anterior, posterior and lateral.

These canals are responsible for coordination and movement control (29). The anterior side of the vestibule contains the main organ of hearing: the cochlea. The cochlea reaches adult size at birth (30). Its shape resembles a small snail, with two and a half turns, covered by a thin layer of bone. It measures about 5 mm from the base to the apex and 1 cm wide. When unraveled it can be 35 mm long and the channel inside each of the turns comprises three chambers: scala vestibuli, scala media and the *scala tympani* (see Figure 3). The two outer layers (*scala vestibuli* and s*cala tympani*) surrounding the *scala media* contain perilymph, a liquid resembling the composition of extracellular fluid with high Na^+ and Ca^{2+} concentrations (31). The scala media contains endolymph, a liquid similar to the intracellular fluid due to high K^+ and low Na^+ concentrations. The endolymph's potential is highly positive, while the perilymph has a potential resembling the surrounding bone (32). To maintain the chemical composition of these chambers, a network of capillaries in the *scala media* regulate homeostasis. This network of capillaries contains three types of cells: marginal, intermediate, and basal cell. The *scala media* is enclosed on top by the Reissner's membrane and on the bottom by the basilar membrane (33).

Above the basilar membrane rests a specialized sensory receptor for hearing, called the organ of Corti (depicted in Figure 4). This structure contains the hair cell receptors with their nerve endings and supporting cells that are covered by the tectorial membrane (32). There are two types of hair cells: outer hair cells (OHC) and inner hair cells (IHC). Both types of hair cells have an elongated shape and stereocilia projecting from their tip. However, the OHC stereocilia are connected to the tectorial membrane while the IHC stereocilia do not touch that membrane. There are approximately 13, 000 OHC placed throughout three rows in the cochlea, and about 3, 500 IHC placed in one single row (17).

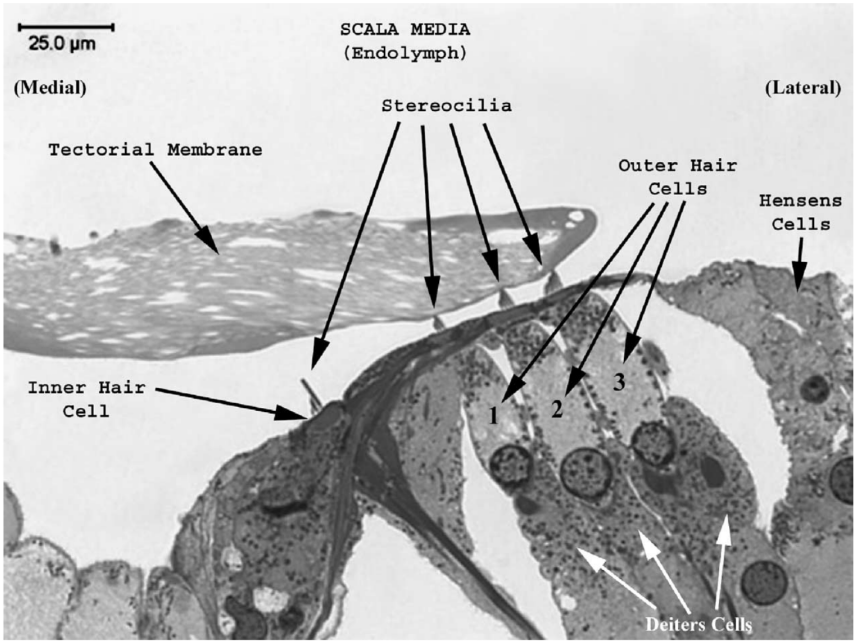


Figure 4. Organ of Corti cross-section of a mole rate cochlea demonstrating no contact between the stereocilia of the inner hair cells and the tectorial membrane. Copied from Raphael & Altschuler (2003) with author's permission for this paper (34).

Physiology:

When the vibrations of the stirrup are received on the oval window, a fluid motion referred to as the traveling wave is created. This wave is intensified due to the difference in electrochemical composition of the endolymph and perilymph liquids. This motion moves the fluid in the cochlea, causing a displacement of the basilar membrane (BM) and organ of Corti (32). For lower frequencies, the movement occurs up to the apical section of the cochlea. For higher frequencies, the wave travels to the end of the cochlea. As the BM position changes, depending on the frequency of the sound, the hair cells connected to it will be displaced (17). This shift cause stereocilia to open K^+ ion channels that in turn allow

K+ ions to flow freely down their electrochemical gradient and depolarizes the hair cells. This will cause the release of neurotransmitters into spiral ganglion neurons, (35) evoking the action potential of the auditory nerve fibers (17).

2.1.5 Auditory Nervous System

After leaving the cochlea, nerve fibers join the VIIIth cranial nerve and enter the cochlear nucleus (CN) in the brainstem. Any signals that the nerve fibers carry will be divided into the ventral CN and the dorsal CN, both linked to the superior olivary complex of the brainstem. The convergence of information from the right and left ear happen at the olivary nuclei, this is an important step for the spatial perception of sound. The signals then travel to the inferior colliculus, which serves as a processing station. Auditory signals are then transported to the thalamus nucleus (medial geniculate body). Finally, from the MGB sound signals are sent to the auditory cortex in the temporal lobe (36).

2.2 Chemotherapeutic Ototoxicity

Ototoxicity is linked to a reduction in hearing sensitivity that can stem from numerous disorders affecting the external, middle and inner ear, result in the following three types of hearing losses: conductive, sensorineural and mixed hearing loss. Ototoxic platinum drugs may create sensorineural hearing loss through a disturbance of the inner ear (37). This type of hearing loss is especially devastating in pediatric populations since children rely on their ability to hear in order to perceive and learn language. If hearing is damaged, children's overall quality of life will be affected (38).

2.2.1 Types of Hearing Loss

In cases of *conductive hearing loss* (CHL), sound is reduced during its travel from the external to the inner ear. This problem is observed when a barrier is present inside either external or middle ear structures (37). Such obstacles can be caused by congenital malformations, infections or trauma (17).

Wide varieties of congenital malformations can cause CHL up to 60 dB. These varieties include malformations to the ossicles, pinna, entrance to the cochlea or even the absence of an ear canal (17). A large study on 4.8 million newborn children revealed that the prevalence of CHL due to congenital impairments is 112 out of 100, 000 pediatric cases (39). Just like congenital malformations, infections may also cause hearing difficulties. The most common infection in children leading to CHL is otitis media, an inflammation caused by a dysfunctional Eustachian tube that allows fluid to enter the middle ear. It is estimated that 76-95% of all children will have at least one episode of otitis media by the age of 6, with higher prevalence rates within the first 2 years of life. Approximately 50% of children who have otitis media in their first year will have 6 or more cases within the next 2 years. Usually hearing loss is transient during this infection and will resolve itself along with the inflammation. However, in cases of chronic infections the middle ear structures can become damaged resulting in CHL. Following otitis media, other acquired disorders can reduce hearing such as: otosclerosis, tympanosclerosis, physical trauma (e.g. barotrauma) or tumors (e.g. glomus tumors) (17).

S*ensorineural hearing loss* (SNHL) results from a failure in the cochlea's transduction of sound along the auditory neural pathway, from the VIIIth cranial nerve into the temporal lobe. This hearing loss causes lower sensitivity of the cochlea’s hair cells,

shorter range of hearing and a reduction in frequency identification (40). A typical SNHL is characterized by superior hearing in the lower frequencies compared to the higher frequencies. Vowels are found in lower frequencies, while consonants are present in higher ones. Therefore, SNHL makes it difficult for consonants to be heard (41). There are many different causes of SNHL such as: hereditary factors, infections, age, trauma, or ototoxic drugs (40).

There are two common hereditary factors linked to SNHL. The first, *syndromic hearing disorders* which occur commonly with another medical disorder such Alport syndrome (42), branchio-oto-renal syndrome (43), Wildervanck syndrome (44), Jervell and Lange-Nielson syndrome (45), Pendred syndrome, Usher syndrome or Waardenburg syndrome (46). The second, *nonsyndromic hearing disorders* are caused by either an autosomal recessive or dominant genetic disorders without other significant features except for hearing loss. This includes X-linked hearing disorders caused by a faulty gene located on the X chromosome (40).

Next, SNHL may result from teratogenic effects caused by congenital infections in cases where mothers are infected during the fetus's development (40). Common congenital infections include: mumps (47), measles (48), syphilis (49), human immunodeficiency virus (HIV) (50) or toxoplasmosis (51) or cytomegalorivirus (CMV) (52). CMV, a herpes virus, is the most frequent infection among infants and young children (42). It provokes progressive hearing loss, which is moderate in one ear and severe in the other. Acquired infections such as mumps and syphilis may also cause SNHL (40).

In adults, the most prevalent cause of SNHL is aging. It is referred to as presbycusis. It is estimated that 25% to 40% of people over the age of 65 have some degree of SNHL.

This problem is increased to approximately 90% for those over 90 years old. Following presbycusis, noise-induced hearing loss (NIHL), also referred to as acoustic trauma, is the most common cause of SNHL (40). NIHL can be induced from either a sudden acoustic trauma or long-term exposure to sound levels over 75 dB to 85 dB (53). Alternatively, SNHL can arise from ototoxicity drugs (see section 2.3.3) or radiation (see section 2.5).

Finally, if patients experiences both sensorineural and conductive hearing loss, they are diagnosied with *mixed hearing loss*. In this situation, sound will travel to an impaired cochlea from either a damaged external or middle ear. There are many sources of mixed hearing loss. In some patients, this loss is simply the addition of a conductive hearing loss (e.g. active middle-ear disease) to a longstanding SNHL. In other cases, a middle-ear disorder can cause a problem in the cochlear resulting in a mixed hearing loss (40).

This thesis focuses on understanding the effects of platinum-induced SNHL. Therefore, all participants where screened for any prior hearing losses and only individals with no prior hearing losses were recruited to participate in the studies of this thesis.

2.2.2 Characteristics of Ototoxicity

Ototoxicity refers to a loss of hearing following the use of therapeutic drugs. There are five general categories of ototoxic medications: loop diuretics (e.g bumetanide, furosemide), salicylates (e.g aspirin), antimalaria (e.g quinine), aminoglycosides (e.g streptomycin, gentamycin) and chemotherapeutic agents (e.g cisplatin, carboplatin). Hearing impairments caused by loop diuretics, salicylates and antimalaria can be temporary. However, aminoglycosides and chemotherapeutic drugs create permanent bilateral SNHL (54).

Incidence rates of chemotherapeutic ototoxicity vary from 11% to 91%. In addition, otoxicity can be accompanied by tinnitus (ringing in the ears) in 2% to 36% of patients. Primary damage is located in the higher frequencies (4, 000 Hz to 8, 000 Hz), before spreading to lower ranges (54). Platinum chemotherapeutic drugs affect patients of all ages with a higher susceptibility in children (6, 56). In these cases the presence of the following genetic markers have been linked to hearing loss: low-density lipoprotein receptor-related protein 2 (LRP2), solute carrier family 31 member 1 (SLC31A1, a copper transporter), solute carrier family 22 member 2 (SLC22A2 a organic cation transporter 2), glutathione S-transferase (GST), excision repair cross-complementing rodent repair deficiency (ERCC), xeroderma pigmentosum complementation group C (XPC), catechol- O -methyltransferase (COMPT) and thiopurine S -methyltransferase (TPMT) (57).

2.2.3 Quality of Life

Given that hearing loss diminishes quality of life in a large number of children, it is important to consider the side effects of using platinum-based drugs. Children are learning language while playing and during social interactions. Therefore, SNHL at a young age has greater consequences than in adulthood or old age (56,58).

