INCIDENCE AND RISK OF COCHLEOTOXICITY AND VESTIBULOTOXICITY IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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ABSTRACT

Incidence and risk of cochleotoxicity and vestibulotoxicity in patients with haematological malignancies

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The risk of ototoxicity accompanying therapeutic use of aminoglycosides is clinically well recognised. However, detailed description of the incidence, degree, severity and specific patient group risk is usually poorly documented. The aim of this project was to establish the incidence and risk of ototoxicity in patients with haematological malignancies recruited during follow up within the Haematology Department at Leicester Royal Infirmary. A total of fifty patients treated for haematological malignancies were recruited; 33 who had received aminoglycoside therapy as part of their treatment and 17 who had not. Following a comprehensive review of medical history for other potential causes of hearing loss or balance disturbance, patients were then tested with: standard pure tone audiometry (PTA); high frequency pure tone audiometry (HFPTA); distortion product otoacoustic emissions (DPOAEs); computerised dynamic posturography (CDP).

There was a considerable incidence of noise exposure in both treatment groups. After accounting for this, the aminoglycoside treated group PTAs returned an incidence of cochleotoxicity of 4/33 or 12%. With HFPTA, there was evidence of hearing loss in the high frequency range for both the non aminoglycoside and the aminoglycoside treated groups. The loss was more marked for the aminoglycoside treated patients.

The DPOAE results were interpreted alongside the PTA results and revealed evidence of mixed sites of damage at the cochlear outer hair cells and at a secondary site at the cochlear inner hair cells and/or cochlear nerve. The CDP results returned unexpected and highly significant evidence of mixed contribution to postural control performance deficit in the visuo-vestibular system with equivalent incidence in both treatment groups.

None of the above findings showed any correlation with the comparatively low exposures to aminoglycosides or to any of the other drugs these patients may have received. The PTA and DPOAE findings are novel providing physiological evidence of involvement at the inner hair cells/cochlear nerve. The evidence from the HFPTA and CDP studies is considered to reflect potential non specific broad toxic effects due to disease/other therapeutics effects manifest in the cochlea and vestibular apparatus.

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I also dedicate this thesis to my son and to my parents who have been very supportive since the early days of my medical schooling.

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CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Patients with haematological malignancy often develop neutropenia as presenting feature (Mc Kenna, 2000). It is also secondary to chemotherapy for the treatment of those diseases. The use of aminoglycosides has been almost universal in the treatment of neutropenic sepsis in these patients with haematological malignancy.

The risk of ototoxicity accompanying therapeutic use of aminoglycosides is clinically well recognised (Forge and Schacht, 2000). However, detailed description of the incidence, degree, severity and specific patient group risk is generally poorly documented. There have been reports of aminoglycoside ototoxicity in neutropenic patients that only gave binary outcome measures of cochleotoxicity in a group of 28 patients, with no accompanying assessment of vestibulotoxicity (El-Bakry *et al*., 1998).

Little or no attention is usually given to hearing loss and/or balance disorder in the haematological malignancy patient group. This may be explained by the fact that at least some of those patients are critically ill at presentation. Their treatment is therefore focused on short term survival, sometimes at the expense of their long term quality of life. With the development of new chemotherapy regimens, however, life expectancy has increased for many

patient groups. It is therefore justified, if possible, to address the problem of hearing loss and unsteadiness and try to improve the patients' symptoms, firstly by trying to avoid giving them potential ototoxic drugs if there is an alternative, and secondly to refer them to a hearing and balance specialist should a problem develop.

Another important factor in this specific patient group is the prior and sometimes concurrent use of other highly potent drugs, together with the aminoglycosides. The use of these includes antineoplastic agents that have a well recognised risk of systemic toxicity. These include: chemotherapeutic agents that act by alkylation of DNA such as cyclophosphamide; antimetabolites such as methotrexate and cytarabine; vinca alkaloids and anthracycline antibiotics, all of which are used as cytotoxic agents. None of those agents is known to cause ototoxicity. Along with the aminoglycosides the structurally unrelated antibiotics vancomycin and erythromycin are also used to treat or prevent infection. Patients with haematological malignancy may receive between 0 and 8 courses of aminoglycoside antibiotics in their neutropenic state.

Along with the potential for systemic toxicity some of these drugs, either alone or in combination with aminoglycosides have been reported to be associated with a risk of ototoxicity. This documented ototoxicity consists of isolated case reports with no real scientific background (Brummet *et al*, 1990; Hughes *et al*, 1984; Lugassy and Shapira, 1990). The vinca-alkaloids are known to be neurotoxic and could therefore affect any nerve in the body. Consequently, an attempt was made to check if these drugs alone or in conjunction with any of the others carried a potential ototoxic risk. In the present study an attempt

was made to establish in detail in a group of fifty patients, the incidence, degree, and specific severity of both cochleo and vestibulotoxicity. This was carried out by measuring; standard pure tone audiometry; high frequency pure tone audiometry; distortion product otoacoustic emissions and computerised dynamic posturography. The above battery of tests was complemented by thorough clinical examination with full inspection of patients' notes and questionnaire (Appendix). The subsequent parts of this chapter first deal with an overview of the pathologies involved in this patient group. A summary of the anatomy and physiology of the inner ear is then given. This is followed by a consideration of the pharmacology of aminoglycosides antibiotics and the other drugs that the patients with haematological malignancy may receive. This is followed by a consideration of the pharmacology of aminoglycosides antibiotics and the other drugs that the patients with haematological malignancy may receive.

1.2 MALIGNANCIES

1. Acute myeloid leukaemia (AML)

AML is a rapidly progressing leukaemia, which causes the bone marrow to produce too many of a certain type of white blood cell. The excess production then suppresses the body's production of normal white blood cells, red blood cells and platelets. This in turn can lead to the patient becoming prone to infection, anaemia, bleeding and bruising (Harrison's Principles of Internal Medicine, 16th edition).

Treatment includes chemotherapy (daunorubicin, cytosine arabinoside, retinoic acid and other drugs such as etoposide or amsacrine) and bone marrow transplant.

Prognosis: The chances of initial remission are between 50 and 80%.

2. Acute Promyelocytic Myeloid Leukaemia (APML)

APML constitutes about 10 percent of the cases of AML. In APML, there is an abnormal accumulation of immature granulocytes called promyelocytes. The disease is characterized by a chromosomal translocation involving the retinoic acid receptor alpha (RARα or RARA) gene and is unique from other forms of AML in its responsiveness to trans retinoic acid (ATRA) therapy.

Epidemiology: The median age is approximately 40 years, which is considerably younger than the other subtypes of AML (70 years).

Treatment: APML is sensitive to all-trans retinoic acid receptors (ATRA), a derivative of vitamin A. ATRA is typically combined with anthracycline based chemotherapy resulting in a clinical remission in approximately 90% of patients.

3. Chronic Myeloid leukaemia (CML)

CML is a form of leukaemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophiles and basophils) and their precursors is the main finding. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome (Faderl *et al,* 1999).

Epidemiology

CML occurs in all age groups, but most commonly in the middle-aged and elderly. Its annual incidence is 1–2 per 100,000 people, and slightly more men than women are affected. CML represents about 15–20% of all cases of adult leukemia in Western population (Faderl *et al*, 1999).

Treatment

The chronic phase is treated with inhibitors of tyrosine kinase (imatinib). The blast crisis can be treated by a bone marrow transplant after high dose chemotherapy.

Prognosis

A follow-up on patients using imatinib published in the New England Journal of Medicine shows an overall survival rate of 89% after five years (Druker *et al*, 2006).

4. Acute Lymphoblastic Leukaemia

This is a cancer of the white cells where immature white blood cells continuously multiply and are overproduced in the bone marrow. ALL causes damage and death by crowding out normal cells in the bone marrow, and by spreading to other organs (Harrison's Principles of Internal Medicine, 16th edition).

Epidemiology: ALL is most common in childhood and young adulthood with a peak incidence at 4-5 years of age, and another peak in old age. ALL is slightly more common in males than females.

Treatment. This includes chemotherapy, radiotherapy, bone marrow transplantation and stem cell transplantation.

Chemotherapy

-For induction of remission: Combination of prednisolone or dexamethasone (in children), vincristine, asparaginase and daunorubicin (adult ALL).

-For intensification: vincristine, cyclophosphomide, cytarabine, daunorubicin, etoposide, thioguanine or mercaptopurines given in different combinations. Since ALL cells sometimes penetrate the Central Nervous System (CNS), most protocols include intrathecal chemotherapy. Intrathecal methotrexate or cytarabine is usually used for this purpose.

-For maintenance therapy: oral mercaptopurine, oral methotrexate, intravenous vincristine and oral corticosteroids are usually used. The length of maintenance therapy is 3 years for boys, 2 years for girls and adults. Central nervous system relapse is treated with intrathecal administration of hydrocortisone, methotrexate, and cytarabine (Hoffbrand, 2006).

Prognosis: The overall cure rate in children is 85%, and about 50% of adults have long-term disease-free survival.

5. Non-Hodgkin's lymphoma (NHL)

NHL is a malignancy originating in the lymphatic system. Tumours develop from lymphocytes.

Epidemiology: NHL is more than five times as common as Hodgkin's disease.

Treatments and drugs

Chemotherapy, radiotherapy, stem cell transplantation, b*iotherapy (r*ituximab is a type of monoclonal antibody that helps the immune system to target and destroy cancer cells. It is frequently used in combination with chemotherapy) and r*adioimmunotherapy*

6. Hodgkin's lymphoma

Hodgkin's lymphoma, also known as Hodgkin's disease (HD), is a type of lymphoma characterized clinically by the spread of disease from one group of lymph nodes to another and by the development of systemic symptoms with advanced disease.

Epidemiology: HD has a bimodal incidence curve; it occurs most frequently in two separate age groups, the first being young adulthood (age 15–35) and the second being in those over 55 years old. Overall, it is more common in males, except for the nodular sclerosis subtype, which is more common in females. The annual incidence of Hodgkin's lymphoma is about one in 25,000 people, and the disease accounts for slightly less than 1% of all cancers worldwide. The incidence of HD is increased in patients with HIV infection.

Treatment

-Chemotherapy: currently, the ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) chemotherapy regimen is the gold standard for treatment of Hodgkin's disease. Developed in Italy in the 1970s, the ABVD treatment typically takes between six and eight months, although longer treatments may be required.

-Radiotherapy

Prognosis: the survival rate is generally around 90% when the disease is detected relatively early (Edelson, 2007), making it one of the more curable forms of cancer. More recent trials are showing much higher five-year survival rates than have previously been seen, up to 98% for patients in earlier stages. Later-stage cancers, though, continue to show a significantly worse prognosis.

7*. Multiple myeloma*

This is a cancer of plasma cells. These are the immune system cells in the bone marrow that produce antibodies.

Treatment

If the disease is completely asymptomatic (no end-organ damage), treatment may be deferred.

-Biphosphonates are usually administered to prevent fractures

-Erythropoietin is usually administered to prevent anaemia

Initial treatment of multiple myeloma depends on the patient's age and comorbidities. In recent years, high-dose chemotherapy with hematopoietic stem-cell transplantation has become the preferred treatment for patients under the age of 65. Prior to stem-cell transplantation, these patients receive an initial course of induction chemotherapy (such as thalidomide-dexamethasone, bortezomib, lenalomide-dexamethasone) (Kyle and Rajkumar, 2008). Patients over age 65 and patients with significant concurrent illness often cannot tolerate stem cell transplantation. For these patients, the standard of care has been chemotherapy with melphalan and

prednisone. Recent studies among this population (Durie, 2008) suggest improved outcomes with new chemotherapy regimens. Treatment with bortezomib, melphalan and prednisone had an estimated overall survival of 83% at 30 months, lenalidomide plus low-dose dexamethasone an 82% survival at 2 years and melphalan, prednisone and lenalidomide had a 90% survival at 2 years.