As mentioned earlier, ototoxicity impacts higher frequencies first. These high frequencies are the one's that children depend on for the understanding and formation of phonetic letters (e.g. s, t and z). This is a problem since the English language is based on almost 50% of these phonemes (59,60). Therefore, early identification (before the age of 6 months) promotes development of picture/oral vocabulary, grammatical comprehension, phonological analysis, sentence combining, word production, syntax and semantics (61).

Without early screening, severe hearing loss can go undetected until the age of 3 (38).

Permanent hearing loss impacts on children's development of literacy, psychosocial functioning and academic performance (62,63). Serious consequences regarding education are present even in mild cases of hearing loss (64). A 1998 study focusing on the education of 1, 218 children found that youth with SNHL experienced greater difficulty in a series of educational and fictional tests than normal hearing children. In that project, 37 children with SNHL failed at least one grade (65). Another study that focused on pediatric neuroblastoma survivors assessed hearing though parental reports and determined that survivors with ototoxicity had at least twice the risk of developing a problem with reading skills, math skills, and/or attention (66).

Apart from language and educational problems, children with hearing loss exhibit poorer behavior and psychological well-being than hearing children (67). Rates of behavioural problems of children with SNHL are between 30-38% (68,69) compared to 3-18% in children with normal hearing (70). These problems may manifest themselves as oppositional behaviour, aggression or violating social rules (55) and often continue themselves often into adulthood (71). Apart from the overall diminished quality of life of pediatric survivors, hearing loss impacts the child's family unit. Parents of hearing-impaired children report higher stress levels than parents with hearing children (14,72).

2.3 Clinical Tests and Assessment of Platinum Ototoxicity

Sensorineural hearing loss can be documented using pure tone audiograms and distortion product otoacoustic emissions tests. The results can be graded using the Chang grading criteria or the American Speech-Language-Hearing Association criteria. Thus, a

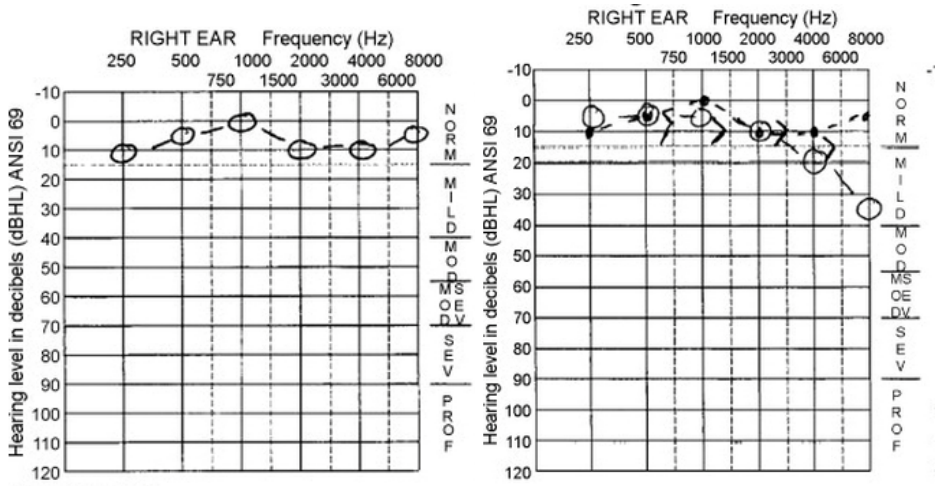
description of these tests with the criteria's used to understand their results are presented in this part (17,73).

2.3.1 Pure Tone Audiograms

Pure tone audiograms (PTA) focus on measuring the lowest intensity of sound, called a pure tone threshold of hearing, that a person is capable to respond to 50% of the time in frequencies important for speech understanding (74) (125 to 8, 000 Hz) (17). There are two types of pure PTA tests: air conduction audiometry (ACA) and bone conduction audiometry (BCA). ACA uses earphones to deliver sound through the ear canal, while BCA places a vibrator on the skull thereby allowing vibrations to enter the inner ear directly and bypassing the outer and middle ear (74). During each audiometry test, the ears are tested separately. Either evaluation begins by familiarizing the patient to 1 Hz, before starting to test at other frequencies. The American Speech-Language Association recommends starting at amplitude of 30 dB before increasing or decreasing the volume by increments of 5 dB or 10 dB until the lowest hearing threshold is determined (75). If the threshold is higher than 20 dB then a hearing loss is identified (76).

In cases of conductive hearing loss, patients demonstrate better hearing in the BCA than in the ACA, since the outer and middle ear channels are replaced by bone conduction. If SNHL is present, both the BCA and ACA tests will demonstrate high thresholds (74). These elevated thresholds are clearly noted in a case report by Troung et al. (2007); where a 16-year old boy showed bilateral SNHL from 5 dB to 35 dB following cisplatin treatment (see Figure 5) (77).

Assessments of audiograms can be accomplished thought the use of different criteria's. The two used in this thesis where the Chang grading system (73) and the American Speech language Association criteria (17). They are both described in sections 2.2.3 and 2.2.4.



(A) (B)

Figure 5. Audiograms of the right ear of a teenager with osteosarcoma. (A) Prior hearing tests demonstrated no issues. (B) Following cisplatin treatment SNHL was perceived trough higher hearing thresholds. Retrieved with permission from Truong et al. (2007) for the use of this manuscript (77).

PTA testing provides information about patient's hearing and can determine different types of hearing loss. However, the test has limitations such as test-retest reliability, human error and poor calibration. Situations have been documented where the BCA and ACA thresholds where different in patients with SNHL demonstrating a lack of test-retest reliability. Other times audiogram readings may be inaccurate in situations of

severe hearing loss, since individuals may mistake sound vibrations as a sense of touch. These vibrations are described as vibrotactile thresholds. Another problem is testing individuals with tinnitus because tinnitus can interfere with the perceptions of pure tones leading to false-positive responses. (19) Another limitation is that PTA relies on the cooperation of patients to identify the sound they hear by clicking a button. For instance, in cases of infants requiring a hearing test the PTA assessment cannot be used. In these situations, otoacoustic emissions test (section 2.2.2) will be used instead (39).

2.3.2 Distortion Product Otoacoustic Emissions (DPOAE)

David Kemp a geophysicits discovered Product Otoacoustic Emissions (OAE) in the 1940s, but a screening test for OAE was only developed for them in 1978 (78). OAE are sounds that initiate as a secondary propulsion from the cochlea's (79) outer hair cells in response to auditory stimulus (78). This test has been used to identify any damage to the hair cells which may arise from the following conditions: ototoxic drugs (80), noise trauma (81), hypoxia (82) and prebycusis hearing loss (83). In normal hearing, the test can be determined in 99% of normal hearing ears; however, if hearing loss is greater than 30 dB no responses can be reported (79). As well, OAE's are fast taking a short time to complete (< 1 minute) and requires physical response from patients (80) thus, making it a popular newborn screening test (84).

OAE are divided into two sections: spontaneous and evoked. *Spontaneous OAEs* (SOAE) occur naturally and *evoked OAEs* (EOAE) appear when added stimulus to the ear is present. One type of evoked OAEs frequently used to assess children's hearing is *Distortion products* OAEs (DPOAE), which are present when stimulating cochlea using two pure tone

sounds. This makes hair cells generate additional frequencies. For example, if the acoustic tones are at frequencies f_1 and f_2, a healthy cochlea may generate several DPOAEs such as the following frequencies: $2f_1$-f_2, $3f_1$-f_2, $2f_2$-f_1, $3f_2$-f_1 etc (80). These newly created frequencies shall move in the opposite direction, away from the basilar membrane though the middle ear and into the outer ear where they are assessed by a specialized microphone (85). Although errors are observed at all frequencies, previous research has demonstrated that DPOAEs are generally accurate in identifying lower auditory frequencies 2, 000 Hz to 4, 000Hz (86).

Ideally one would have preferred to obtain data form both PTA and DPOAE tests for every patient. But, in the hospital setting non-infant patients commonly only receive the PTA test. Therefore, to standardize this thesis we focused on audiogram measurement in all patients.

2.3.3 Chang's Grading Criteria

Clinical practice suggests that patients receive a baseline audiogram before beginning any chemotherapeutic ototoxic treatment in order to monitor changes. Audiograms measure the following range 250 Hz to 8, 000 Hz. However, assessing a pediatric population is a challenge because there are discrepancies between the theoretical and clinical frequencies measured. When children have attention problems or refuse to accept earphones, the audiologist may only be able to test two or three of the full range of frequencies. Not only is it difficult to receive a complete baseline audiogram, but clinical trials report on several different numeric grading systems making it difficult to access national hearing loss (74).

The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) criteria focuses on subjective hearing changes in two conjoint frequencies and losses, which are graded on a scale of 1 to 4 (87). However, such measures lead to under reporting cases of chemotherapeutic-induced hearing loss due to the lack of sensitivity of the criteria (88,89). Due to these inconsistencies, certain clinical trials prefer to use the Brock's criteria to measure platinum ototoxicity (12,90).

The Brock criterion is based on the audiometric patterns of hearing in children exposed to high-dose cisplatin (56). In most cases, hearing thresholds that fell below 40 dB rarely impacted the lower frequencies. Based on the understanding that hearing would not be affected below this point, 40 dB is used as a cut-off level. Thus, the major limitation of this criterion is that it does not distinguish between normal hearing and mild hearing loss. This makes it impossible to tell whether a child has had a slight deterioration in hearing subsequent to cisplatin treatment. In addition, the Brock criteria does not apply for extended high frequencies or losses at 3, 000 Hz and 6, 000Hz (91).

Chang & Chinosornvatana (73) understood the limitations of past identification criteria (Brock and CTCAE) and the difficulty of obtaining complete audiograms. They proposed a new and more specific grading system called the Chang criteria, which allows for the severity of ototoxicity to be distinguished (see Table 1). The Chang criterion focuses on assessing hearing loss beginning from high to low frequencies. It uses a simple grading scale (grade 0, 1a, 1b, 2a, 2b, 3 and 4) that helps identify the severity of hearing loss. Individuals with grade 0 demonstrated no hearing loss while individuals with grade 2a and above had significant hearing loss (73). This thesis used the Chang grading system to

identify hearing loss and rate the severity of ototoxicity from mild (grade 1a & 1b) to moderate/severe hearing loss (grade ≥ 2a).

Chang Grade	Sensorineural Hearing Threshold (dB HL) bone conduction or air conduction with normal tympanogram
0	≤ 20 dB at 1, 2, and 4 kHz
1a	≥ 40 dB at any freq 6 to 12 kHz
1b	> 20 and < 40 dB at 4 kHz
2a	≥ 40 dB at 4 kHz and above
2b	> 20 and < 40 dB at any freq below 4 kHz
3	≥ 40 dB at 2 or 3 kHz and above
4	≥ 40 dB at 1 kHz and above

Table 1. Chang's grading criteria to determine platinum-induced ototoxicity retrieved with permission to use in this thesis from Chang & Chinosornvatana (2010) (73).

2.3.4 American Speech-Language-Hearing Association (ASHA) Criteria

Using specific criteria to define drug-induced hearing loss is controversial (92). For this reason, the American Speech-Language-Hearing Association (ASHA) attempted to create a standard criterion to identify ototoxicity. The ASHA believes an occasional false-positive diagnosis is preferable to a delayed detection of ototoxic hearing loss. Therefore, the ASHA focuses on decreases of adjacent frequencies as an indicator of ototoxicity (93). Moreover, this criterion assesses high frequencies (> 8, 000 Hz) for hearing losses, even if these frequencies are not primordial for speech recognition (94). The ASHA criterion examines changes from baseline measures to determine hearing loss if either of the following three situations are present:

(A) 20 dB or greater hearing loss in pure tone threshold in at least one frequency, OR

(B) 10 dB or greater decrease at two adjacent test frequencies, OR

(C) Loss of responses at three consecutive frequencies where responses were previously obtained (95).