Prognosis

The International Staging System can help to predict survival, with a median survival of 62 months for stage 1 disease, 45 months for stage 2 disease, and 29 months for stage 3 disease (Greipp et al, 2005).

A summary of the haematological conditions of patients taking part in the current study is shown in table 1.1.

Table 1.1. Summary of the haematological conditions of the patients who took part in the present study.

Condition	**Age**	**Possible hyper viscosity**	**Chemotherapy at time of study**	**IT therapy**	**possible neutropenia**	**BMT**	**Survival**
ALL	Childhood adulthood	-	Vincristine,anthracyclines, steroids,asparaginase, cytarabine methotrexate, and mercaptopurine	+	+	+	At 5 yrs 80% child 18% adult
AML and APML	>40	+	Cytarabine anthracycline	-	+	+	At 5 years 9%adult
CML	Adult	+	bisulfan cyclophosphamide IFN alpha, hydroxycarbamide	-		+	At 5 years 50%
HD	15-40 and >55	-	doxorubicin, bleomycin vinblastine, dacarbazine			+	
NHL	All	-	cyclophosphamide, doxorubicin, vincristine prednisone	+	+	+	At 5 years 80%child 52%adult
MM	>50	+	bortezomib, melphalan , lenalidomide, prednisolone vincristine, anthracyclines	-		+	80% at 2 years

1.3. ANATOMOPHYSIOLOGY OF THE INNER EAR

A general description of the main anatomical and physiological features of the cochlea and vestibular apparatus up to the transduction along the eighth nerve is now given. A comprehensive treatment of these areas is outside the immediate scope of this thesis and is limited to what is necessary to understand the results. The reader is referred to the many excellent texts that cover these areas from which the overview given below was drawn.

1.3.1. General cochlear anatomy

The cochlea is embedded deep in the temporal bone. It is shaped like a snail shell. It stands about 1 cm wide and 5mm from base to apex. It contains a coiled basilar membrane about 35mm long. There are 3 compartments within the cochlea: two of these, the scala vestibuli and the scala tympani are associated with the oval and round windows. The third compartment is the cochlear duct. The scalae spiral together along the length of the cochlea. The osseous spiral lamina divides the scala vestibuli from the scala tympani on the side near the modiolus. The scala media is separated from the scala vestibuli above by Reissner's membrane and from the scala tympani below by the basilar membrane. The 2 outer scalae, the scala vestibuli and the scala tympani are joined at the apex of the cochlea by an opening known as the helicotrema. The vibrations of the stapes are transmitted to the oval window, a membranous window opening onto the scala vestibuli.

Fluid in the cochlea is displaced to a second window, the round window, opening onto the scala tympani. The flow causes a wave-like displacement of the basilar membrane and the structures attached to it. It is this that is responsible for the stimulation of the hair cells, and the first stage of the

analysis of the incoming sound is performed by the spatial distribution of the resulting displacements.

The organ of Corti on the basilar membrane constitutes the auditory transducer and it is here that the nerve supply ends. The nerve supply and blood vessels of the cochlea enter the organ of Corti by way of the central gravity of the cochlea, the modiolus, the spiral structure of the cochlea imparting a corresponding twist to the nerve and blood vessels during development

1.3.2. The Organ of Corti

The highly specialised structure of the organ of Corti contains the hair cells, which are the receptor cells, together with their nerve endings and their supporting cells. The hair cells consist of one row of inner hair cells on the modiolar side of the organ of Corti, and between three and, towards the apex, five rows of outer hair cells. The organ of Corti is given rigidity by an arch of rods or pillar cells along its length, the upper ends of the rods ending in the reticular lamina which forms the true chemical division between the ions in the fluids of the scala media and those of the scala tympani. Supporting cells are involved in the ionic microenvironment and reuptake of neurotransmitter (Santi, 1988). It is shown in figure 1.1.

Figure 1.1

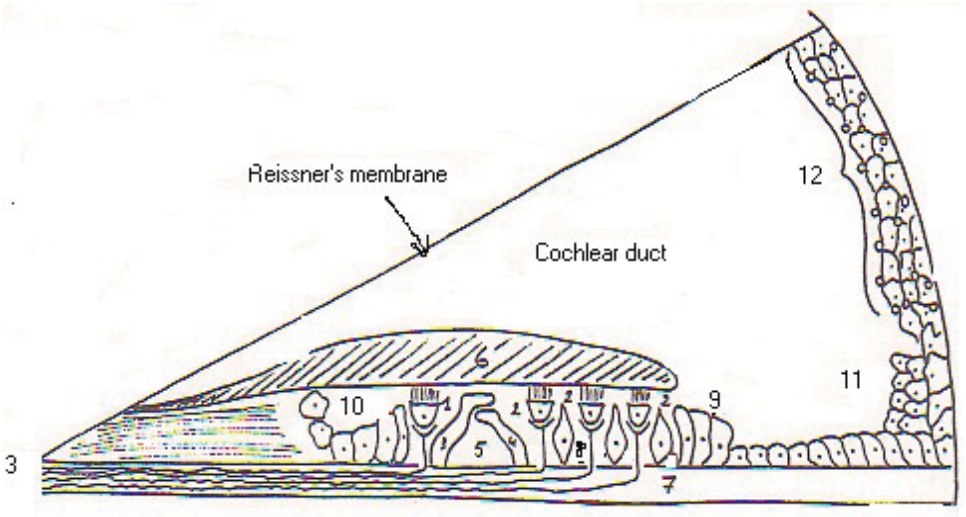


Figure 1.1. Organ of Corti. Adapted from " Anatomophysiologie de l'oreille et des fosses nasales". Professeur Van den Eeckhaut. Centre d'impression benevole. Cercle medical St Luc. Universite Catholique de Louvain, 1990-1991

1. Inner hair cell
2. Outer hair cell
3. Inner pillar
4. Outer pillar
5. Tunnel of Corti
6. Tectorial membrane
7. Basilar membrane
8. Supporting cells (Deiter's cells)
9. Hensen's cells
10. Subtectorial space
11. Spiral prominence
12. Stria vascularis
13. Afferent and efferent nerve fibres

1.3.3. Inner Hair Cell (IHCs)

An inner hair cell is flask-shaped, 35 micrometers in length, about 10 micrometers in diameter at its widest point. It contains a central nucleus surrounded by numerous mitochondria (Furness *et al.,* 1990). The nerve endings are situated near the base of the cell. These terminals are associated

with the afferent fibers of the auditory nerve, conveying information from the cochlea to the brainstem. The primary neurotransmitter at the IHC synapse is glutamate (Pujol, 1994).

1.3.4. Outer Hair Cell (OHCs)

In contrast with the IHCs, OHCs have a poor afferent innervation but possess a large and well-developed efferent supply. They comprise densely packed interconnected actin filaments arranged in a bundle, some of which go down into the cell body and are anchored in the cuticular plate (Hirokawa and Tilney, 1982, Neugbauer and Thurm, 1984). These bundles are responsible for transduction. Displacement of these hair cell bundles gates cation-conducting transduction channels, which are located near the stereociliary tips.

1.3.5. The innervation of the organ of Corti

The sensory innervation of the cochlea consists of an afferent and efferent supply (Morrison, 1975). The efferent supply divides into two components: the lateral and medial efferents (Warr, 1978). One efferent fibre supplies many afferents in any given region (Spoendlin, 1978). The efferent fibres also act to reduce the activity of the afferent fibres, and may consequently have a role in modulating against excessive metabolic activity in these fibres (Comis, 1970, Ruel *et al*., 2001). Some medial efferents innervate the OHCs and again, significant branching occurs with one efferent supplying up to 25 OHCs. The physiological role of the efferents is to decrease sensitivity of the OHCs to presumably provide some minor degree of protection against noise damage (Wiederhold, 1970).

The afferent fibres convey the auditory information from the cochlea to the central nervous system. The afferent fibres are classified as Type I and Type II fibres (Spoendlin, 1967). About 90-95% of the afferent fibres connect directly with the inner hair cells, and are called type I fibres. The remaining 5 % go to the more numerous outer hair cells and are called type II fibres. The role of these OHCs afferent fibres is subject to speculation as no physiological activity has been recorded from them (Robertson, 1984).

1.4. PHYSIOLOGY OF THE COCHLEA

From the 70s onwards, it became clear that sensitivity and selectivity were dependent on physiologically active processes. Some of the key experiments carried out by Rhodes (1971, 1978) found the tuning characteristics of the basilar membrane to be as sharp as those of the cochlear nerve fibres. This was subsequently confirmed by other workers (Khanna and Leonard, 1982). A major step in understanding the active process underlying cochlear sensitivity and selectivity was made by Kemp (1978) who applied discrete acoustic stimulation into the sealed auditory meatus in humans. This produced a delayed acoustic response, of which the timing and amplitude could not be explained by passive echo. From this, Kemp proposed that there was an active transduction process in the cochlea that produced a coherent release of mechanical energy that was transmitted back through the basilar membrane, the middle ear ossicular chain and the tympanic membrane. This was termed the otoacoustic emissions and triggered further work on OHCs, leading to the discovery by Brownell *et al*. (1985) and Ashmore (1987) that OHCs were electromotile and were responsible for the generation of otoacoustic emissions. Subsequent experimentation has shown that the OHCs act as electromechanical couplers in the cochlea and use the

endocochlear potential as an immediate source of energy to drive their activity (Patuzzi and Robertson, 1988).

It is widely held that thanks to their ability to perform bidirectional transduction (Weiss, 1982; Brownell *et al*., 1985; Dallos and Evans, 1995), OHCs are the key elements of a mechanical feedback loop at the origin of the so-called "cochlear amplifier" (Gold, 1948; Davis, 1983). In short, the activity of OHCs enhances basilar membrane vibrations in a frequency-selective manner, thereby increasing both the sensitivity of the cochlea to low-level sounds and its tuning. Disruption of OHC function either by acoustic overexposure or by any other OHC-specific pathology leads to impaired auditory thresholds as well as bad frequency selectivity (Liberman and Dodds, 1984; Patuzzi *et al.,* 1984; Hamernik *et al*., 1989; Davis *et al.,* 1993). Since Kemp (1978) discovered otoacoustic emissions as sounds being reemitted in the external ear canal and representing a by-product of mechanical activity inside the cochlea, otoacoustic emissions have been proposed as an objective and non-invasive means of assessing cochlear damage.

Most of the recent research has been devoted to the distortion product otoacoustic emissions (DPOAEs), which are evoked in response to stimulation by two pure tones or primaries at f1 and f2. The DPOAEs are typically found at a frequency of twice a first frequency called f1 minus a second frequency called f2 (f2 is ideally 1.22 times the first frequency (2f1 - f2)), and most of the following presentation will be restricted to the properties of the 2f1 - f2 DPOAEs. Obviously, its existence requires some nonlinear mechanism in the cochlea and appropriate mechanical feedback. Whenever the cochlear function is mechanically normal, DPOAEs are found not only in the external ear canal (Kim et al., 1980), but also in basilar membrane

movements (Robles et al. 1991) and in scala vestibuli (Avan et al, 1998). By contrast, whenever the sensitivity and tuning of the cochlea have been altered in such a way that a hearing loss >30 dB is observed around f2, DPOAEs decrease or disappear at 2f1 - f2 (review in Probst *et al*., 1991). That OHCs should be healthy and motile for otoacoustic emissions to exist has been clearly acknowledged (Brownell, 1990).