2.4 Platinum Chemotherapeutics Drugs

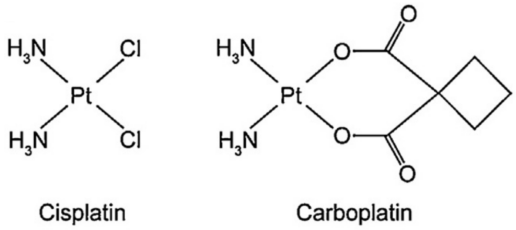


Figure 6. Compositions of cisplatin and carboplatin retrived and modifed with permission from Karasawa & Steyger (2015) for the use in this thesis (96).

The main purpose of chemotherapeutic agents (cisplatin and carboplatin, see Figure 6) is to stop the tumor cell proliferation and reduce tumor growth. Although they possess unique chemical and pharmacological properties capable of helping in chemotherapy, one of their main side effects is ototoxicity (97-99).

2.4.1 Cisplatin & Carboplatin

Cisplatin, also called *cis*-diamminedichloroplatinum (II), is a standard platinum compound. It has a molecular weight of 300.1 gm/mol and a density of 3.74 g/cm^3. The structure of cisplatin consists of an inorganic platinum (Pt) molecule and four ligands of *cis* positioned pairs of chlorine atoms or amine groups (see Figure 6). M. Peyrone first

synthesized cisplatin in 1844. However, it only gained scientific recognition in the 1960s, when M. Rosenberg discovered that certain electrolysis products from platinum mesh electrodes are able to inhibit cell division in *Escherichia coli*, which lead to the use of platinum compounds in chemotherapy (97,98).

Once administered, cisplatin spreads to the liver, kidneys, large and small intestines with low penetrations to the central nervous system. Within the first 24 hours, 25 % of the cisplatin dose will be eliminated via renal clearance (100). According to a 6-year follow-up screening, it was demonstrated that cisplatin has a first elimination half-life ($t_{1/2}$) of 5.02 months and the second $t_{1/2}$ of 37.0 months (101). Presently, cisplatin is widely used in the treatment of a variety of cancers: lung, ovarian, bladder, testicular, esophageal, gastric, colon, pancreatic head and neck (102). Its side effects include nephrotoxicity, neurotoxicity and ototoxicity (4).

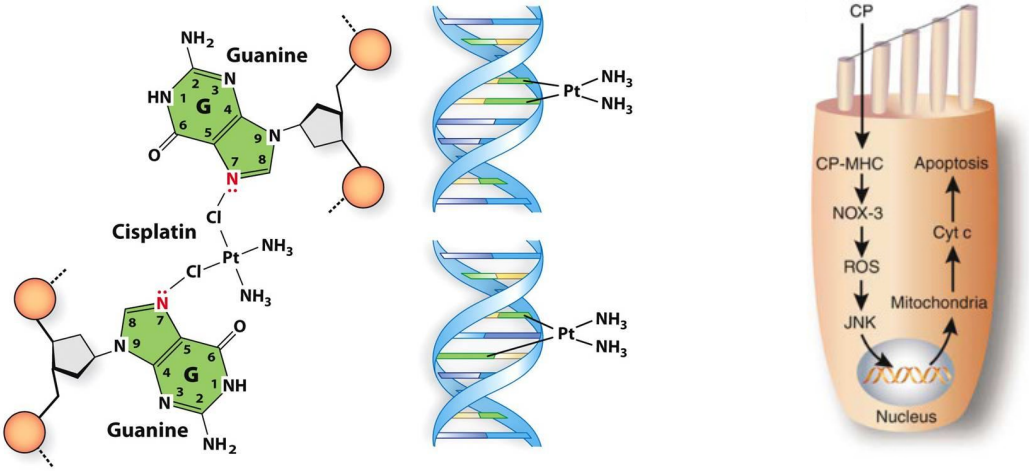
Cisplatin is the most common ototoxic compound used in clinics with hearing loss rates from 11%-91% (55). The degree of hearing loss varies depending on each patient, but when present auditory difficulties tends to occur in high frequencies. Li et al. (2004) focused on the effects of cisplatin (median cumulative dose 397 mg/m^2) in 153 children (6 months to 18 years) for germ cell tumors, hepatoblastoma, neuroblastoma or osteosarcoma. They found that mild hearing loss developed in high frequencies for 26 patients (17%) and moderate to severe hearing loss was present in 54 patients (35%). Even with many patients suffering from secondary hearing loss, cisplatin continues to be used with no available alternative because it is one of the most powerful drugs in treating solid and hematological cancers (8).

Due to the side effects of cisplatin, the Institute for Cancer Research in the United Kingdom focused on developing an alternative, less toxic drug, by reducing cisplatin's leaving groups (molecular fragments with a pair of electrons). This resulted in the introduction of *cis*-1,1-cyclobutanedicarboxylatodiammineplatinum(II), referred to as carboplatin, (103) in 1981 (see Figure 6). Following intravenous administration, carboplatin binds to the plasma proteins with an efficacy > 85%. Within the first 24 hours following administration, as much as 70% is eliminated though urine. Just like cisplatin, carboplatin has an equivalent biochemical selectivity and therapeutic ability in treating certain cancers such as: ovarian (104), lung (105) and retinoblastoma (12). However, cisplatin remains more effective in treating testicular cancer (103), bladder cancer, germ cell tumors, head and neck cancers (106).

Carboplatin is considered less toxic than cisplatin; it causes less nausea, vomiting and neurotoxicity (103). While carboplatin has less devastating side effects, it still causes severe bone marrow toxicity and ototoxicity. In the case of bone marrow toxicity, the issue can be resolved by integrating autologous stem cell rescue treatments (107). However, unlike bone marrow toxicity, ototoxic remains untreatable. This is a problem even when carboplatin incidence levels are lower than cisplatin. Montaguti et al. (2002) determined that incident rates of hearing loss were decreased in pediatric patients treated with carboplatin (33%) compared to cisplatin (86%) for malignant neoplasms (108). However, when carboplatin is administered with or following other ototoxic agents, the rate of ototoxicity can increase to 82% (109).

2.4.2 Mechanisms of Platinum-Induced Ototoxicity

Cisplatin is ototoxic since it damages to the auditory system by triggering apoptotic cell death though several different mechanisms (110). The first mechanism is due to cisplatin's *cis* geometry that allows it to become cytotoxic (103). The structure permits cisplatin's chlorine atoms to react and be exchanged with DNA guanine base 7-nitrogen atoms. Cisplatin will form inter-and intra-strand cross-linked Pt-DNA bonds. High mobility group (HMG) proteins will recognize this formation and bind to the DNA at the 1,2-d (GpG) cross-link position. This new complex of cisplatin-DNA-HMGB1 can block transcription, thus preventing DNA transcription and replication. This action may force the DNA to send out damage signals resulting in cell apoptosis (see Figure 7a) (103-105). Like cisplatin, carboplatin induces platinum-DNA destruction, but requires a 10-fold higher drug concentration and a 7.5-folds longer incubation time (111).



(A) (B)

Figure 7. (A) Cisplatin forming inter- and intra- strands cross-linked DNA though an exchange with guanine. (B) Cisplatin (CP) pathway resulting in apoptosis retrieved with

permission from Brock et al. (2012) paper on platinum-induced ototoxicity in children (112).

The second mechanism (see Figure 7b) that is widely discussed focuses on the production of reactive oxidative species (ROS). ROS increase lipid peroxidation, alter enzymes and structural proteins creating cell apoptosis (110). ROS are created following the entry of cisplatin though mechanotransducer channels. Cisplatin brings about a monohydrate complex (MHC) and activates unique isoform of nicotinamide adenine dinucleotide phosphate oxidase (NOX-3) causing the production of ROS. ROS activates the c-Jun N-terminal kinase (JNK) pathway allowing molecules to enter into the cell's nucleus to activate genes. These genes can then pass to the mitochondria, causing the release of cytochrome c (cyt c), which can trigger apoptosis though caspase-dependent mechanisms (2).

Cisplatin has the ability to enter the cochlea though different routes (see Figure 8) as summarized in a review from Brock et al. (2012). They believe that it is likely cisplatin passes from the strial capillaries into the marginal cells following clearance into the endolymph liquid of the cochlea (shown as number 1 in Figure 8). Another pathway involves cisplatin crossing the blood-labyrinth barrier into the perilymph liquid and then entering into the endolymph via transcytosis across the epithelial perilymph and endolymph barriers (shown as numbers 2 and 3 in Figure 8). Once in the endolymph, cisplatin enters the hair cells of the organ of Corti through latter's apical membranes (shown as number 4 in Figure 8). Cisplatin is also believed to penetrate from the scala

tympani into the basilar membrane by the extracellular fluids inside the hair cells (shown as number 5 in Figure 8) (112).

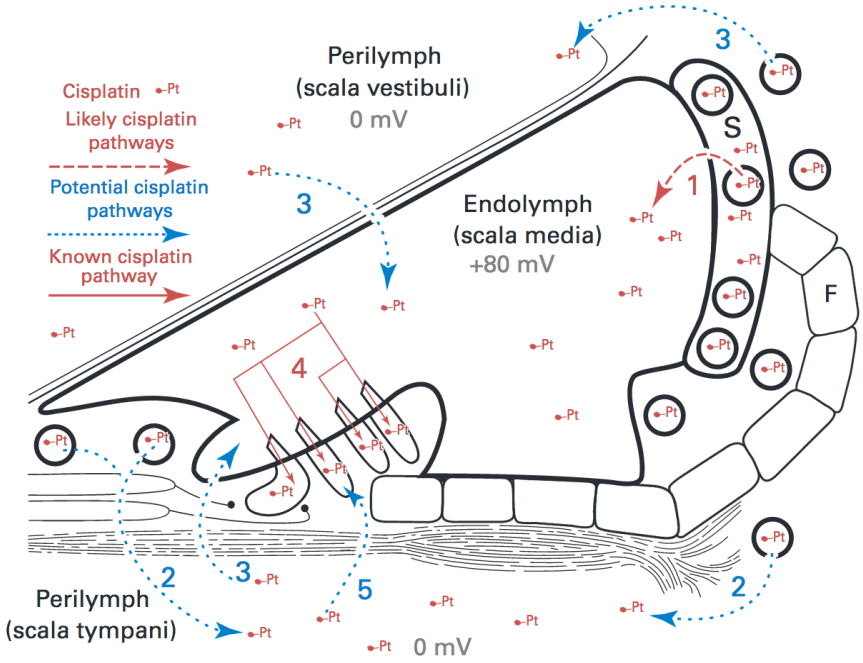


Figure 8. Diagram demonstrating the trafficking routes of cisplatin (depicted as Pt) into the hair cells of the organ of Corti retrieved with permission from Brock et al. (2012) paper on platinum-induced ototoxicity in children (112).

2.4.3 Platinum Damage to the Inner Ear

Platinum induces ototoxicity by targeting three major areas in the cochlea: the stria vascularis, the spiral ganglion and the organ of Corti. Cisplatin creates damage to the stria vascularis trough the thinning of the tissue and the reduction of marginal cells. It may also induce myelin sheath detachment in the spiral ganglions due to both perikaryal shrinkage and swelling of the myelin sheaths (113-115). Platinum can destroy the organ of Corti's outer (OHC) and inner hair cells (IHC). Cisplatin is more susceptible to damaging hair cells

depending up to a certain dose (over 400 μmol/l in mice) (116). Carboplatin hair cell destruction depends on the dosage and animal model. In a guinea pig experiment, daily administration of 50 mg/kg carboplatin for 2 to 3 days lead to elevated action potential thresholds and damages to the OHC, but not in IHC (107). In chinchilla model's, low-to moderate doses (e.g. 38-150 mg/kg) of carboplatin can selectively destroy some or all of the IHC in the entire cochlea but spare the OHC (117). For example, Lobarinas et al. (2013) demonstrated that chinchillas receiving 75 mg/kg of carboplatin exhibited extensive IHC damage greater than 80% (118). High doses of carboplatin (e.g. 200 mg/kg) will completely destroy all hair cells from the base towards the apex of the cochlea (117).

2.5 Combination of Ototoxic Chemotherapeutic Treatments

This section contains additional information about other ototoxic cancer treatments that are combined with cisplatin or carboplatin drugs and radiation. An overview of the uses of these treatments and their impact on hearing when combined with platinum drugs is discussed in this section (119, 120).

2.5.1 Radiation

In treating cancer, radiotherapy can be utilized after surgical tumor dissection either by itself or with chemotherapy (119, 121). For head and neck cancer, total radiation ranged from 59.5 to 76.5 Gy for adults (122) and 25 to 55 Gy for children (123). A combination of both chemotherapeutic drugs and radiation leads to an increased potential of killing tumor cells and the chance of survival. The overall 5-year survival rate of patients treated with cisplatin and radiation is 53% compared to 40% for patients treated only with

radiation. These promising results have made this combination a preferred treatment method for patients with head and neck cancer called chemoradiotherapy (124,125).

Unfortunately, both cranial radiation and chemotherapeutic drugs cause hearing loss following damages to the external, middle and inner ear. Radiation can create acute and delayed skin reactions to the pinna, external auditory canal and periauricular regions. Acute events may include: erythema, dry and moist desquamation or in rare cases ulceration of the auricule skin and external ear causing pain. Any epithelial damage or destruction of the sebaceous and apocrine glands can lead to diminished wax secretion. Later reactions can include: atrophy, ulceration, external otitis (126). High dose external-beam radiation therapy alone does not predispose patients to external auditory canal stenosis but when combined with surgery to the area, treated patients are at higher risk for developing stenosis to the external ear (127).

Irradiation to the middle ear can cause acute middle ear side effects in up to 40% of patients. The most common is otitis media, which can develop into conductive deafness. This can be permanent (loss of up a 60 dB) (126, 128) or temporary. In a study with 58 carcinoma head and neck patients, 28% regained normal hearing within 6 months following radiotherapy (129).

As well as causing problems to the external and middle ear, radiation can also cause SNHL if it radiated the cochlea. The incidence rate following radiation ranges from 0 % to 50 % and retrospective reports have reported cases where 30 Gy to > 65 Gy were administered (130). Though lower frequency tones are better preserved, hearing loss can reach 80 dB at 4, 000 Hz in children (131). Therefore, even if radiation therapy is less ototoxic then platinum-based chemotherapy, it still causes major SNHL. This particular

issue is more prominent when radiation is combined with cisplatin (132-134). Although radiation is given before chemotherapeutic drugs, hearing loss may take time to develop making it a confounding variable when trying to assess platinum-induced hearing loss.

CHAPTER 3: Manuscript 1 - Long-Term Hearing Assessment of Platinum-Induced Ototoxicity in Pediatric Patients

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Stephanie Fay Lenhart presented this manuscript as a podium presentation at the **American Society of Pediatric Otolaryngology (ASPO)** Annual Conference in Austin Texas, US on May 18-21th 2017.

3.2 Abstract

Objectives: The aim of this study was to assess the long-term effect of platinum-induced ototoxicity in pediatric patients by evaluating the incidence of progression of ototoxicity after completion of treatment.

Methods: 98 participants treated with cisplatin or carboplatin were recruited from two pediatric hospitals (CHU Sainte-Justine and Montreal Children's Hospital). Hearing thresholds were examined at the following stages: pre-treatment, end of treatment, first follow-up (3-9 months), second follow-up (15-24 months), third follow-up (24-60 months), fourth follow-up (60-96 months) and final follow-up (96 or more months) after platinum-based chemotherapy. During each visit, audiograms assessed hearing for the frequency ranges of 250 to 16000 Hz. The Chang's and the American-Speech-Hearing-Association (ASHA) grading systems were used to assess Ototoxicity.

Results: Following the end of treatment, 58% (57/98) of children had hearing loss. In 14% of these cases, hearing loss progressed in the next 3-9 months. 2 more cases of ototoxicity appeared 18-24 months after treatment and 1 case after 7 years. Patients that received radiation to the head and neck region or cisplatin had higher rates of ototoxicity. Children under the age of 10 had higher rates of hearing loss. Gender and cumulative cisplatin doses were not linked to ototoxicity.

Conclusion: Platinum-induced ototoxicity following chemotherapy with cisplatin or carboplatin is permanent and can progress or appear long after the completion of treatment. Long-term follow-up is strongly recommended.

Keywords: Long-term, Oncology, Ototoxicity, Pediatric, Platinum

3.3 Introduction

Platinum-based chemotherapy agents such as cisplatin and carboplatin, are commonly used for the treatment of various cancers. These antineoplastic agents are known to be highly effective in the treatment of a variety of pediatric cancers such as head and neck, lung, bladder, ovarian, testicular cancers (2). However, there are adverse events associated with these drugs such hearing loss and renal damage. Unlike nephrotoxicity, that can be reduced using hyperhydration (135), there is currently no prevention for platinum-induced ototoxicity and no available acceptable alternative drugs. Cisplatin and carboplatin exert their ototoxic effects through apoptosis, triggered by reactive oxygen species and activation of an inflammatory response to the following areas of the cochlea: the spinal ganglial cells, the stria vascularis, and the outer hair cells in the basal turn creating the hearing loss (136, 137).

Platinum compounds cause bilateral irreversible hearing loss (138, 139) in 22% to 71% of pediatric patients treated for childhood cancer (9). This leads to devastating consequences on the quality of life of cancer survivors since this ototoxicity is prevalent in children under the age of five (89) who are relying on hearing to develop their speech, vocabulary, sentence structure and language comprehension. This hearing loss will also impact children's school performance, social development and overall quality of life (13, 140).

Though this hearing loss can often be identified following only one administration of chemotherapeutic platinum drug (141), ototoxicity has been seen to develop over 2 years after treatment (142). Studies have determined different lengths of time that hearing loss may occur following treatment therefore, this project focused on determining the long-

term effect in a cohort of patients in order to provide hospitals a timeframe for hearing assessments after treatment (10, 11, 143).

3.4 Methods

3.4.1 Recruitment and Participant Inclusion/Exclusion Criteria

A prospective cohort group (≤ 18ys) that received cisplatin and/or carboplatin treatment between 2000 and 2016 from the Montreal Children's Hospital and the CHU Sainte-Justine were recruited. Participants were excluded if they had any of the following: prior exposure to cisplatin and/or carboplatin, congenital hearing loss, tympanic perforation, prior head injuries resulting in hearing loss, or past auditory problems. All participants were required to have a valid baseline audiogram of their hearing to ascertain if any hearing loss was present prior to platinum treatment.

3.4.2 Assessment of Hearing Function

Audiological assessments were conducted to determine patients hearing thresholds at frequencies ranging from 250 to 1600 Hertz (Hz). Audiograms revealed hearing status before treatment, post-treatment, follow-up 1 (FU) (3-9 months), FU2 (15-24 months), FU3 (24-60 months), FU4 (60-92 months) and FU5 (92 or more months) after platinum-based chemotherapy. Ototoxicity was identified using two criteria: the Chang's grading system and the American Speech-Language-Hearing Association (ASHA) evaluation (73).

3.4.3 Statistical Analyses

Descriptive statistics were used to demonstrate demographics data. Quantitative variables were expressed in means, ranges and percentages. Proportions were analyzed using the Fisher Exact Test and the Pearson Chi-Square. An ANOVA was used to determine if drug cumulative dose was associated with hearing loss.

3.5 Results

3.5.1 Demographics

There were 203 participants who consented to the study; 34 before their treatment commenced and 169 who had begun or completed chemotherapy. Of these participants, 105 were excluded: 68 had no baseline audiogram, 12 did not have post hearing assessments, 11 passed away during treatment, 3 were over 18 yrs and 11 are still on treatment, therefore 98 patients where recruited. Demographic data are listed in table 2. Mean age at diagnosis was 8.2 yrs (range 0.3-18 years, N = 98). There was a higher prevalence of males (67.3%) compared to females. 66.3% of patients did not receive radiation therapy to the head and neck region. The cohort represented 15 types of cancers. The following where the most frequent diagnoses: medulloblastoma (20.4%), neuroblastoma (19.4%), germ cell tumor (17.3%) and osteosarcoma (13.3%). The survival rate one year after the end of treatment was 94%. The average cumulative dose of cisplatin was 412 ± 132 mg/m^2 (range 91-794 mg/m^2) and was the primary chemotherapy agent for 63% of patients. For carboplatin, the average cumulative dose 27223 ± 1770 mg/m^2 (range 765-7500 mg/m^2) was given to 25% of children. 11% of the participants received both

drugs. All participants received an audiometric hearing test prior and 1-2 months following platinum treatment (see Table 1).

	n	%
Characteristics	98	100
Sex		
Male	66	67.3
Female	32	32.7
Age at Diagnosis		
0-3 yrs	21	21.4
4-7 yrs	27	27.6
8-11 yrs	20	20.4
12+ yrs	30	30.6
Radiation		
None	65	66.3
Head and Neck	33	33.7
Present Status		
Alive	89	90.7
Deceased	9	9.3
Diagnosis		
Medulloblastoma	20	20.4
Neuroblastoma	19	19.4
Germ Cell	17	17.3
Osteosarcoma	13	13.3
Astrocytoma	5	5.1
Other	26	24.5

Table 1. Patient demographics with percentages demonstrating gender, age, radiation, diagnosis and ototoxic drugs.

3.5.2 Post-Treatment Hearing Loss

Ototoxicity was present in 58% of patients using the ASHA criteria. This hearing loss was predominant in 75% of patients who received radiation to the head and neck region (p = 0.017). A higher risk of developing hearing loss was associated with participants who

received cisplatin (73%) and a combination of both drugs (73%) then compared to only carboplatin (16%) Gender did not influence ototoxicity. No difference was found in comparing hearing loss in children under the age of three. However, children under the age of 10 had the higher rates of hearing loss determined using the Fisher Exact Test (p = 0.017) (see table 2).

Ototoxicity Post-Treatment With ASHA Criteria	No Hearing Loss		Hearing Loss		*P*
	n	%	n	%	
Total Participants	41	42	57	58	
Sex					**0.281***
Male	25	26	41	42	
Female	16	16	16	16	
Age at diagnosis					
≥ 36 months	29	30	48	49	**0.137***
< 36 months	12	12	9	9	
Radiation					**0.017***
None	33	35	32	21	
Head and Neck	8	8	25	26	
Drug					**0.00****
Cisplatin	17	17	45	46	
Carboplatin	21	21	4	4	
Both	3	3	8	8	

* Fisher Exact Test

**Pearson Chi-Square

Table 2. Hearing loss following treatment breakdown depending on gender, age, radiation and platinum drug.