The primary site for DPOAE generation must be the place on the basilar membrane where maximum interaction occurs between the vibrations produced by the two primary tones, accordingly, it is widely thought to be at or near the place tuned to f2 (Fahey and Allen, 1997). Direct confirmation has been obtained from normal cochleas by Avan *et al*. (1998) who checked that the phase of acoustic pressure at 2f1-f2 inside scala vestibuli is minimum at the place tuned to f2 as long as the stimulus level does not exceed 70 dB SPL. It indicates that the emission is generated at f2, then exhibits a progressive phase lag along with its intracochlear propagation. Damaged cochleas also provide clear evidence of the role of the place tuned to f2: Martin *et al.* (1987) then Puel *et al*. (1995) showed that, in animals exposed to auditory fatigue, the map of DPOAE decrease tends to coincide with that of audiometric alterations when DPOAEs are plotted against f2. The idea that DPOAEs are a "frequency specific" analyser of cochlear mechanics relies upon these considerations.

Nevertheless, several secondary DPOAE sources have been discovered. It has been admitted for years that, once emitted, otoacoustic emissions behave as regular external sounds while they propagate along the cochlear scalae. The DPOAE at 2f1 - f2 is generated at a place tuned to the higher frequency

f2, thus the corresponding acoustic pressure is expected to propagate toward to the more apical place tuned to 2f1-f2. Robles *et al*. (1991) directly showed that the basilar membrane vibrates at 2f1 - f2 as a result of DPOAE propagation from f2 toward its characteristic place. In turn, when it is healthy, the place tuned to 2f1 - f2 can emit an acoustic contribution to the overall emission at 2f1 - f2 that interferes with the signal directly coming from the place tuned to f2.

In theory, the existence of secondary sources may confuse the issue of frequency specificity, that is of the frequency-to-place dependence of DPOAE levels on OHC function. However, the most common pathologies apparently lead to simpler situations. In human ears with different profiles of hearing loss, Mauermann *et al*. (1999) reported that DPOAE generation mainly depended on the cochlear status at the place tuned to the primary tones, in other words on the ability of the primary source of DPOAEs to work properly. If restricted to the places tuned to 2f1 - f2, cochlear impairment only flattened the DPOAE fine structure without influencing much the DPOAE level. This observation was backed up by a theoretical model of intracochlear sound propagation in active or less active cochleas.

Therefore, it seems conservative to state that the primary source of DPOAE is near the place tuned to f2 and is predominant in many experimental situations. Secondary sources undoubtedly exist elsewhere in the cochlea, one of them with characteristic frequency 2f1 - f2, and the cochlear status at this place influences the fine structure of DPgrams. A summary of the main steps involved in transduction is schematized in figure 1.2.

Figure 1.2

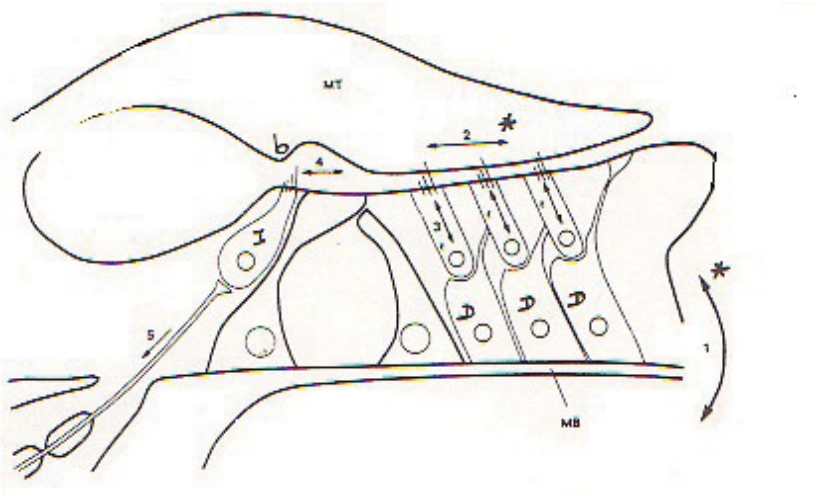


Figure 1.2. Different steps due to active mechanisms. The numbers indicate the chronology of different events. Adapted from " Anatomophysiologie de l'oreille et des fosses nasales". Professeur Van den Eeckhaut. Centre d'impression benevole. Cercle medical St Luc. Universite Catholique de Louvain, 1990-1991.

1. Sound vibration is transmitted to the basilar membrane with a maximal excitation close to the base a high frequency. Basic tonotopy.
2. The tectorial membrane moves on the hair cells.
3. Active mechanisms; the depolarized OHCs (due to shearing of the ciliae) contract and modify the relationship basilar membrane/tectorial membrane. This gives rise to an amplification of the displacement of the basilar membrane into a very restricted area and this gives a very finely tuned tonotopy.

4. Transduction. The ciliae of the IHCs are displaced by the movement of the tectorial membrane and the fluids. The opening of the ion channels at the level of the ciliae depolarises the IHCs.
5. Beginning of the auditory message. At the synaptic level, a message goes into the fibres of the auditory nerve that transmits it to the CNS.

When a sound reaches the ear, the tympano-ossicular complex is mobilised and the vibrations of the stapes footplate are transmitted to the perilymph of the scala vestibuli (pressure wave), producing a mobilisation of Reissner's membrane, basilar membrane and organ of Corti. These vibrations of the basilar membrane increase in amplitude from the base of the cochlea to a point variably distant from the base (depending on the frequency of the incoming sound), after which this amplitude rapidly decreases and the vibrations stop. This wave is called Bekesy wave. This wave is fusiform, has a maximal amplitude (proportional to the sound intensity), and a localisation of maximal amplitude (base of the cochlea for high frequency sounds, apex for low frequency sounds).

Vibration of the basilar membrane results in a sliding, or shearing movement between the tectorial membrane and the reticular lamina. The hairs of the hair cells are thus displaced relative to their cell bodies in the direction corresponding to excitation of the outer hair cells. This initiates the bioelectric stimulation of the outer hair cells that depolarizes the cell.

The active contractions of the outer hair cells increase the vibration of the basilar membrane in a very restricted area, therefore finely tuning the selectivity of given frequencies. These contractions are thought to be

responsible for the otoacoustic emissions. When acoustic energy is introduced into the system, the oscillation starts up at the basal end of the cochlea and is passed down towards the apex. Normally, in a passive system, this oscillation would simply fade away. However, the OHCs act as phase and frequency-sensitive positive feedback elements acting to inject additional energy into the arrays of resonators. Thus, for a given frequency, the oscillation is maximal at its natural point of passive resonance along the cochlear partition (Neely and Kim, 1983 and 1986).

The vibrations of the basilar membrane, amplified and selectively narrowed, mobilise the stereociliae of the inner hair cells, which depolarises the cells. The mecano-electrical transduction is started by the opening of the ciliary canals where potassium from the endolymph enters, which depolarises the inner hair cell. The opening of the ciliary canals results from the traction exerted on the transverse and apical bonds. IHCs contain negatively charged ions inside and there are positively charged ions outside. Moving the stereocilia causes positive ions to enter the cell, and this gives rise to depolarisation. Through a series of biochemical steps, this depolarization causes the hair cell to release neurotransmitters. Contact with the receptors depolarizes nerve fibers and starts an electric signal moving down the auditory nerve.

Clinically, if there is no intervening damage to the rest of the auditory pathway, the PTA and HFPTA represent the output at the level of the cochlear nerve.

1. 5. PHARMACOLOGY OF THE COCHLEA

Glutamate is the most important afferent neurotransmitter within the inner ear. A massive glutamate release induced by cochlear damage may result in excitotoxicity and irrevocable cell death. Efferent cochlear neurotransmitters include dopamine, gamma aminobutyric acid (GABA), acetylcholine (ACh) and serotonin (5-HT). Dopamine and GABA are inhibitory transmitters that may protect the cochlea from excitotoxicity. ACH, like GABA, reduces the stiffness of the outer hair cells and increases their motility. Serotonin is a neuromodulator of the cholinergic and GABAergic innervation within the cochlea and can inhibit glutamatergic impulses (Mazurek *et al*, 2007).

Recent models describe active processes within the cochlea that amplify and sharpen the mechanical response to sound. Although it is widely accepted that outer hair cells (OHCs) contribute to these processes, the nature of the medial efferent influence on cochlear mechanics needs further clarification. Acetylcholine (ACh) is the major transmitter released onto OHCs during the stimulation of these efferents. The inhibitory influence of this system is mediated by post- and presynaptic nicotinic and muscarinic receptors. The inner hair cells (IHCs) that transduce the mechanical displacements into neural activity, release glutamate on receptor-activated channels of AMPA, kainate, and NMDA types. This synapse is in turn controlled and/or regulated by the lateral efferents containing a cocktail of neuroactive substances (ACh, GABA, dopamine, enkephalins, dynorphin, CGRP). This glutamatergic nature of the IHCs is responsible for the acute destruction of the nerve endings and subsequently for neuronal death (Puel, 1995).

Halmos *et al* (2008) provided evidence that nitric oxide can modulate the release of dopamine from the cochlea following NMDA receptor activation, but does not affect the uptake of dopamine.

1.6 ANATOMOPHYSIOLOGY OF THE VESTIBULE AND SEMI-CIRCULAR CANALS

1.6.1 Anatomy

(I) General Anatomy

The vestibular labyrinth is situated behind the cochlea within the petrous bone. It consists of a system of the utricule, saccule and three semi-circular canals. Each semicircular canal is an osseous tube containing perilymph, which in turns surrounds the membranous tube containing endolymph. The ends of each semi-circular canal open into the utricule. The three semi-circular canals are known as the horizontal, the superior and the posterior semi-circular canals. The canals are oriented in such a way that when the horizontal canals on each side are in the same plane, the superior canal on one side is in the same plane as the posterior canal of the opposite side.

Close to the opening into the utricule, each semi-circular canal is enlarged. This enlargement is known as the ampulla and contains a transverse elevation. The crista, which is a projection of hair cells embedded into a thick gelatinous material called the cupula, projects into the ampulla. The anatomy of the vestibular apparatus is shown in figure 1.3.

Figure1.3

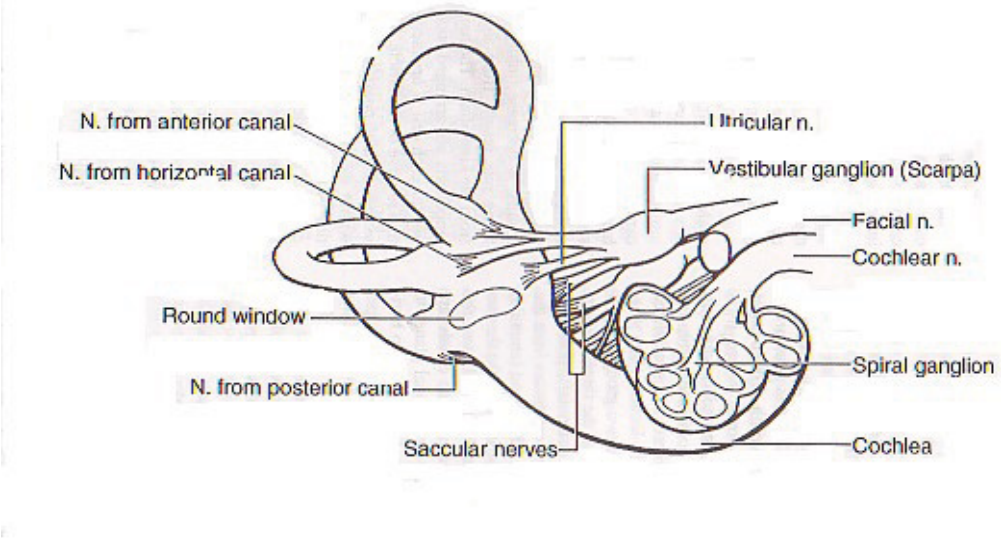


Figure 1.3: Illustration of the labyrinth showing the relative orientation of the 3 semi-circular canals to the cochlea and the neural innervation. Adapted from " Practical management of the balance disorder patient". N.T. Shepard, S.A. Telian. Singular Publishing group, Inc, 1996.

The utricule and the saccule also contain sensory epithelium called the macula. The macula is a mass of hair cells attached to the wall of the membranous duct. Small granules of calcium carbonates lie on the hair cells and are called otoconia. They are believed to initiate the static responses due to the effect of gravity. Because of the fact that the utricule is affected by static equilibrium, stimulation of the utricule produces compensatory eye positions, head straightening reflex and alterations in muscle tone.

The semi-circular canals are stimulated by rotation or acceleration in any direction, with inertia being the primary result. The right and left canals always function together. The function of the saccule is not clearly known. The axons from the utricule and semi-circular canals join to form a vestibular portion of

the eighth cranial nerve. When they reach the brainstem, the cochlear and vestibular portions of the 8th nerve divide and terminate in their respective nuclei.

(2) Central connections and innervation

The axis cylinders of the nerve fibres ramify round the hair cells of the receptor organs. As in the cochlea, these fibres are of 2 types: type I fibres, which are probably afferent and type II fibres, which are richly granular and probably efferent. The fibres are gathered together to form the vestibular nerve, which passes through the internal auditory canal. The neurones pass in the 2 main subdivisions of the vestibular nerve, through the internal auditory canal, to the large bipolar cells of the vestibular (Scarpa's) ganglion. The superior branch (which has anastomotic connections with the facial nerve) innervates the cristae of the superior and lateral semicircular canals, and the macula of the utricle; the inferior branch innervates the posterior semicircular canal and most of the macula of the saccule. The nerve then enters the lower border of the pons, where it separates from the cochlear nerve. The vestibular nerve passes backwards into the medulla, to reach the vestibular nuclei in the pons and medulla, close to the floor of the 4th ventricle. There are 4 main vestibular nuclei: lateral (in the lateral portion of the medulla), superior (above the lateral and in the angle of the 4th ventricle), medial (medial to the lateral and superior) and inferior.

The vestibular nuclei have connections with nuclei of the third, fourth and sixth cranial nerves through the medial longitudinal bundle and with the ventral motor gray cells of the spinal cord through the vestibular spinal tract. Fibres also connect to the cerebellar cortex through the inferior cerebellar peduncle.

1.6. 2. Physiology

The vestibular system can be split into component parts and each part treated more or less separately. There is a sensory input that tells the central processing unit how the posture and the visual field are changing in relation to the environment. The motor output from the central unit then acts to maintain the stability of the body and direction of gaze. The vestibular labyrinth is located in the inner ear and gives information about the angular and linear acceleration of the head.

(i)Physiology of the semicircular canals

During angular acceleration of the head, the crista moves with the head. However, the endolymph by way of its inertia tends to stay still and is therefore forced through the space between the cupula and the crista therefore deflecting the stereociliae. When constant rotation is reached the endolymph quickly gains momentum so that it is rotating at the same speed as the head and further stimulation does not occur as the stereociliae return to their resting position. With deceleration the endolymph carries on moving for a short while because of its recently acquired momentum and causes the reverse deflection of the stereociliae and the opposite neural output. Since the 3 canals in 1 ear are at right angles to each other, they effectively perform a vector analysis of the applied rotation. The 3 canals in the other ear are complementary in position and output of the 6 canals allows complex angular acceleration and deceleration to be analysed. Each of the vestibular nuclei also has a separate functional efferent role. The subjective sensation of

movement is due to vestibulocortical projections. The threshold of subjective angular acceleration is in the range of 0.1 to $0.5^0/s^2$ (Clark, 1967).

(ii) Physiology of the otolithic organs

The otolithic organs (saccule and utricule) also function in pairs, with the two utricular maculae in the horizontal plane and the two saccular maculae in the vertical plane. Otolith stimulation is responsible for the perception of linear movement in the horizontal plane and tilt. Horizontal linear acceleration results in the perception of both types of motion. At low amplitudes of oscillation, the subject feels motion without specific direction. Increasing the intensity of stimulation will result in the perception of direction of linear movement. When the intensity is increased further, the subject will have the perception of tilting.

(iii) Polarisation

This polarisation within the horizontal canals causes an excitation of neural activity when cupular movement creates a deviation of the stereociliae toward the utricule (called utriculopetal flow) and inhibition of neural activity (utriculofugal flow) for shearing the stereociliae away from the utricule. The situation is reversed for the superior and posterior canals. Therefore, stimulation of any of the three functional pairs by angular acceleration in their plane of orientation causes an increase in neural firing rate on one side and a decrease on the contralateral side. The asymmetrical neural input from the vestibular nerves is interpreted by the CNS as either linear or angular acceleration. In addition, the asymmetry resulting from action of the semi-circular canals causes a compensatory reflex eye movement in the plane of the canals being stimulated (Baloh and Honrubia, 1990). This compensatory

reflex movement of the eye is produced by the vestibulo-ocular reflex (VOR) and is opposite to the direction of acceleration.

(iv) The vestibulo-ocular reflex

The VOR of the horizontal canals is mediated by a simple three-neuron arc involving the vestibular nuclei and cranial nerves III and VI. Stimulation of the vertical pair of canals also produces a VOR along analogous brain stem pathways. The VOR helps to stabilise images of the visual surroundings on the retina during head movements. Angular head accelerations are detected by three pairs of semicircular canals, and linear head accelerations by two pairs of otoliths. These sensors induce compensatory eye movements (slow phase) in the opposite direction to head acceleration by a three-neuron reflex arc (vestibular afferents-vestibular nucleus-ocular motor nuclei). Sensory motor transformation occurs from canal planes to the planes of eye movements, so that the neurons always contact their two respective extraocular eye muscles. Horizontal VOR can be stimulated either by thrusting the head in one direction (causing contralateral slow phase) or by caloric irrigation. As the measurements are easy and yield quantitative results, testing is a common diagnostic tool. With the VOR, the clinician can test labyrinthine function for a unilateral or bilateral hypofunction or loss of function (caloric irrigation); cerebellar function for a lesional disinhibition; and brainstem function in comatose patients (Dietrich and Brandt, 1995). A detailed description of the brain stem and cerebellar pathways and the physiology of the VOR is found in Baloh and Honrubia (1990).

(v) Somatosensors

The sensors within the body detect the relationship of various parts of the body to each other, and of the whole body to the outside. Touch and pressure receptors in the skin, the spindles and the joint position sensors all feed this information along the spinal cord to the brain and are called the somatosensors. They are very well developed in the muscles and joints of the neck. Information from all 3 systems passes into the brainstem and cerebellum. Together, these coordinate sensory input, determine the appropriate response to alteration in the incoming information, and send out the correct instructions to compensate smoothly for the changes that have occurred. These instructions are directed to the muscles that maintain the body posture and eye position. The vestibular nuclei appear to be of major importance in the integration of the sensory information. The brainstem has a blood supply derived almost completely from the 2 vertebral arteries and their subsequent branches, most of which are end arteries. The brainstem and cerebellum are therefore influenced not only by the general disorders of the circulation, but also by disorders related specifically to the course of the vertebral arteries within the cervical spine.

(VI) Postural and Motor system control

The cerebral cortex influences lower motor centres via projections through two pathways:

(I) The Pyramidal system consists of long fibres of the corticospinal tract. It plays a major role in controlling fine, isolated movements. The cells and axons of the pyramidal system are called “upper motor neurones”.

(ii) The Extrapyramidal system consists of four large brainstem nuclei and the basal ganglia. These patterns provide large gross movement patterns that are primarily reflexive and constitute major postural adjustments.

Three tracts are heavily contributed by the vestibular nuclei: the medial and lateral vestibulospinal tracts and the reticulospinal tract.

(i) The medial vestibulospinal tract enters the spinal cord as part of the medial longitudinal fasciculus (MLF). This tract plays a major role in the cervical-vestibulo-ocular reflexes, coordinating eye-head movements.

(ii) The lateral vestibulospinal tract originates in the lateral vestibular nucleus. The principal influence of the vestibular system and the specific areas of the cerebellum on the spinal motor activity are carried through this tract. An influence of the lateral vestibulospinal tract is noted by activity present during a threatened fall following rapid accelerating rotation.

(iii) The reticulospinal tract originates from the pontine and medullary reticular formations in the brainstem. Stimulation of the reticular formation of the brainstem has been shown to influence muscle tone and cause facilitation or inhibition of cortically generated movements and reflex activities.

The central vestibular pathways are represented in figure 1.4.

The pathways involved in the control of limb and body movements are shown in figure 1.5.

Figure 1.4

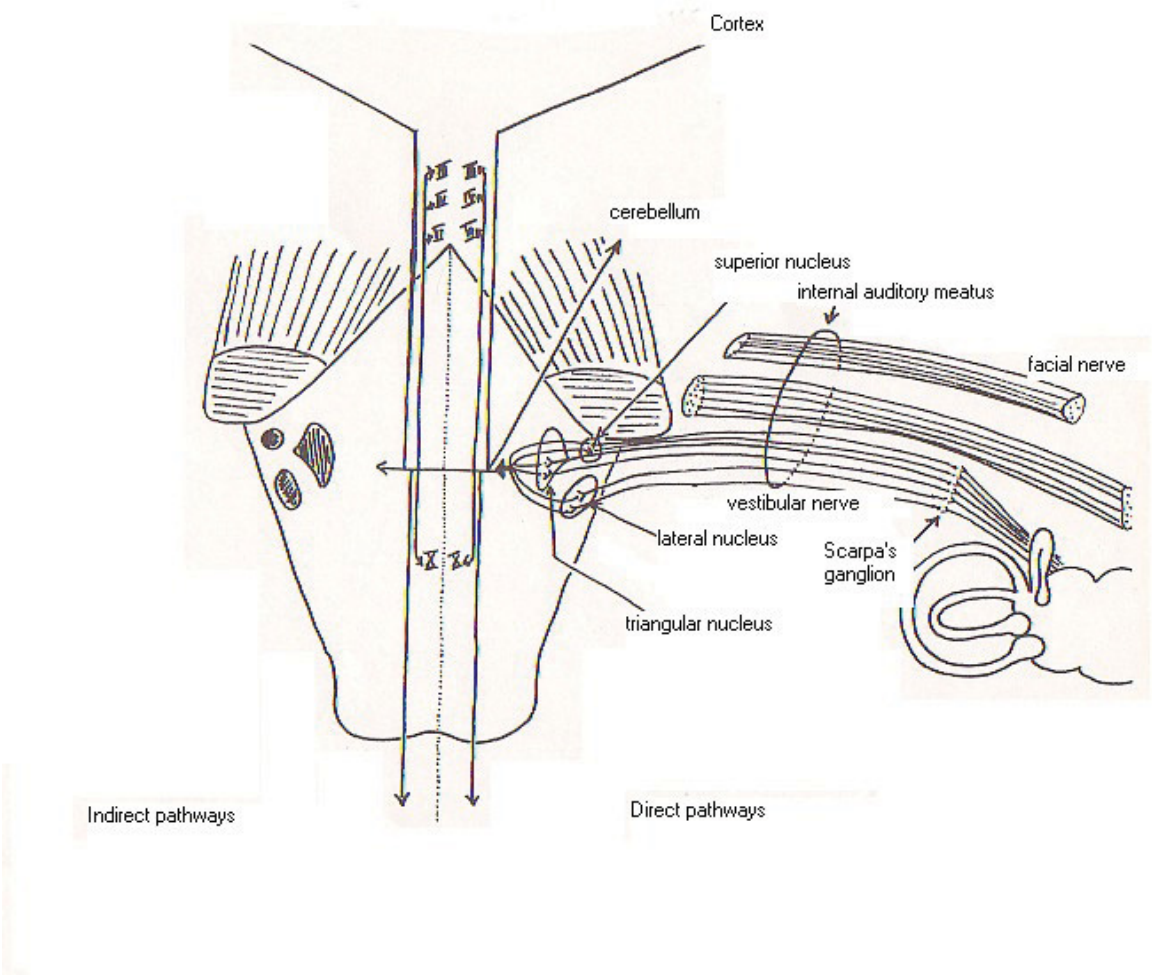


Figure 1.4. Central vestibular pathways. . Adapted from "Anatomophysiologie de l'oreille et des fosses nasales". Professeur Van den Eeckhaut. Centre d'impression benevole. Cercle medical St Luc. Universite Catholique de Louvain, 1990-1991.