3.5.3 Hearing Loss Due to Platinum Cumulative Dose

An ANOVA was used to analyze the incidence of hearing loss compared to their cumulative dose of cisplatin. 51% of patients received a dosage less than 400mg/m^2. Some

(37%) received between 400 to 500 mg/m^2 while a small group (12%) received over 500 mg/m^2 (see Table 3). Though 72% of patients received ototoxicity, the amount of cisplatin administered did not impact the participants hearing loss identified with the ASHA criteria (see table 4, $F(2)= 0.897$, $p = 0.412$).

	No Hearing Loss		**Ototoxicity**	
Cisplatin*	**n**	(%)	**n**	(%)
≤ 400	**11**	15	**27**	36
400 - 500	**6**	8	**22**	29
≥ 500	**4**	5	**5**	7

* Cumulative Dose of Cisplatin Expressed in mg/m^2

Table 3. Ototoxicity Depending on Cumulative Dose of Cisplatin using the ASHA Assessment Criteria

3.5.4 Long-Term Hearing Loss

Progression of hearing loss continued from 3 to 9 months, following treatment in 14% of children (see Table 4). Out of 73 participants that did not opt out during this time frame; 4 went from grade 0 to 1a (Chang grading), 2 from grade 1a to 2a, and 4 increased from grade 2a to 3. Two cases improved during that time frame (grade 1b to 0 and 1b to 1a). Hearing loss progression in 54/98 patients who completed both the first and second follow-up is depicted in table 5 using the Chang criteria. Out of these participants, 50% developed a hearing loss after treatment and the number increased to 53.7% after 15-24 months. Of those patients, hearing worsened going from grade 0 to 2a. ASHA criteria

demonstrated a hearing loss of 70% after treatment in all patients (N = 98) that remained consistent in all follow-ups. In the 13/98 that was followed for over eight years, one patient developed hearing loss after 7 years. No changes were observed in any audiograms obtained over 8 years after treatment in 2/98.

Time After Treatment	All Participants	No. Cases of Hearing Loss Progression	No. Cases of Hearing Improvements
Post [1-2 months]	98	57	–
FU1 [3-9 months]	73	10	2
FU2 [15-24 months]	54	2	0
FU3 2-4 yrs	26	0	0
FU4 5-7 yrs	13	1	0
FU5 8 yrs+	2	0	0

Table 4. Progressive Hearing Following Treatment using Chang Criteria

	Chang Grading System						
	0	**1a**	**1b**	**2a**	**2b**	**3**	**4**
Post	27(50%)	15(27.8%)	1(1.9%)	8(14.8%)	1(1.9%)	2 (3.7%)	0 (0%)
FU1 [3 ± 9 months]	25(46.3%)	16(29.6%)	0(0%)	8(14.8%)	1(1.9%)	4(7.4%)	0 (0%)
FU2 [15 ± 24 months]	23(42.6%)	16(29.6%)	0(0%)	10(18.5%)	1(1.9%)	4(7.4%)	0 (0%)

Table 5. Follow-up Evaluations of Ototoxicity After Chemotherapy (n =54)

3.6 Discussion

3.6.1 Impact of Age and Prior Radiation on Ototoxicity

In this study, 73 of the remaining participants (58%) showed a hearing loss following the use of platinum compounds. The identified deficit level and incidence is in accordance with prior studies (10, 144). It is known that exposure to radiotherapy to the head and neck area increases children's susceptibility to develop hearing loss. In our project, patients who received radiation to the head and neck area in combination to platinum based chemotherapy had a higher rate of hearing loss.

Due to the increase of head and neck cancer survivors, over the past 20 years an increased awareness regarding the ototoxic side effect of cranial radiation to the head and neck region has been noted (145). Since these pediatric and adult long term studies have documented this effect, it is recognized that radiation is linked to delayed sensorineural hearing loss (134, 146). An early study by Baranak et al. (1988) using a chinchilla model depicted that cranial radiation increased the susceptibility of hearing loss following the administration of cisplatin. These findings are similar to the ones that we found in our pediatric population (147).

The correlation between the age of pediatric patients receiving platinum-based chemotherapy and hearing loss is not yet delineated. Certain studies have demonstrated that the younger the patient the higher their risk is of developing this ototoxicity following exposure to platinum agents (148). While Fetoni et al (2016) did not report a link between age and platinum ototoxicity (149). Li et al. 2004 demonstrated that children under the age of 5 years are 21 times more likely to develop hearing loss (150). Liberman et al. (2016) found that patients over the age of 6 showed an increase of hearing loss compared to those

less than 6 years old (144). Bertolini et al. (2004) demonstrated that 25% of children (out of 120 children) diagnosed and treated with cisplatin before the age of 36 months had higher rates of hearing loss (151). Our results demonstrated that found that children ≤ 10 years instead of 36 months are at a higher rate of developing a hearing loss.

3.6.2 Chemotherapeutic Agents Dosage

In a study by Knight et al. (2005) bilateral hearing loss was seen in 61% of patients treated with cisplatin. (89) This rate was higher than children exposed to only carboplatin. In a study by Nitz et al. (2013), 6 out of 13 children (46%) receiving only carboplatin developed a hearing loss. (152) A study on 488 North American male germ cell tumor patients cumulative cisplatin dosage over 400 mg/m^2 was related to hearing loss at 4, 6, 8, 10 and 12kHz (153). The same results were seen in 59 pediatric patients of Castelan-Martinez et al. (2014) (154). Other studies have reported similar findings in smaller size group (56, 155).

3.6.3 Progression of Hearing Loss

Progression of hearing loss may occur early on in treatment. In these cases, treatment regimen changes can be made in regards to cisplatin and carboplatin dosages (10). Nonetheless, the time required for permanent hearing loss to present is not yet determined. Therefore, there is no clear consensus on audiology follow-up's. Studies have focused on the long-term effects of hearing loss using follow-up's ranging from 2-13yrs (see Table 6). However few have been able to follow the same group of patients throughout many years and assessed them at the same follow-up point.

Peleva et al. (2014) noted that 148 of 306 children (48%) had hearing loss post treatment and progressive hearing loss still developed after 5 yrs of hearing loss (10). Kushner et al. (2006) showed stabilization of hearing over 2 or more years (142). In a study by Yasui et al. (2014), 6 out of 55 children (11%) developed hearing loss ≥ 2 yrs post end of treatment (90). Patients in this study were entered into a prospective cohort at various times post platinum treatment. All patients had to have a normal baseline hearing and upon entering the cohort where followed prospectively with audiograms over a minimum period of 2 yrs. Most patients progressive hearing loss stabilized between 15-24 months post-platinum administration. However, as one patient developed hearing loss 7 yrs post-treatment, a long-term follow-up is important in all patients exposed to platinum.

3.7 Conclusion

Based on this cohort of documented normal hearing patients exposed to platinum chemotherapy followed prospectively with audiology monitoring over a minimum 2 yrs following, hearing loss seems to stabilize after 15-24 months of follow-up. Therefore, all children exposed to platinum chemotherapy should have auditory monitoring for a minimum of 2 yrs post-treatment. Unfortunately, this is currently not the standard of care in many pediatric hospitals where monitoring ends at the completion of chemotherapy. Also, patients should be warned to report any long-term hearing loss as one patient developed ototoxicity 7 yrs post chemotherapy.

Table 6. Long-Term studies on the Effects of Cisplatin and Carboplatin on Hearing Loss
ASHA = American Speech and Hearing Association, FU = Follow-up, HL = Hearing Loss, kg = Kilogram, NCI-CTCAE = National Cancer Institute Common Terminology Cri
Adverse Events, SIOP = The International Society of Pediatric Oncology

Author	Drug	Participants	Dose	Hearing Loss Assessment	Hearing Loss	Details Long-Term
Peleva et al. (2014)[10]	cisplatin carboplatin	out of n=466 children, n=306 were from 2000-2012	cumulative dose of cisplatin not sig for HL	audiograms evaluated with ASHA Chang Criteria	148 had HL post treatment	progression of HL continued in FU months category
Yasui et al. (2014) [90]	cisplatin carboplatin	n = 55 children between 1983 and 201	10/18 who received < 360mg/m2 cis had HL at a min dose of 200mg/m2	Brock criteria Chang criteria	35 (64%) had HL according to Brock	medium time onset after cisplatin days, 6 patients had hearing loss a 2yrs
Al-Khatib et al. (2010) [143]	cisplatin	out of n = 49 patients n=31 were included and from 2000-2005	minimum ototoxic dose 302.06mg/m2	high-frequency audiograms, ASHA	42% suffered otoxicity, 33% worsened in the long-term. Under 5yrs most vulnerable and RT associated with HL	n=21 were followed long-term per to 6.6yrs (median 3.4). and 4(required hearing aids
Geurtsen et al. (2016) [11]	carboplatin	n = 22 with retinoblastomas, *M* =11.9yrs at first administration	*M* = 2240 mg/m2 range = 900-5600 mg/m2	high-frequency audiometry Brock criteria, SIOP Boston scale	one child had bilateral low-grade high-frequency HL	Mean FU 12yrs with median 11.6yrs. No observed ef carbo and HL in young child
Qaddoumi et al. (2012) [12]	carboplatin viscristine	n=60 children with retinoblastoma	median cumulative dose carbo 3590mg/m2	audiometric tests using: NCI-CTCAE Broke criteria ASHA	20% had HL after treatment but it was resolved in 2 patients, age sig predictor of HL (younger at risk)	FU ranging from 3.5-13.3yrs m 6.1yrs
Jehanne et al. (2009) [137]	carboplatin	n=175 children with retinoblastoma, mean age 8 months	either 3days (200mg/m2/day) or 5days (160mg/m2/day)	pure-tone audiometry Broke criteria	ototoxicity detected in 8 children, two of which had bilateral high frequencies deterioration considered secondary to carbo	median FU was 5yrs (1.8-11
Bertolini et al. (2004) [151]	cisplatin carboplatin	n=120 mean age at diagnosis 2.6yrs	cumulative 400mg/m2 for cis and 1600mg/m2 for carbo	Broke criteria and hearing impairment defined as a change in hearing threshold	HL in 37% children treated with cis and 43% with cis and carbo	carbo at standard dose does not g risk and children with 400 mg/n should be followed LT FU ranged from 2-13yrs
Bergeron et al. (2005) [156]	carboplatin vincristine	n=30 children medium age 4,7 months at diagnosis of neuroblastoma	carbo (6.6mg/kg/day) viscristine (0.5mg/kg/day, days 1-5)	pure tone audiometry Broke criteria	1/30 had HL	In a 6yr follow-up carbo and VP1 seen to be safe for hearing

CHAPTER 4: Manuscript 2 –

The Role of Parents' Evaluation of Aural/Oral Performance of Children (PEACH) following the use of ototoxic agents

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4.2 Abstract

Introduction: Permanent hearing loss is a side effect of ototoxic chemotherapeutic drugs. Despite the profound impact of hearing impairment, to date, there has been no study of the impact of chemo radiotherapy induced hearing loss on the Quality of Life (QoL) of pediatric cancer survivors. It is therefore necessary to determine the impacts of chemo radiotherapy ototoxicity on the day to day functioning of children.

Methods: From 1998 to 2016 parents of children at the Montreal Children's Hospital and CHU Sainte-Justine Hospital receiving chemotherapy and/or radiotherapy completed the Evaluation of Aural/Oral Performance of Children (PEACH) questionnaire either before or after treatment. Their hearing status was determined using the Chang grading criteria.