Figure 1.5

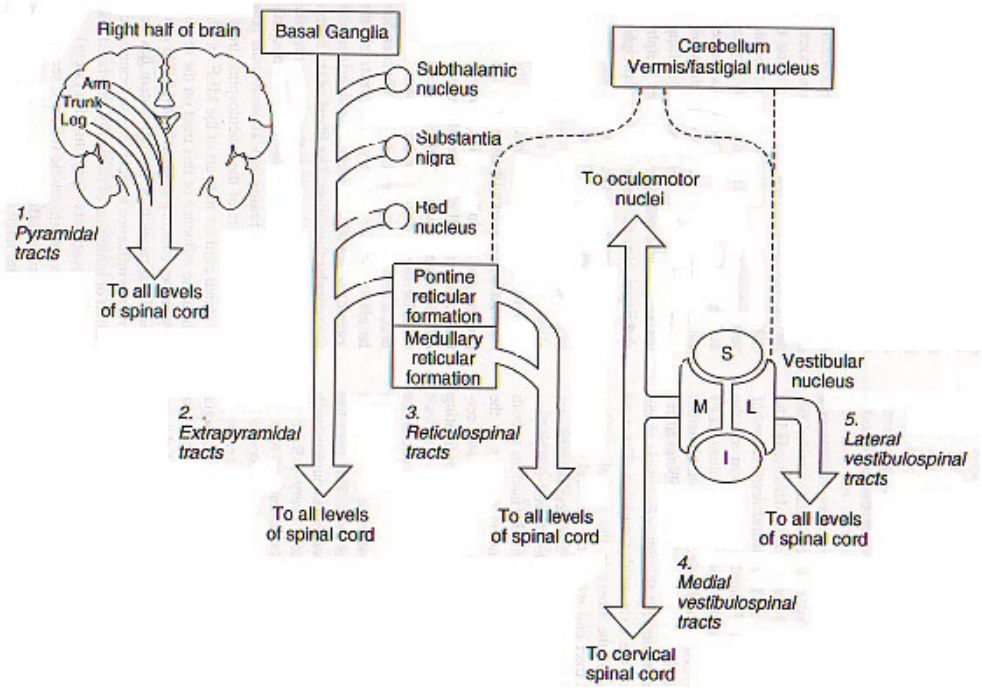


Figure 1.5 Pathways involved in the control of coordinated limb and body movements. S=superior vestibular nucleus. L=lateral vestibular nucleus, I=inferior vestibular nucleus. Adapted from " Practical management of the balance disorder patient". N.T. Shepard, S.A. Telian. Singular Publishing group, Inc, 1996.

1.6.3. Pharmacology of the vestibular system

The vestibular nerve and the vestibular nuclei use glutamate, aspartate, acetylcholine and in addition gamma-aminobutyric acid (GABA) in the vestibular nuclei. Functions of the different neurotransmitters have been determined experimentally through the use of their particular agonists and antagonists (Kurzan *et al*, 1993). Inhibitory pathways within the vestibular projections mediating the vertical vestibulo-ocular reflex (VOR) use glycine (Spencer and Baker, 1992). Glycine is also a neurotransmitter of pause

neurons, which inhibit saccadic burst neurons in the paramedian pontine reticular formation. Licata *et al* (1993) demonstrated that noradrenaline modifies the level of neuronal activity in the vestibular complex by acting mostly, but not exclusively, through α_2-adrenergic receptors. This means that, in addition to serotonin (Johnston, 1993) and dopamine, noradrenergic systems may also influence the vestibular function by a direct action of noradrenaline inside the vestibular nuclei.

The effects of histamine and a variety of histamine receptors agonists and antagonists were investigated in the guinea-pig (*in vitro* and *in vivo*) through the modulation of the firing rate of medial vestibular nuclei neurons (Serafin *et al,* 1993; Yabe *et al*, 1993). The studies show that lateral and medial vestibular nuclei neurons contain both H_2 and H_3 receptors and histamine effectively modulates static vestibular reflexes. Botta *et al* (2008) investigated the expression of H_1 and H_2 histamine receptors in the frog and mouse semicircular canal sensory epithelia. Their data show that both frog and mouse vestibular epithelia express H_1 receptors, but that there is no evidence for H_3 receptors expression.

Scheffer *et al* (2008) identified two novel acetylcholine (ACh) receptor subunits in the inner ear. They suggest that hair cells transiently express alpha 1 gamma-containing ACh receptors in addition to alpha 9 and alpha 10 and that these may have a role during the development of the inner ear innervation. Muscarinic acetylcholine receptors (mAChRs) are widely expressed in the CNS and peripheral nervous system and play an important role in modulating the cell activity and function. Li *et al* (2007) showed that

there is considerable co-expression of the subtypes of muscarinic receptors on the neural elements of the labyrinth.

Long *et al* (2008) tried to determine whether the atrial natriuretic peptide receptor (NPR-A) is present in the secretory regions of the membranous labyrinth of the adult mouse inner ear. They demonstrated that NPR-A was expressed in the mouse stria vascularis as well as in the non strial tissue of the cochlear lateral wall and vestibular organ. This suggests that natriuretic peptides may play an important role in maintaining the fluid homeostasis of inner ear endolymph through their interaction with NPR-A.

Choi *et al* (2008) suggest that excitatory afferent signals from the peripheral vestibular receptors, resulting from acute hypotension, release glutamate into postsynaptic neurons in the vestibular nuclei and the excitatory signals are transmitted through the GluR1 subunit of the AMPA (glutamate alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors and the NR2B subunits of the NMDA (N-methyl-D-aspartate) receptors in the vestibular system.

Sheng Li *et al* (2008) reveal the existence of efferent neurotransmitter receptors on vestibular hair cells (VHCs) type I. This helps in understanding the function of vestibular efferent nervous system, and may provide some useful information on guiding the clinical rehabilitative treatment of vertigo.

GABA and glutamate have been postulated as afferent neurotransmitters at the sensory periphery inner ear vestibule in vertebrates (Meza, 2008). GABA has fulfilled the main criteria to act as afferent neurotransmitter but may also

be a putative efferent neurotransmitter, mainly due to cellular localization of its synthesizing enzyme glutamate decarboxylase derived from biochemical, immunocytochemical, in situ hybridization and molecular biological techniques, whereas glutamate afferent neurotransmission role is supported mainly by pharmacological evidence. GABA and Glu could also act as afferent co-neurotransmitters based upon immunocytochemical techniques. This multiplicity was not considered earlier and postulates a peripheral modulation of afferent information being sent to higher vestibular centers. In order to make a definitive cellular assignation to these putative neurotransmitters it is necessary to have evidence derived from immunocytochemical and pharmacological experiments in which both substances are tested simultaneously.

Di Mauro *et al* (2008) studied the effects of noradrenaline (NA) on the inhibitory responses to GABA. It was concluded that NA enhances GABA responses by acting on noradrenergic alpha (2) and to a lesser extent beta receptors, whereas depressive action involves beta receptors only. These results confirm the hypothesis that the noradrenergic system participates in the regulation of the vestibulospinal and the vestibulo-ocular reflexes and suggest that conspicuous changes of NA content in brain due to aging or stress could lead to deterioration in the mechanisms of normal vestibular function.

Previous studies have confirmed the existence of vestibulo-sympathetic pathways in the central nervous system. However, the exact pathways and neurotransmitters underlying this reflex are unclear. Cai *et al* (2008) undertook a study to investigate whether the vestibulo-cardiovascular

responses are a result of activated glutamate receptors in the caudal vestibular nucleus. They also attempted to verify the indirect excitatory pathways from the vestibular nucleus (VN) to the rostral ventrolateral medulla (RVLM) using a tracing method combined with a vesicular glutamate transporter immunofluorescence. Their results suggested that activation of caudal vestibular nucleus neurons could induce pressor response and NMDA receptors might contribute to this response in the medial vestibular nucleus. The glutamatergic vestibular nucleus (VN)-nucleus of the solitary tract (NTS) and vestibular nucleus-parabrachial nucleus pathways might exist, and the projections from the VN to the rostral ventrolateral medulla relayed by the NTS comprise an indirect vestibulo-cardiovascular pathway in the brain stem.

A summary of the main vestibular neurotransmitters is shown in table 1.2.

Table 1.2.

Transmitters	Action	Location
Glutamate	excitatory	Vestibular nuclei and vest nerve
Aspartate		Vestibular nuclei and vest nerve
Acetylcholine		Vestibular nuclei and vest nerve
GABA		Vestibular nuclei
Glycine	inhibitory	Pontine reticular formation
Noradrenaline	Ä$_2$ adrenergic receptors Vestibulo-spinal and vestibulo-ocular reflexes	Vestibular nuclei
Serotonine		Vestibular nuclei
Dopamine		Vestibular nuclei
Histamine		Vestibular nuclei

1.7. DRUGS USED IN TREATMENT OF PATIENTS WITH HAEMATOLOGICAL MALIGNANCY; GENERAL PHARMACOLOGY, CLINICAL THERAPEUTICS AND OTOTOXICITY

1.7.1. Drugs taken by patients with haematological malignancy

The list of drugs taken by patients suffering from haematological malignancy who took part in the current study is shown in table 1. 3.

Table 1. 3

ANTIBIOTICS	1. Aminoglycosides(AG): Gentamicin , Streptomycin 2. Glycopeptide antibiotics: vancomycin and teicoplanin 3. Macolide antibiotics: Erythromycin
CYTOTOXIC AGENTS	1. Alkylating drugs: cyclophosphamide , melphalan ,ifosfamide, busulphan 2. Cytotoxic antibiotics: Daunorubicin , doxorubicine, mitozantrone 3. Antimetabolites: Methotrexate , Cytarabine , Fludarabine 4. Vinca-alkaloids and etoposide: Vinblastine , vincristine , etoposide 5. Other antineoplastic drugs: amsacrine, dacarbazine , cisplatin (n=1)
MISCELLANEOUS	1. H_2-receptor antagonists: cimetidine 2. Drugs for treatment of gout: allopurinol 3. Antimalarials: quinine 4. Gucocorticoids: solumedrol

1.7.2. General considerations of ototoxicity of drugs used in the treatment of patients with haematological malignancy.

In the following sections for each drug where relevant a consideration of their general pharmacology, mechanism of therapeutic action, their clinical use and ototoxicity is given. In particular the incidence, risk, severity, patterns of pathology and synergy with the other drugs is discussed. In the literature, these factors have been best described for the aminoglycosides. The

literature for the other drugs is generally much less extensive and for some consists of isolated case reports. The drugs where no evidence of ototoxicity is found in the literature will not be discussed.

1.7.2.1. Antibiotics 1 ; aminoglycoside gentamicin

General pharmacology

Gentamicin belongs to the aminoglycoside group of antibiotics and, as the name implies, they contain aminosugars linked to an aminicyclitol ring by glycosidic bonds. Aminoglycoside antibiotics were first discovered in 1944 the first being streptomycin (Schatz *et al.,* 1944)). Out of this group gentamicin, which was first introduced in 1963, and tobramycin are the most frequently used, at least in the United Kingdom. In this study gentamicin was exclusively used to treat the neutropenic patients. Gentamicin has a molecular weight of 477 daltons and at physiological pH is poorly soluble. This is due to its polycation charge and this polarity is largely responsible for their pharmacokinetic properties, having a half life in plasma of about 2-4 hrs and being excreted unmetabolised by the kidneys. The structure of the gentamicin is shown in Figure 7. As it is inadequately absorbed by oral administration it is almost always given intravenouslv (iv) or intramuscularly (im) (Sande and Mandell, 1980).

Mechanism of therapeutic action

The mechanism by which all aminoglycosides appear to act is by preferentially binding to bacterial ribosomes thus interfering with protein synthesis in susceptible aerobic gram negative bacteria (Kapusnik *et al*, 1988; Davis, 1988).

Clinical use

The aminoglycosides are used primarily to treat infections caused by microorganisms. Normally aminoglycosides including gentamicin are given as a single course of 3-8mg/kg/day given over 7-10 days. These yield peak plasma levels falling between 4-8 µg/ml (Bendush, 1982; Cone, 1982; Govaerts *et al*., 1990; Kahlmeter and Dahlager, 1982 and Neu and Bendush, 1976). Typically, patients with heamatological malignancy receive 7 mg/kg/day once or over two doses daily for about 5-7 days, although individual cases can be longer or occasionally shorter than this. During therapy peak and trough levels of the drug are estimated as plasma levels exceeding 1 or 2 µg/ml can lead to toxicity (Bendush, 1982).

Gentamicin ototoxicity - cochleotoxicity and vestibulotoxicity

Cochleotoxicity: Clinical manifestation

Since the introduction of streptomycin as a successful antituberculous agent in 1944, serious nephro and ototoxicity were recognised as a major limitation to the usefulness of the aminoglycosides. (Hinshaw and Feldman,1945). The clinical use of streptomycin was tempered by a high incidence of ototoxicity manifest in deterioration of both vestibular and auditory function (Hinshaw and Feldman, 1945). This led to the development of derivatives with the specific aim of reducing this risk to a clinically acceptable minimum. One of these derivatives was gentamicin, which was introduced in 1963 and gentamicin is the agent of choice for patients with haematological malignancy within the LRI. Whilst it has proved to be very successful as an antibiotic it still carried a risk of ototoxicity. The per course risk was reported in many studies as falling between 5-15% (Cone, 1982; Govaerts *et al*., 1990; Kahlmeter and Dahlager, 1982). Gentamicin along with tobramyicn is considered to fall

midway in the ototoxicity spectrum (Cone, 1982; Kalhlmeter and Dahlager, 1982, Jackson and Arcieri, 1971). This single course incidence and severity is offset by its efficacy in treating gram negative infection.

Morphological changes

It has been repeatedly demonstrated in experimental studies (Aran *et al*., 1982; McCormick *et al.,* 1985) that the principal structural lesion caused by aminoglycosides is an irreversible degeneration of sensory hair cells. In these earlier morphological studies, the pattern of loss seen involved the hair cells at the base of the cochlea (ie at the high frequency end). This was found to progress towards the apical lower frequency turns of the cochlea. This loss was found to be permanent.

Biochemical mechanism underlying irreversible damage

The most recent experimental studies suggest that the main biochemical mechanism underlying ototoxicity involves the delayed generation of free radical species by AGs. These free radicals disturb normal cell function which if not successfully dealt with by detoxicant enzymes, leads to cell death and irreversible hearing loss. Support for this theory was explained by the fact that protection from ototoxicity was provided by glutathione, which is a free radical scavenger and a cellular antioxidant (Garetz and Schacht, 1992; Hoffman *et al.,* 1988). In this model gentamicin is proposed to chelate cytosolic free iron. The gentamicin-iron complex can then catalyse the formation of active oxygen species. This then leads to the formation of other radicals. It is these free radicals that are capable of damaging biological membranes, DNA and proteins. A schematic illustration of the mechanism of gentamicin ototoxicity is shown in figure 1.6.

Figure 1.6

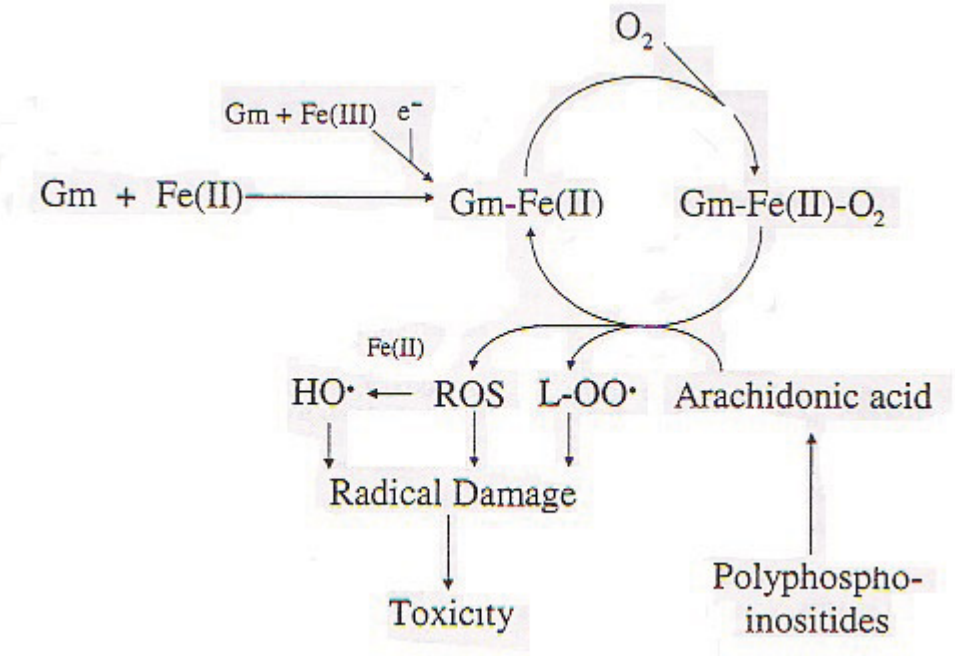


Figure 1.6. Mechanism of AG ototoxicity. Gentamicin chelates iron to form a redox-active Gm-Fe(II) complex. The intracellular labile iron pool consists mainly of ferrous iron Fe (II). However, if iron is initially chelated as ferric iron Fe(III), a one-electron reduction can lead to Gm-Fe(II) complex. This complex activates molecular oxygen and reduces with electrons from an electron donor such as arachidonic acid. As a direct result, lipid peroxides(L-OO-) and superoxide (indicated as ROS, reactive oxygen species) are generated. Lipid peroxides can initiate a chain reaction of peroxidation, and superoxide can undergo a Fenton-type reaction to hydroxyl radical (HO-). The sum of these reactions is a Gm-Fe(II) catalyzed reduction of oxygen and an ensuing chain reaction of formation of different ROS which go on to damage the cell. Adapted from Forge and Schacht: Aminoglycoside antibiotics. Audiol Neuro-Otol 5:3-22, 2000.

Normally, as in all cells, this cascade of reactions is halted by cellular antioxidants. But if the rate of free radical formation overwhelms and leads to the excessive depletion of cellular antioxidants cell damage will occur. Glutathione depletion is also thought to be the trigger for the activation of gene expression in the way of cell death by apoptosis (Forge and Li, 2000).

Biochemical mechanism underlying reversible damage.

Reversible damage to the cochlea is considered to be due to aminoglycoside antibiotics acting as non selective inhibitors at voltage gated calcium and potassium channels (Sokabe *et al*., 1982). This arises due to the aminoglycosides cationic nature, which enables them to displace calcium from many of its binding sites (Dulon *et al*., 1989; Nakagawa *et al*., 1992). It has also been shown to bind with phospholipids which result in disturbances to metabolism. As these interactions with the aminoglycosides are weak they are generally considered to be reversible.

Pharmacokinetics underlying toxicology

The pharmacokinetics and toxicology derived from animal models may underlie the pathology seen in man. The key features from this experimental work appear to include the following. As mentioned above, the plasma half-life is very short (2-4 hrs), but there is evidence that the half life in the cochlea is much longer. Aminoglycosides seem to penetrate cochlear endolymph and perilymph in the μM range by an active selective uptake process. This process also seems to occur in the cochlear OHCs and IHCs. There also is a possible acceleration in uptake by OHCs and IHCs with increasing length of exposure. There is a biphasic release process from the IHCs and OHCs, a fast phase ($t_{1/2}$=days) and a second slow phase ($t_{1/2}$ = months). Within the

OHCs and IHCs, the main features determining cochleotoxicity are an AG 'storage' threshold, below which AG remains as a dormant 'protoxin'. When this is exceeded, AG metabolism generates an 'active toxin' species (Aran, 1995; Crann and Schacht, 1996; Dulon *et al*., 1993; Hiel *et al*., 1993 and Tran Ba Huy *et al*., 1986); As outlined above detoxicant processes involving free radical scavengers may ameliorate the severity of damage but if it is overcome it leads to cell dysfunction and eventual death (Satoh *et al*., 1998; Tan *et al*., 1998; Tong *et al*., 1998).

Effects of aminoglycosides on cochlear electrophysiology

The effects on cochlear function following AG exposure have been well described. Normally, there is deterioration in threshold that follows the pattern of OHC and IHC loss (Aran, 1995 and Dulon *et al*., 1993). This loss of sensitivity is due to the loss of OHC function as amplifiers and by the IHCs to transduce this amplified signal. The varying degrees of this loss in man are reflected in the elevation of PTA thresholds (Kahlmeter and Dahlager, 1984).

Limitations in extrapolation of experimental work to humans

The detail about the pharmacology physiology and toxicology provided from these animal models appears to be much greater for aminoglycosides than for any of the other compounds that patients with haematological malignancy receive. Whilst these animal based experiments have been very useful in increasing the understanding of the mechanism they are limited by the use of very high dosing levels in vivo to accelerate cochlear damage. These are typically 50–400 mg/kg/day for two weeks ie at least 5 to 40 times greater than the doses given in man (Mulheran *et al*., 2001).

Aminoglycoside Cochleotoxicity: Clinical studies

Morphological studies

Post mortem studies in patients with a known deafness arising from AG therapy have shown the same pattern of loss as in the animal studies. Namely, in the organ of Corti, pathology is first evident as loss of outer hair cells at the base of the cochlea. With continued drug treatment, the damage will spread further towards the apex. Superimposed on this progression, is a lateral gradient whereby outer hair cells of the innermost row are affected before those of the second and third rows (Hawkins, 1976). Inner hair cells appear to be more resistant than the outer hair cells to loss (Forge and Schacht, 2000).

Pure tone audiometry

The results from experimental studies are largely reflected in the results seen in man. Clinically, patients cochlear status is usually measured using a pure tone audiogram (an example of which is shown in chapter 4 introduction).

Usually the criteria for hearing loss are an elevation in the post course audiogram of two or more frequencies elevated by 15 –20 dB HL (International Organisation for Standardisation, 1984; Lutman and Davis, 1994). AG ototoxicity is again typically seen as a steep high frequency loss spreading to lower frequencies over time (Forge and Schacht, 2000). There is normally some delay after exposure, but in many studies elevated PTA thresholds are reported soon after the AG course has finished (Tange, 1998). It was from these studies using PTA that estimates of single course risk were obtained. The range of cochleotoxic incidence for gentamicin fell between

about 0-16%. The overall median incidence of cochleotoxicity reported for gentamicin was about 7.5% (Cone, 1982; Govaerts *et al*., 1990 and Kahlmeter and Dahlager, 1984). Three primary factors identified with increased cochleotoxic risk were: total daily dose (mg/kg), course length; and repeated courses of therapy (Bendush, 1982; Govaerts *et al*., 1990 and Moore *et al*., 1984). These three factors are found in the treatment of patients suffering from haematological mailgnancy with some patients receiving up to 10 courses.