Results: A total of 56 children were included; the mean age was 7.6 (range 4 month-18 years). The baseline group (before treatment) of 16 patients demonstrated that their total PEACH scores vary from to 86 to 100 and normal hearing. In the post treatment group, 22% of the 40 children had mild hearing loss based on the Chang and 33% suffered from severe hearing impairment following chemo radiotherapy. The total PEACH scores in the post treatment group ranging from 42 to 100. 75% of these children had a decreased PEACH scores compared to the baseline group.

Conclusion: This is the first chemo ototoxicity study using the PEACH questionnaire to assess hearing difficulties following cancer treatments. In the pilot study, we demonstrate that the modified PEACH questionnaire is easy to use by parents for relevant information with regard to hearing functions and is linked with a severity of hearing loss.

Keywords: Chemotherapy, Ototoxicity, PEACH Questionnaire, Platinum

4.3 Introduction

Chemotherapy and radiotherapy are widely used chemotherapeutic agents in the treatment of several pediatric malignancies. The platinum compounds Cisplatin and Carboplatin are two of the most successful and widely used chemotherapy drugs available. They are highly effective against a variety of childhood malignancies (151). However, despite their effectiveness, their use is limited by their ototoxicity. Hearing loss is often permanent following therapy with platinum chemotherapy and/or cranial radiation in pediatric malignancies. There is currently no prevention or treatment for ototoxicity, which maybe permanent and bilateral (2). Hearing impairment has been associated with significant morbidity and might have a detrimental effect on speech, language, and social development outcomes (89). Morbidity is greater when hearing loss remains undetected and/or untreated, particularly for the developing child. In addition, therapy-related ototoxicity can initially arise or progress years after completion of treatment (157).

All childhood cancer survivors should undergo yearly evaluation with appropriate risk-based screening for potential cancer-related complications. A complete audiological evaluation consisting of air conduction, bone conduction, speech audiometry, and tympanometry. Infants and survivors of any age who are difficult to test may require electrophysiological assessment such as auditory brainstem response measurement. Otoacoustic emissions provide objective information about outer hair cell function in patients treated with cisplatin. Patients who receive cranial irradiation are at risk for delayed onset hearing loss that may progress over a period of years, and thus need longer follow-up (157).

Although they are useful, these tests are presented in a quiet or soundproof room. These conditions do not reflect the subject's normal environment, and children who do well on these tests may still have difficulty locating sounds or hearing in noisy environments, such as the classroom or workplace. To measure how hearing abilities affect daily living, subjective assessment tests are preferred over other hearing tests. Furthermore, subjective monitoring of hearing is much more accessible using a questionnaire than performing a conventional audiology test.

Of the methods, which are currently available for the subjective assessment of hearing loss in children, most are designed for children of certain ages only (such as preschool or older children), or for children with severe hearing loss only. The PEACH questionnaire was developed to assess functional auditory performance in everyday life in children with hearing loss ranging from mild to profound (158). In our study, we would like to evaluate the correlation between the PEACH score and audiometry hearing levels in pre- and post-chemo-radiotherapy in the pediatric population over an extended period of time.

4.4.1. Methods

4.4.2 Subjects

The participants were primary caregivers of 56 children. An audiogram was conducted on all children prior to chemotherapy and/or radiotherapy. Families of children with prior hearing problems or who were unable to complete the PEACH scale due to age, language barriers, or cognitive impairments were excluded from the study. Chemotherapy treatment included platinum based drugs provided at the CHU Sainte-Justine (n = 46) or the Montreal Children's Hospital (n = 10) from 1998 to 2016. Over three-quarters of

participants (n = 40) completed their PEACH questionnaire up to 2 years following treatment, with the remainder (n = 16) filling the survey only prior to treatment.

4.4.3 PEACH Questionnaire

To include the Francophone population of Quebec, the PEACH questionnaires (see figure 7 in Appendix) were adapted from English into French to make both languages available to the participants. The translation took into account the original sentence structure, vocabulary and syntax to preserve the meaning of the test. Each child's caregiver was provided with a PEACH scale and children ≥ 14 years old were allowed to complete the questionnaire on their own. The questionnaire took 10-15 minutes to complete and consisted of 13 questions scored scale from 0 to 4. The first two questions, which relate to the child's use of hearing aids, were not relevant in our study group and where removed modifying the questionnaire.

4.4.4 Hearing Assessments

Before and after treatment, audiologists performed pure-tone audiometry tests at thresholds of 250-8,000 Hz. The Chang Grading Criteria, which evaluates severity of ototoxicity, was used to compare post-treatment audiograms with follow-up audiograms. Mild hearing loss was assessed as grade 1a-1b and substantial hearing loss as over ≥2a.

4.4.5 Statistical Analysis

Descriptive statistics were prepared across all variables, using means and standard deviations for continuous outcomes such as the PEACH scores, cisplatin dose and age, and

counts and percentages across all dichotomous and categorical variables. Cross tables were created to describe the numbers and percentages of subjects receiving each possible combination of cisplatin, carboplatin and radiation therapies. Averages doses of cisplatin, carboplatin and radiation were calculated with 95% confidence intervals. Similarly, mean PEACH scores with 95% confidence intervals were calculated for each of the Quiet, Noise, and Total subscores. Boxplots were created to visually compare the PEACH scores across the different Chang Grades. The rates of hearing loss following treatment as measured by the Chang Score were compared at baseline vs. follow-up. Logistic regression models were fit to estimate the effects of treatment by cisplatin, carboplatin and radiation on hearing loss, defined as a non-zero Chang Score. All logistic regression results are reported as odds ratios (ORs) with 95% confidence intervals. Similarly, linear regression models were fit to estimate the effects of treatment by cisplatin, carboplatin and radiation on the three PEACH scores. ROC curves were calculated to estimate the sensitivity and specificity of different cut-off values on the PEACH Scores in predicting hearing loss. These are reported graphically and with the corresponding area under the curve, which represents the overall association of the PEACH scores with hearing loss as graded by the Chang Criteria. All analyses were completed using R statistical software (version 3.3.2, available from cran.r-project.org/).

4.5 Results

Descriptive characteristics of the 56 pediatric patients are reported in Table 8 and overall characteristics in table 9. Close to two-thirds where males and the age at diagnosis ranged from infancy to late teens (the youngest being 3 months and the oldest 18 years).

The majority of children received only cisplatin (n = 33), while a small sample received both cisplatin and carboplatin (n = 10).

	N	(%)
Gender		
Female	17	30
Male	39	70
Drugs		
Cisplatin	33	59
Carboplatin	13	23
Both	10	18
Radiation		
Yes	19	34
No	37	66
Diagnosis		
Medulloblastoma	13	23
Germinal	9	16
Neuroblastoma	8	14
Osteosarcoma	8	14
Wilms Tumor	8	14
Retinoblastoma	4	7
Other	6	11

	Mean	Range
Radiation (Gy)	53.99	[30 - 59.4]
Age (Years)	7.63	[0.3 - 18]
Drugs (mg/m^2)		
Cisplatin	421.6	[91 - 930]
Carboplatin	2791	[914 - 6632]

Table 8. Demographics of the patients

Gender	Age (Years)	Treatment	Dose (mg/m2)	XRT to Head and Neck	XRT Dose (Gy)	Total PEACH score	Chang Criteria (Grade)	Time
M	17	Cisplatin	900			93.18	0	Baseline
M	13	Cisplatin	572	+	55.8	100	0	Baseline
M	2.5	Both	91, 1541			90	3	F/U
M	1.1	Carboplatin	1116			75	4	F/U
M	8	Cisplatin	490	+	54	100	0	F/U
F	16	Cisplatin	410			100	0	Baseline
M	14	Cisplatin	292			61.3	0	F/U
M	1	Both	476, 914			100	0	Baseline
M	8	Cisplatin	350	+	54	70	1a	F/U
F	3	Carboplatin	6562.5			77	1b	F/U
M	5.5	Carboplatin	5397			100	0	F/U
M	10	Both	394, 1050	+	55.8	88	1b	F/U
F	1.5	Both	600, 3130			72	1a	F/U
F	7	Both	400, 1500			84	3	F/U
M	3.3	Both	400, 1700			100	1a	F/U
F	2.67	Both	400, 1700			86	3	F/U
F	2.1	Carboplatin	3360			47	0	F/U
M	3	Both	300, 1200			100	3	F/U
M	8.6	Cisplatin	480			97	0	F/U
M	15	Cisplatin	480			61	0	F/U
F	8.5	Cisplatin	412.5	+	55.8	84	3	F/U
M	7.9	Cisplatin	375	+	54.5	86	0	Baseline
M	16	Cisplatin	480			97	1a	F/U
M	16	Carboplatin	1800	+	54	90	0	F/U
M	6.3	Carboplatin	6632.5			91	0	F/U
F	5.5	Cisplatin	225	+	55.8	81.81	3	F/U
M	3	Carboplatin	2500			100	0	Baseline
M	4.1	Cisplatin	450	+	54	75	1a	F/U
M	9	Cisplatin	200	+	59.4	61	1a	F/U
F	0.3	Cisplatin	420			100	1a	F/U
M	9	Both	400, 1700			77.27	3	F/U
M	5.5	Cisplatin	450	+	54	80	0	F/U
M	11.7	Cisplatin	480			100	0	F/U
F	16	Cisplatin	400			100	0	F/U
M	3	Cisplatin	500			88.64	2a	F/U
M	9	Cisplatin	450	+	55.8	100	0	F/U
F	4	Cisplatin	480			100	0	F/U

M	5	Cisplatin	400			59.09	2a	F/U
F	18	Cisplatin	300	+	55.8	86.36	2a	F/U
M	3	Both	700, 1700			100	3	F/U
M	7.6	Cisplatin	450	+	54	100	0	Baseline
M	2.6	Cisplatin	600			100	0	F/U
F	3	Cisplatin	930			36.36	2b	F/U
F	10	Carboplatin	2400			100	0	Baseline
F	3	Cisplatin	100	+	59.4	100	0	Baseline
M	5	Carboplatin	5906			93.18	0	F/U
M	2	Carboplatin	3360			81.81	0	F/U
M	5.5	Cisplatin	142	+	54	90.9	0	Baseline
M	10	Cisplatin	360			39	1a	F/U
M	16	Carboplatin	2250	+	55.8	95.45	0	F/U
M	6	Cisplatin	450	+	54	100	0	Baseline
M	5	Carboplatin	4375			88.63	0	Baseline
M	17	Cisplatin	240			100	0	Baseline
F	5.5	Cisplatin	400			90.9	0	Baseline
F	13	Cisplatin	300			88.63	0	Baseline
M	13	Carboplatin	2400	+	30	100	0	Baseline

Table 9. Characteristics of Patients

Both platinum drugs where associated with hearing loss ($OR_{cisplatin}$ = 49.50, 95% CI = 3.83 - 638.31, $OR_{carboplatin}$ = 15.75, 95% CI = 1.77 - 139.91) however, the population was to small to determine which of the two drugs lead to higher rates of hearing loss following treatment. In the post-treatment group, 42% did not develop hearing loss, 25% had mild hearing loss (1a, 1b) and 33% had severe hearing loss (grade 2a, 2b, 3, 4).