One patient group that receives frequent courses of AG therapy consists of patients with cystic fibrosis (CF). Several studies have been done on this patient group (Mulherin *et al*., 1993). Most of these studies did not comment on any relationship between the amount of AG received and hearing loss or return any estimates of the per course risk of AG cochleotoxicity in this group. The issue of per course incidence and risk was more fully addressed in a report by Mulheran *et al* (2001) who found that 12 out of 70 CF patients suffered a hearing loss based on normative criteria provided by Lutman and Davis (1994). It was proposed in this paper that the incidence of per course risk was low in this patient group at about 1-2%. This contrasted with the findings in the non CF groups. Moreover from the clinical literature it appears that this paper was the only one that attempted to clearly address the issue of per course risk and severity of loss. This in part formed the stimulus for the current study to see if another defined patient group requiring AGs returned similar or differing estimates of ototoxic risk.

DPOAEs have also been carried out in CF patients who receive repeated courses of AG therapy and this is covered in the introduction of Chapter 5 (Mulheran and Degg, 1997 and Katbamna *et al*., 1998).

Aminoglcyoside Vestibulotoxicity: clinical manifestation

Vestibular function is not often monitored effectively after treatment with aminoglycosides (Halmagyi, Fattore and Curthoys, 1994). Despite new hope of regenerating cochlear and vestibular hair cells after destruction by aminoglycosides (Forge, Li and Nevill, 1998), or protecting them against such toxicity using drugs such as neurotrophines (Staecker *et al*., 1996 and Kimura *et al*., 1999), currently there is no pharmacological therapy which can be used to restore cell function once it is lost. Although the destruction of vestibular hair cells is permanent, the duration of vestibular symptoms is usually limited because of central compensation (Griffin, 1988). This compensation however is often compromised in the dark.

Clinical ototoxicity is manifest in two-thirds of patients as isolated vestibular dysfunction and as combined vestibular and cochlear or isolated cochlear dysfunction in the remainder (Gorbach and Bartlett, 1974). Aminoglycoside vestibulotoxicity is experienced by patients as an unusual form of dizziness, where the objects around them seem to bounce up and down. This is called bobbing oscillopsia (Halmagyi, Fattore and Curthoys, 1994). Gentamicin is considered to be more vestibulotoxic than cochleotoxic in man (Halmagyi, Fattore and Curthoys, 1994) and is therefore sometimes used for “chemical vestibulectomy” in patients with Meniere’s disease (Monsell *et al*., 1993).

Some of the mechanisms by which AGs cause vestibulotoxicity are the same as the ones involved in cochleotoxicity, ie they disrupt mitochondrial protein synthesis in hair cells as a result of the similarity between mitochondrial ribosomes and bacterial ribosomes (Hutchin & Cortopassi, 1994). Another possible mechanism of ototoxicity is that AGs promote the formation of free radicals (Schacht, 1998) by binding to iron and the formation of oxidative compounds. Another hypothesis includes the idea that AGs reversibly block sensory transduction by blocking Ca^{2+}-sensitive K^{+} channels in the tubulo-vesicular cell system (Hess, 1996). The most recent hypothesis is that AGs may act as agonists at the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, resulting in excessive NMDA receptor activation and excitotoxicity (Basile *et al*., 1996). The action of glutamate on NMDA receptors has been discussed earlier in this introduction (Choi *et al*, 2008).

Aminoglycoside Vestibulotoxicity: Clinical studies

The subliminal nature of vestibular processes, coupled with the debilitated condition of many patients receiving aminoglycosides, can make vestibulotoxicity difficult to detect. A review of 1976 patients receiving gentamicin or another aminoglycoside showed that about 3% developed some form of vestibular injury (Kahlmeter and Dahlager, 1984).

In those patients, the diagnosis was most often made after the patient noted gait ataxia when trying to return to normal activity after leaving the hospital. The most common vestibular deficit in these patients was bilateral vestibular hypofunction.

Vestibulotoxicity typically occurs without accompanying injury to the auditory system. Patients can also develop vestibulotoxicity even when their serum peak and trough levels are well controlled.

Halmagyi *et al* (1994) reviewed 36 patients with gentamicin vestibulotoxicity to determine its relationship to gentamicin dosage, serum gentamicin levels, and the development of gentamicin nephrotoxicity. Thirty of the patients had received intravenous or intramuscular gentamicin; six had received intraperitoneal gentamicin. Sixteen of the 30 patients treated with intramuscular or intravenous gentamicin had received less than the recommended maximum dose of 5 mg/kg/day for less than the recommended maximum period of 10 days. Nephrotoxicity as well as vestibulotoxicity developed in 16 of these 30 patients. It was concluded that as far as the vestibular system is concerned there is no safe gentamicin dose and no safe serum gentamicin level, and there is an increased risk of vestibulotoxicity in patients in whom nephrotoxicity develops.

Vestibulotoxicity: Mechanisms of toxicity – experimental studies

The vestibular system has also been subject to experimental investigation, though not as extensively as the cochlea. Essentially the biochemical mechanisms and pharmacokinetics underlying the reversible and irreversible damage to the vestibular sensory cells is considered to be similar to that in the cochlea. In relation to vestibulotoxicity, it is generally accepted that only the sensory hair cells are affected and any changes in the vestibular nerve and vestibular nucleus are secondary to hair cell damage (Hess, 1996; Li *et al.*, 1995). Like their cochlear counterparts, vestibular ganglia may be protected by nerve growth factors (Zheng *et al*., 1995). Damage appears to develop first in the central part of the semi-circular canal ampulla cristae, with

Type I cells more vulnerable than Type II cells. Hair cell loss then progresses towards the periphery of the vestibular receptor organ with type I cells affected earlier than type II cells (Wersall *et al*., 1968, 1973). Although certain areas of the otolithic maculae are lesioned, hair cells in the utricule and saccule seem to be affected to a lesser extent than those in the ampullae (Hess, 1996). In contrast to the situation in the cochlea, regeneration of vestibular hair cells has been observed in mammalian species (Forge *et al*., 1993, 1998).

As mentioned above, evidence is gradually emerging that aminoglycoside vestibulotoxicity is partially mediated by an excitotoxic process (Basile *et al*., 1996; Strumpo *et al*., 1991; Pullan *et al*., 1992; Yamakura *et al*., 1999; Segal *et al*., 1998; Segal *et al*., 1999; Basile *et al*., 1999; Shigomori *et al*., 1998). Inhibition of mitochondrial protein synthesis and the formation of free radicals are also likely to contribute to hair cell destruction following AG treatment. However, cell death which is mediated by excitotoxicity is a result of overactivation of glutamatergic receptors which are part of the normal process of neurotransmission (Smith and Darlington, 1996) and therefore recognition of their involvement is important because it suggests a number of therapeutic possibilities for reducing aminoglycoside ototoxicity.

1.7.2.2.Antibiotics 2; Vancomycin and its analogues.

General pharmacology

Vancomycin is another antibiotic sometimes used either prophylactically or in the presence of an established Gram positive infection. Vancomycin is produced by Streptomyces Orientalis.

Mechanism of therapeutic action

Vancomycin has a unique mode of action inhibiting the second stage of cell wall synthesis of susceptible bacteria. There is also evidence that vancomycin alters the permeability of the cell membrane and selectively inhibits ribonucleic acid synthesis.

Clinical Therapeutics

Vancomycin is primarily active against Gram positive organisms. Virtually all strains of Staphylococcus aureus and staphylococcus epidermidis, including the methicillin-resistant species, are almost always susceptible to vancomycin. The drug is also extremely valuable in severe staphylococcal infections in patients who are allergic to penicillins and cephalosporins.

Ototoxicity, Clinical evidence

The ototoxicity of vancomycin is controversial: in a prospective study (Mellor *et al*., 1985), 2 of 34 patients developed tinnitus and dizziness during vancomycin therapy. These symptoms resolved on withdrawal of the drug.

In a prospective study with high frequency auditory monitoring (Van der Hulst *et al.,* 1991) no serious ototoxicity could be demonstrated in patients receiving vancomycin in the treatment of peritonitis.

From the above studies, it would appear that ototoxicity due to vancomycin would be reversible.

On the other hand, it appears that administration of gentamicin in conjunction with vancomycin enhances the potential ototoxicity of vancomycin.

Experimental evidence, relevant mechanism

Tange *et al.* (1989) studied the ototoxic liability of vancomycin at a dose of 80 mg/kg/day for 2 weeks in the mongolean gerbil and found no evidence of ototoxicity.

Lutz *et al.* (1991) studied the effects of vancomycin alone in guinea pigs. These were given doses as high as 200 mg/kg for 11 to 17 days. They found no changes in cochlear hair cell counts. There were no changes in auditory brainstem thresholds in response to tone pulses from 1 to 8 kHz.

In a multicentre animal study (Tange *et al*., 1989) they could not demonstrate microscopical hair cell damage in gerbils receiving high doses of vancomycin. Teicoplanin, a structural analogue of vancomycin did not result in ototoxicity used at doses of 75 mg/kg per day for 28 days. From both the clinical and the animal experimental studies, it is still not clear about the ototoxicity of vancomycin and analogues. There is no real evidence that vancomycin alone produces permanent ototoxicity as manifested by hair cell counts.

Brummet *et al.* (1990) reported on the ototoxicity of vancomycin alone and in combination with gentamicin in guinea pigs. The addition of a small dose of gentamicin (50mg/kg/day for 2 weeks) plus vancomycin in doses up to 200 mg/kg/day for 2 weeks resulted in a greatly increased ototoxic effect.

It is clear that vancomycin can greatly increase the ototoxicity of aminoglycoside antibiotics in experimental animals. The permanent ototoxicity

could be the result of a vancomycin-induced augmentation of aminoglycoside ototoxicity.

1.7.2.3. Antibiotics 3; Erythromycin

General pharmacology

Erythromycin is an antibiotic agent frequently used in patients with blood malignancies.

Structure

Erythromycin is one of the macrolide antibiotics. They are so called because they contain a many-membered lactone ring to which are attached one or more deoxy sugars.

Mechanism of therapeutic action

Macrolides exert their antibiotic effect by binding irreversibly to the 50S subunit of bacterial ribosomes. By binding to the ribosome, macrolides inhibit translocation of tRNA during the production of proteins under the direction of DNA. Although human cells also have ribosomes, these differ in size and structure from the ribosomes of prokaryotes.

This action is mainly bacteriostatic, meaning that bacterial growth and reproduction are inhibited, in contrast to bactericidal antibiotics which directly kill bacteria. Macrolides can be bactericidal in high concentrations.

Clinical Therapeutics

It is useful against infections by Gram positive cocci and bacilli, Mycoplasma and Legionella species in which it can inhibit bacterial ribosomal protein synthesis.

Ototoxicity: clinical evidence

Several clinical studies (Mintz *et al* (1983); Eckman *et al* (1975); Karmody and Weinstein (1977); Van Marion *et al.* (1978); Meyer *et al.* (1980); Hughes *et al.* (1984)) show the reversible cochleotoxicity of erythromycin, with an elevation of thresholds for all frequencies readily reversible after withdrawal of the drug. Ototoxicity associated with erythromycin is primarily reversible and recovery starts approximately 24 hours after cessation of the drug (Cramer, 1986).

Ototoxicity: Experimental evidence

The mechanism of reversible ototoxicity due to erythromycin is not known. Brummet *et al*. (1984) reported an experiment on guinea pigs. The guinea pigs were anesthetised and received a dose of 125mg/kg/hour of erythromycin lactobionate. An auditory brainstem response (ABR) was performed on the guinea pigs. The guinea pigs showed a change in the ABR to 8 kHz tone pulses. The first effect to be found was an increase in latency of the 4^{th} wave, followed in sequence by the other waves. When the drug administration was stopped, the ABR returned to baseline levels in the reverse order. The hearing loss due to erythromycin seems to affect frequencies used for everyday voice communication at the same time that it affects high frequencies. It is for this reason that patients can easily detect a change in hearing. This is therefore significantly different from the pattern of aminoglycosides ototoxicity primarily affecting high frequencies and, for this reason, remains unnoticed for a long time by the patient.