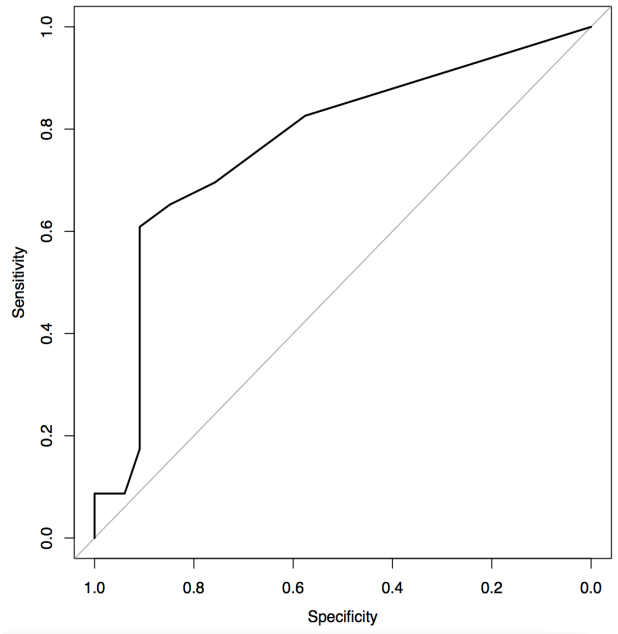
19 patients (33.9%) received cranial radiation (mean: 54.50 Gy, range: 30 - 59.40Gy) with platinum compounds. Out of the 19 participants who received radiation, 15 received only cisplatin, 3 where treated with only carboplatin and one was prescribed both drugs. Due to the small sample size, it was not possible to determine if patients with

radiation demonstrated higher levels of hearing loss ($OR_{\text{radiation present}}$ = 0.77, 95% CI = 0.24 - 2.39).

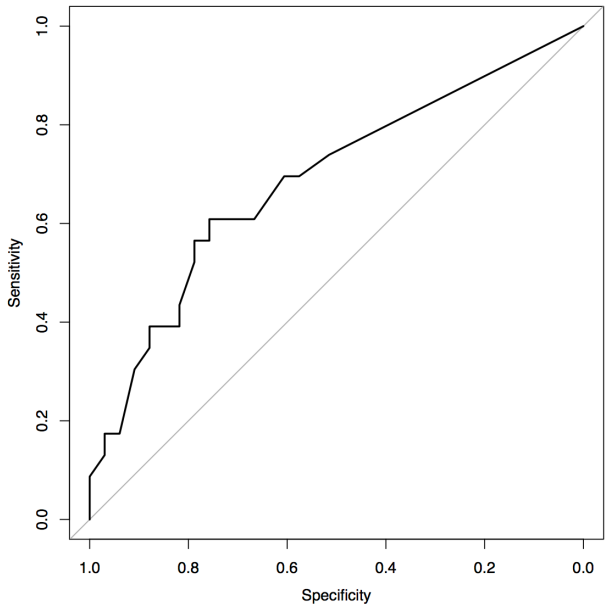
There are 16 children (11:5 males: females) with results of PEACH score and audiology tests before treatment. The baselines mean PEACH score was 96.1 (SD-5.3). All participants in the baseline group (n = 16) had no hearing loss prior to treatment (mean Chang score-0). There are 40 children (26:14 males: females) with results of PEACH score and audiology tests after treatment. The baselines mean PEACH score was 83.1 (SD-17.5). There were 10 children with Chang score of 1, 4 children with a score of 2, 8 children with a score of 3 and one child with a score of 4.

Total questionnaire results where high (Mean = 86.85, range = 36.36 - 100.00), due to increased scores for the quiet (M = 88.77, range = 42.00 - 100.00) and noisy settings (M = 84.64, range = 30.00 - 100.00). Patients with ototoxicity had lower PEACH questionnaire results (OR_{noise} = 0.95, 95% CI = 0.91 - 0.98, OR_{quiet} = 0.95, CI = 0.91 - 0.99 and OR_{total} = 0.94, CI = 0.90 - 0.98). Almost three-quarters of cases with hearing loss had lower quiet, noisy and total PEACH scores (Figure 9).

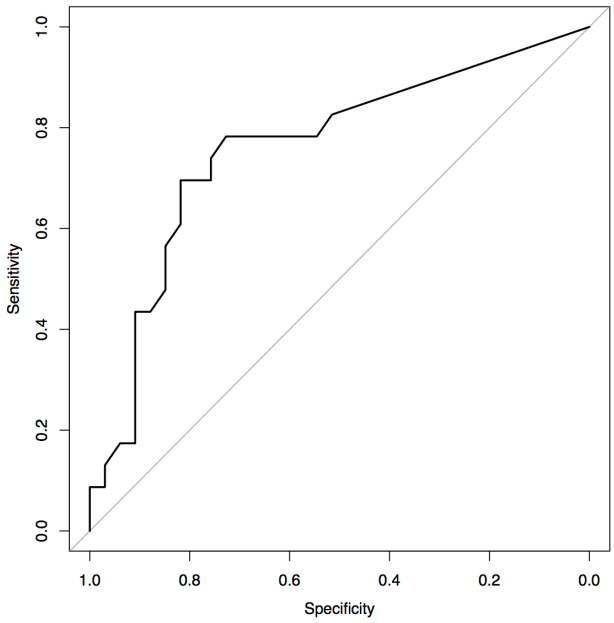
Probability of hearing loss based on the PEACH score received is depicted in Figure 10. The majority of patients with no hearing loss following treatment submitted questionnaires with sores of 100, however 2 patients had a Chang score of 1a and 2 with a Chang score of 3.



(A)

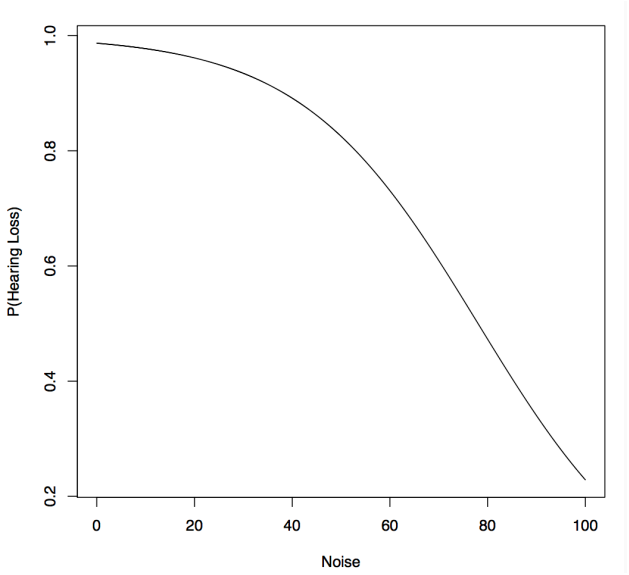


(B)

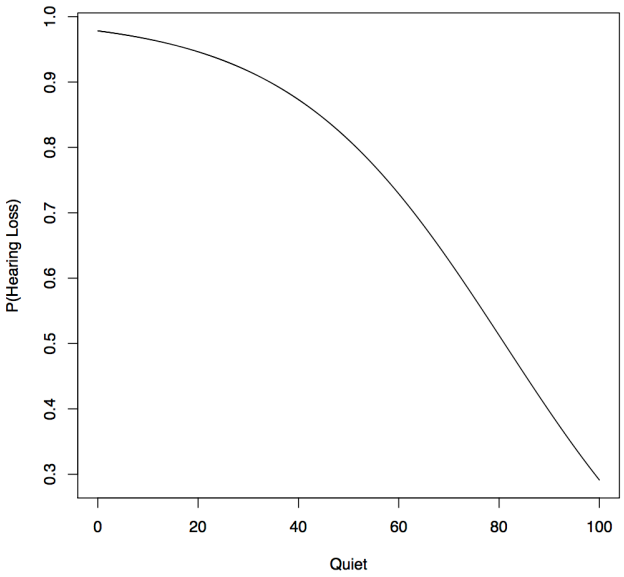


(C)

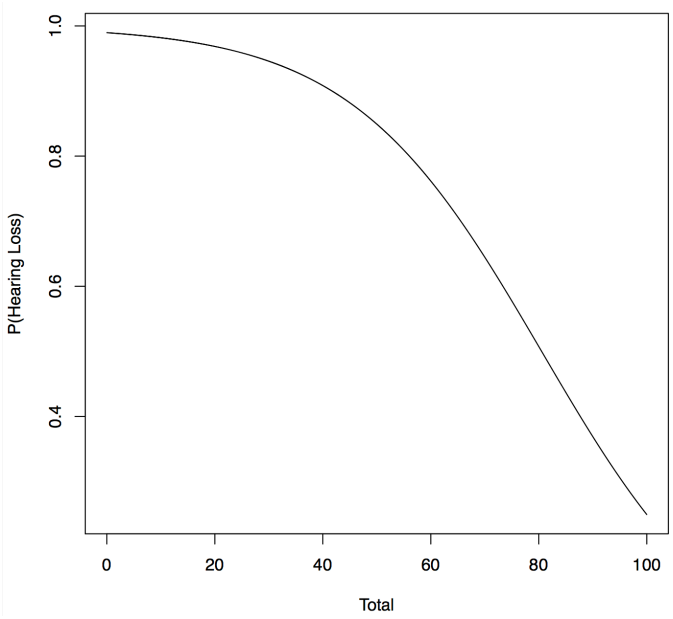
Figures 9. Area under the receiver operating characteristics curve's (ROC) of the Peach questionnaires settings: (A) Quiet (69%), (B) Noisy (77%) and (C) Total (76%).



(A)



(B)



(C)

Figures 10. Graphs demonstrating the probability of hearing loss depending on the PEACH results of (A) the quiet setting, (B) the noisy setting and (C) overall total.

There was only one patient diagnosed with grade 2b and one with grade 4 hearing loss. A wide variability within each Chang Grade score was noted however, an overall trend remained apparent, as confirmed by the linear regression analyses which show that as the severity of hearing loss increases, the PEACH scores decreased in both the noisy and quiet settings (Figure 11). The age of patients did not highly correlate with lower total PEACH scores (correlation = 0.10).

There were 10 patients on follow up tests post chemotherapy with normal hearing measured by conventional audiology tests but with lower PEACH scores (mean-79.8) than expected.

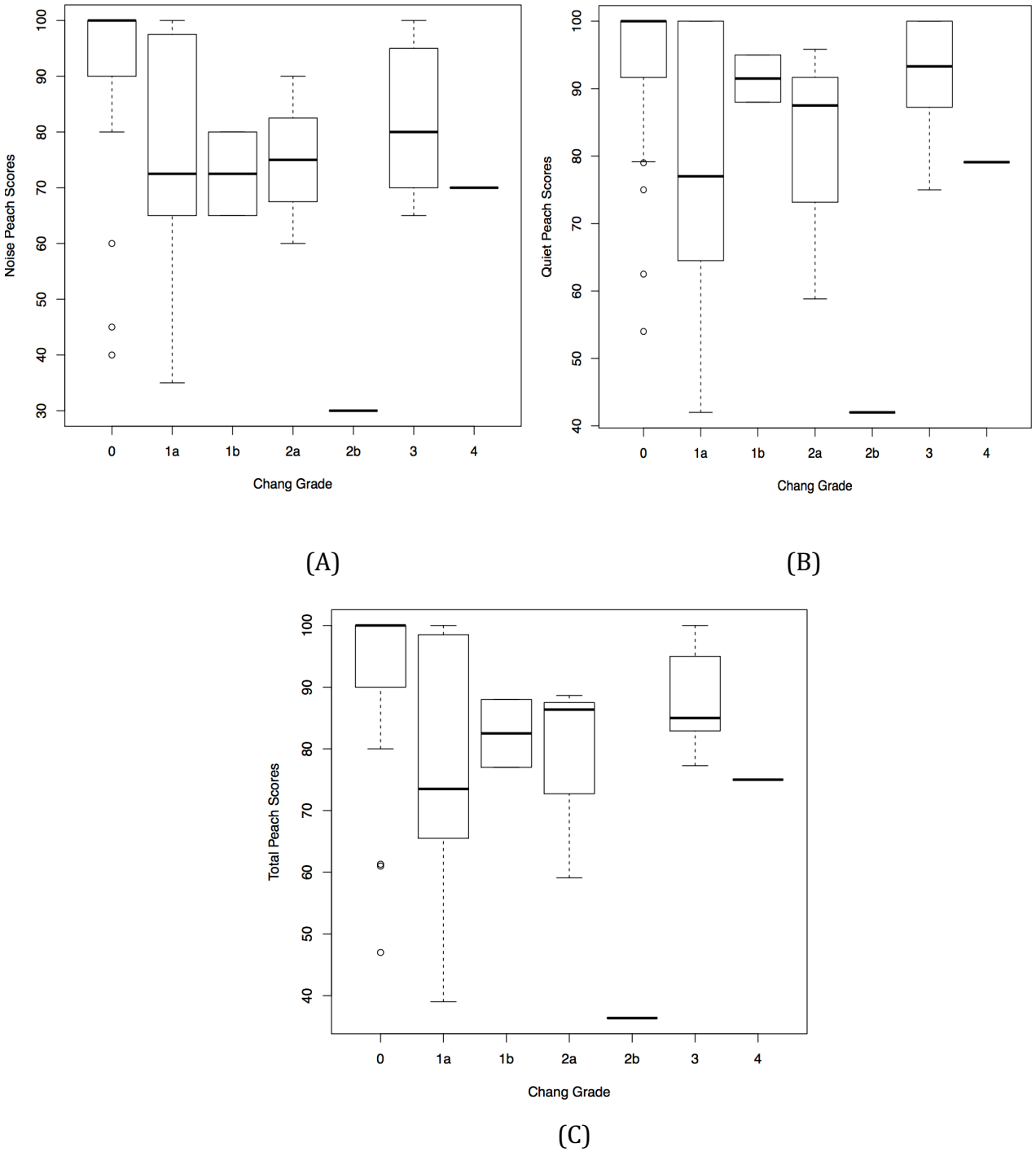


Figure 11. Box plots for PEACH scores depending on the severity of hearing loss as measured by the Chang Grading Criteria. Results combined for the (A) noisy setting, (B) the quiet setting and (C) combined settings.

4.6 Discussion

The present study was designed to compare the everyday functional language performance and audiology test of children who received ototoxic treatment. The performance was assessed using a PEACH questionnaire. The results reveal that children who received chemotherapy showed a correlation between hearing loss measured by conventional audiology tests and the PEACH score.

Cisplatin and carboplatin are crucial chemotherapeutic components in a variety of pediatric malignancies but are associated with ototoxicity leading to a poor quality of life, especially for children.

In our study the children were treated with a mean cumulative cisplatin dose of 421.6[91 - 930] mg/m^2. Increased hearing thresholds were detected in more then half of our patients (52.2%) treated only with cisplatin, which confirms the relationship between cisplatin therapy and ototoxicity. Stöhr al el in accordance with our study showed hearing loss of 51% patients treated with a median cumulative dose of 360 mg/m^2 (159).

Our average age of children with cisplatin induced ototoxicity was 7.5 years. It was found that hearing loss after cisplatin treatment was greater in patients aged < 12 years compared to older patients (159). Li al el. showed that children younger than 5 years were at a greater risk of sustaining cisplatin ototoxicity than children older than 15 years (150).

There is controversy regarding the effect of carboplatin on hearing loss. Many reports show no effect while others report carboplatin induced toxicity with incidence up to 54% (137). In our study only 2 children (15.4%) developed hearing loss after treated merely with carboplatin. One child was 3 years old and the other 1.1 years old. Patients with retinoblastoma who were treated with systemic carboplatin had a higher incidence of

ototoxicity when starting treatment before the age of 6 month (12). Although we have only two patients with hearing loss (Chang score 1B & 4) in the carboplatin group they are much younger then the mean cisplatin group.

All the children (100%) receiving a combination of cisplatin and carboplatin had hearing loss on follow up. Dean et al. reported a high incidence of hearing loss (70%) when receiving a combination of carboplatin and cisplatin. As in our study Carboplatin, when used without cisplatin, was rarely associated with severe hearing loss (160).

The long time effect of chemo radiation has been shown by few studies. A study by Al-Khatib at el. from our hospital revealed that there is a need for a long follow up post platinum chemotherapy due to the fact that ototoxicity can present or worsen years after completion of therapy (143). Deterioration of high frequencies was found in a quarter of the examined ears in the period following completion of chemotherapy with cisplatin. Hearing loss was documented up to three years post treatment (161). Progression of hearing loss has been shown up to 136 months after termination of chemotherapy and worsening of hearing was not only evident in patients who sustained ototoxicity during treatment, it was also seen in patients who had normal audiometry at the end of chemotherapy (151). These prolonged follow ups may need not only conventional hearing tests, but perhaps also questionaries' which are more accessible.

19 children received radiation therapy to the head and neck but only 12 had a follow up hearing evaluation. Radiation causing hearing loss was found in 7 children which received also cisplatin. The probability of ototoxicity is higher when combining radiation and chemotherapy. The dose of cisplatin when combined with cranial radiation has an

increased effect on hearing loss (162). Kortmann et al. reported lower ototoxicity when receiving neoadjuvant chemotherapy before radiation compared to post radiation (163).

Prior research studies have been able to identify children's auditory abilities and quality of life though parental assessment by using the PEACH questionnaire (158). PEACH was originally designed for children with hearing loss ranging from a mild to a profound (158). Two examples of questions are does your child respond to his/her name in a quite situation and does your child follow simple instructions or do a simple task in a noisy situation (see attached). Parents and their children convey in the routine environment and therefore their reports are often considered more reliable and representative of the child's behavioural response than assessments conducted in structured settings (164,165).

Since platinum-induced hearing loss impact children's quality of life using the PEACH questionnaire would make sense as an early diagnostic tool in chemotherapeutic patients treated with platinum drugs.

In our study there seems to be a correlation between conventional hearing test and the PEACH score at the extremities. There is a high probability of having a low PEACH score when the child has profound hearing loss is high. Conversely, the probability of having a high PEACH score when the child has normal hearing is also high. Children with mild-moderate hearing loss and an average PEACH score, however, showed little correlation. The validity of questionnaire from the Swiss Childhood Cancer Survivor study reported hearing in childhood cancer survivors was good. Among those who reported hearing loss, it was confirmed in 80%, and among those who reported normal hearing, it was confirmed in 87% (166). When giving the questionnaire to caregivers before chemo radiation treatment (baseline) the audiology test revealed normal hearing and the PEACH score was very high

(mean-97.0). Weiss et al. showed normal hearing was correctly assessed in 92% of those with normal hearing when using a questionnaire (166).

One interesting finding in our study is normal hearing in the audiology test but a relative low peach score (mean-87.1). Does the lower PEACH score in a normal environment enable us to find difficulties in hearing, which are not revealed by the conventional hearing test? Validation of the benefits of amplification for children with hearing impairment, particularly for speech perception, should be examined in the clinical setting as well as in the child's typical listening environments (167). Our results show that examining a child treated with ototoxic agents should be assessed in the clinical setting as well as in the child's environments for us not to miss out on a new clinical or environmental hearing loss.

Limitations of this study include its retrospective nature and possible selection bias. Our baseline and follow up groups are two different groups of children, which may create a selection bias.

Future studies should examine if low PEACH scores can predict future audiology hearing loss and if this may be used as a screening tool. The option of a questionnaire, which is accessible to the parents, might enable faster diagnoses of hearing loss.

4.7 Conclusions

Pediatric cancer patients may develop ototoxicity from platinum compounds and/or radiation and are at risk of sensorineural hearing loss. These children require careful monitoring over a long period of time. Screening of survivors at risk for hearing loss should be applied.

Parental questionnaires are very useful instruments for physicians to obtain meaningful information regarding children's auditory performance in real life. Our study indicates that the PEACH score might be good as a complementary screening tool in children receiving chemo radiation with an ototoxic drug. It would therefore be helpful to integrate the questionnaire in auditory assessments of hearing loss.

CHAPTER 5: Conclusion

5.1 Summary of Findings

Chemotherapeutic treatment including platinum agents are linked to higher success rates however, over half of pediatric patients will suffer from hearing loss as a secondary effect. This hearing loss is bilateral, irreversible and in certain situations progressive. Such auditory decrease leads to lower quality of life since children's ability to communicate and acquire language shall be impaired. As well, hearing loss paces a socio-economic burden on families of impaired children

The first study in Chapter 3 focused on assessing the auditory functions of a cohort of cancer patients throughout and after their platinum treatments. The outcomes revealed that over half of patients suffered from hearing loss following drug administration. Hearing loss continued to decline in less than one fifth of patients throughout their follow up assessments. Apart from three cases, progression of hearing loss occurred 3-9 months after treatment and no major hearing loss manifested itself 2 years post platinum treatment.

The second manuscript in Chapter 4 discussed the possibility that PEACH questionnaire may help identify platinum-induced hearing loss. It was demonstrated that PEACH overall scores correlated negatively with patients diagnosis of hearing loss, thus making it an interesting tool to integrate in the present detection, treatment and follow up of hearing loss such as pure tone audiograms and distorted production otoacoustic emissions tests.

5.2 Future Directions

The McGill Auditory Laboratory has been studying the effects of INTA tympanic dexamethasone. As an anti-inflammatory agent to prevent platinum-induced ototoxicity. However, until this treatment can move its efficiency and be integrated into clinics it is important for children hearing to be monitored after chemotherapy. Such monitoring should be conducted using all disposable methods from the PEACH modified questionnaire to audiograms.

CHAPTER 6: Appendix - PEACH Questionnaire

Question	Never 0%	Seldom 1-25%	Sometimes 26-50%	Often 51-75%	Always 75-100%
1. How often has your child worn his/her hearing aids and/or cochlear implant? ☐ Child does not have aids/implant	0	1	2	3	4
2. How often has your child complained or been upset by ***loud*** sounds? ☐ Child does not have aids/implant	0	1	2	3	4
3. When you call, does your child respond to his/her name in a ***quiet*** situation?	0	1	2	3	4
4. When asked, does your child follow simple instructions or do a simple task in a ***quiet*** situation?	0	1	2	3	4
5. When you call does your child respond to his/her name in a ***noisy*** situation when he/she can't see your face? (examples of responses include looks up, turns, answers verbally)	0	1	2	3	4
6. When asked, does your child follow simple instructions or do a simple task in a ***noisy*** situation?	0	1	2	3	4
7. When you are in a ***quiet*** place reading with your child, how often does he/she pay close attention to what you are saying? OR if your child is listening to stories/songs on the TV or CD when there is no other background noise how often can he/she follow what is being said?	0	1	2	3	4
8. How often does your child initiate/ participate in conversation in a ***quiet*** situation?	0	1	2	3	4
9. How often does your child initiate/ participate in conversation in a ***noisy*** situation?	0	1	2	3	4
10. How often does your child understand what you say in the car/bus/train?	0	1	2	3	4
11. How often does your child recognise peoples' voices without seeing who was talking?	0	1	2	3	4
12. How often does your child successfully use a phone?	0	1	2	3	4
13. How often does your child respond to sounds other than voices?	0	1	2	3	4

		Raw score		% Score
QUIET	(Q3+4+7+8+11+12) A		(A/24) x100	
NOISE	(Q5+6+9+10+13) B		(B/20) x100	
OVERALL	(A+B) C		(C/44) x100	

Table 7. Complete PEACH questionnaire. This manuscript modified this questionnaire by removing the first two questions from the overall calculation.

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