1.7.2.4. Cytotoxic agents (chemotherapy)

(i) Alkylating agents

1.Cyclophosphamide

General pharmacology

The main effect of cyclophosphamide is due to its metabolite phosphoramide mustard. This metabolite is only formed in cells which have low levels of ALDH. Phosphoramide mustard forms DNA crosslinks between and within DNA strands at guanine N-7 positions. This leads to cell death.

Cyclophosphamide has relatively little toxicity as ALDHs are present in relatively large concentrations in bone marrow stem cells, liver and intestinal epithelium. ALDHs protect these actively proliferating tissues against toxic effects by converting aldophosphamide to carboxyphosphamide that does not give rise to the toxic metabolites (phosphoramide mustard and acrolein).

Clinical Therapeutics

The clinical spectrum of activity for cyclophosphamide is very broad. The drug is effective in Hodgkin's disease and other lymphomas. Complete remission and presumed cures have been reported in acute lymphoblastic leukaemia of childhood when cyclophosphamide is used concurrently with other agents.

2.Melphalan

General pharmacology

This is a phenylalanine derivative of nitrogen mustard

Mechanism of therapeutic action

Melphalan is a bifunctional alkylating agent. Formation of carbonium intermediates from each of the two bis-2-chloroethyl groups enables alkylation through covalent binding with the 7-nitrogen of guanine on DNA, cross-linking two DNA strands and thereby preventing cell replication.

The general spectrum of action of melphalan resembles that of other nitrogen mustards, but melphalan has the advantages to be administered in a gradual but continuous manner by the oral route. This drug is useful in the treatment of multiple myeloma. The clinical toxicity of the drug is mostly hematological.

Clinical evidence of ototoxicity of nitrogen mustards.

Lawrence (Cummings, 1968) reported hearing loss in all 13 patients who underwent regional perfusion of the thorax for carcinoma of the lung. Retrograde aortic administration of nitrogen mustard and a tight pelvic tourniquet gave protection to the pelvic marrow and lower extremities. The dose of nitrogen mustard received was in the range of 0.6-1 mg/kg. The actual blood levels were higher because of the decreased circulating blood volume.

Conrad (Cummings, 1968) reported 3 cases of sensorineural hearing loss out of 8 patients with Hodgkin's disease who received large doses of nitrogen mustard. Three of the four extremities of each patient were protected from exposure by the use of tourniquets. 6 out of 8 patients complained of tinnitus.

Young (Cummings, 1968) reported on 12 patients with non resectable carcinoma of the lung who received selective perfusion with nitrogen mustard. The drug was administered in the superior vena cava in doses of 0.6-1 mg/kg.

Five patients out of 12 had objective hearing losses. Two additional patients had tinnitus.

Mahaley (Cummings, 1968) used C14 tagged nitrogen mustard to see where the effect took place. The maximum non lethal dose of 0.75 mg/kg in dogs was administered either via the inferior vena cava or the carotid artery. The dogs were sacrificed 45 minutes following the injection. The intravenous injections showed a higher degree of radioactivity in the 8th cranial nerve than in any other. Regional head perfusion via the anterior circulation or carotid system shifted the preference from the 8th to the 2nd cranial nerve. Regional perfusion via the carotid arteries apparently spares the cochlea and the cochlear nerve.

Experimental evidence of ototoxicity

Diamant (Cummings, 1968) administered nitrogen mustard to guinea pigs via the carotid route. He noted auditory and vestibular changes, but was not able to identify with certainty the site of action of the drug.

Schucknecht demonstrated the only reported histological evidence of nitrogen mustard ototoxicity in a 30 year-old woman treated with 0.8 mg/kg of nitrogen mustard (this lady also had radiation therapy and thiotepa) with subsequent immediate tinnitus, vertigo and a bilateral sensorineural hearing loss to 60 dB at 4 kHz. Temporal bone histopathology demonstrated total outer hair cell loss from the first 6mm of the basal turn of the cochlea. From 8mm inward to the apex hair cell, nuclei were visible, but with normal cochlear innervation and presumed normal vestibular hair cells (Miller JJ, 1985).

Research studies in cats following single dose of IV nitrogen mustard (1mg/kg) demonstrated near total loss of outer hair cells in the basal and middle turns of the cochlea and absence of cochlear microphonics 14 days later at the time of sacrifice. Additional studies in the guinea pig(1mg/kg) demonstrated a rapid decrease in endocochlear potential (EP) and cochlear microphonics (CM) 24 hours after injection of nitrogen mustard, and then gradual recovery of the EP at 1 week but with a large negative summating potential after nitrogen mustard injection (Rybak, 1986). The electrophysiologic effects of the absence of cochlear microphonics with outer hair cell loss and reversible endocochlear potential suggests a transient effect of nitrogen mustard upon the source of endocochlear potential (Rybak, 1986).

From the literature seen above, it appears that nitrogen mustard gives rise to irreversible ototoxicity. The incidence of ototoxicity seems to be around 50%. However, it is unclear whether those patients had nitrogen mustard beforehand. There is no mention of the concomitant use of other potentially ototoxic drugs and the “per course” risk is not clearly defined. It appears that the ototoxic risk may be associated with a total dose of 0.6-1 mg/kg. The dose used in the present study appears to be much higher than 0.6-1 mg/kg as patients may receive up to 4-5 mg/kg.

(ii) Vinca alkaloids

General pharmacology

Vincristine and vinblastine are antimitotic alkaloids derived from the Vinca Rosea plant. They are commonly called the vinca alkaloids.

Mechanisms of action

Vincristine and vinblastine produce nuclear changes in a variety of cells. The mechanism of action probably resides in arrest of cell division in metaphase by preventing the formation and function of microtubules. Vinca alkaloids inhibit DNA and RNA synthesis and inhibit protein formation. In addition, they interfere with cellular respiration, membrane transport systems, and phospholipid synthesis. Microtubule interactions may account for many of these effects.

Therapeutic actions

Vincristine can induce remissions in acute leukaemia in children. It is also indicated in the treatment of Hodgkin's disease and other lymphomas. Vincristine has neurotoxic and gastrointestinal effects which can cause more severe injury than its action on normal bone marrow. Vinblastine produces less neurotoxicity than vincristine. Vincristine induces a dose related peripheral and autonomic neuropathy.

Ototoxicity: clinical evidence

The neurotoxicity of vinca-alkaloids is well recognised (Turpin et al, 1982). As well as producing a peripheral neuropathy and a paresis or paralysis, vincristin can also affect the cranial nerves in different ways: oculomotor palsies, facial palsies, trigeminal neuralgias and paralysis of the 8th nerve. There are few reported cases of vincristine-induced ototoxicity.

Mahajan (1981) described a case in a 73 year old woman with 2 documented episodes of severe bilateral sensorineural hearing loss. This lady had a 60 dB

average loss across all frequencies. After the 6th and 7th 2mg doses of vincristine sulphate, which resolved 2 months after treatment.

Following their finding of a patient treated with vincristine and who had hearing loss (Lugassy and Shapira, 1990), Lugassy and Shapira (1996) conducted a prospective cohort study of the possible ototoxic effect of vincristine among patients treated for lymphoproliferative malignancies. No deleterious effect of moderate doses of vincristine on pure tone audiometry for air and bone conduction and on speech audiometry could be found. Nevertheless, the isolated finding of sensorineural hearing loss in the only patient who received a high dose of vincristine raises the issue of ototoxicity as a possible dose-related and dose-limiting side effect of vincristine.

Wright *et al* (2005) studied 99 subjects previously treated for ALL and their results showed that the children and youth who had ALL had poorer balance than the comparison subjects. They suggested that balance abilities in patients treated for ALL were compromised and that several factors were associated with this deficit. They also suggested that cranial irradiation may be partially responsible for this deficit. They also incriminated impaired conduction of sensory and motor pathways, due to vincristine-induced neuropathy may have occurred. Harila-Saari *et al* (1998) concluded that treatment of ALL in children principally with vincristine and methotrexate causes long-standing axonal injury throughout the nervous system and demyelination within the spinal cord. These changes are associated with clinical neurologic findings.

Ototoxicity: experimental evidence

Vincristine has been shown to destroy the sensory cells of the organ of Corti in rabbits as well as spiral ganglion cells and fibres (Serafy, 1981). Vinblastine, however, has been shown to destroy only the sensory cells of the organ of Corti, with sparing of the cells and fibres of the spiral ganglion (Serafy, 1982).

(III) Antimetabolites: Methotrexate

General pharmacology

Methotrexate (MTX) is a folic acid analogue. The enzyme dihydrofolate reductase (DHFR) is the primary site of action of most folate analogues. Inhibition of DHFR leads to partial depletion of the tetrahydrofolate cofactors that are required for the synthesis of purines and thymidylate. MTX is a potent inhibitor of DHFR. MTX is also capable of inhibiting RNA and protein synthesis. It slows the entry of cells into S phase.

Mechanisms of therapeutic action

MTX is useful in the management of ALL in children. However, it is of limited value in the treatment of adult leukaemias. It is also of value in non Hodgkin's lymphomas. It is also a powerful immunosuppressive agent and has been used for the prevention of graft versus host reactions that result from marrow transplant.

Ototoxicity

The only report of clinical toxicity was the study by Harila Saari *et al* (1998) mentioned above who concluded that treatment of ALL in children principally with vincristine and methotrexate causes long-standing axonal injury

throughout the nervous system and demyelination within the spinal cord. The demyelination was supposed to be due to MTX.

(IV) Cisplatin

General pharmacology

Rosenberg *et al* (1965) demonstrated that electrolysis products from a platinum electrode caused an inhibition of the cell division process in E. Coli bacteria.

Structure

Cisplatin is a neutral water-soluble complex with two labile chloride groups and two relatively inert amino-groups in a cis-configuration.

Mechanism of action

Cisplatin diffuses passively across the cell membrane into the cells. Cisplatin can react with a variety of molecular sites in the cell and the drug demonstrates a dose-dependant inhibition of DNA synthesis, indicating that DNA is the primary target of cisplatin (Harder and Rosenberg, 1970). The major biochemical effect of cisplatin on tumour cells is inhibition of replication.

Therapeutic actions

Cisplatin, when combined with bleomycin and vinblastine, is curative for 85% of patients with advanced, non seminomatous testicular cancer. Cisplatin is also beneficial for carcinoma of the ovary, cancers of the bladder, head and neck and endometrium; small cell carcinoma of the lung and lymphomas.

Ototoxicity: clinical evidence

Elevation of auditory thresholds at the high frequencies was an early observation after treatment with cisplatin (Rossof *et al*., 1972). Ototoxicity has subsequently been reported in several clinical studies with a considerable variation in the incidence and severity of hearing loss. The hearing loss typically affects the high frequency range from 8 to 12 kHz (Tange *et al*., 1985) and is usually detected only by audiometric evaluations. The importance of monitoring high frequencies was emphasised by a study of Fausti *et al* (1984). Repeated administration of cisplatin affects successively lower frequencies with progressive hearing loss and difficulty in perception of speech in the presence of background noise (Koppelman, 1988) but sudden hearing loss can also occur. The hearing loss may be uni or bilateral and is more likely to occur with a high-dose regimen. The incidence values of cisplatin ototoxicity vary from 40 to 65% (Van Zeyl *et al.,* 1984; Vermorken *et al.,* 1983).

Ototoxicity: experimental evidence

Permanent hearing loss caused by ototoxic drugs always seems to be associated with hair cell loss in the cochlea. Studies in the guinea pigs (Fleischman *et al*., 1975; Estrem *et al.,* 1981; Nakai *et al*., 1982) have shown that the cochlear lesion caused by cisplatin is very similar to the one caused by aminoglycosides.

Cisplatin ototoxicity may result from oxidative stress, DNA damage, and inflammatory cytokines (Schmitt *et al*, 2009).

Wang *et al* (2004) already showed that the pattern of hair cell loss correlated with the electrophysiologic determination of a high-frequency hearing loss.

Cisplatin-induced apoptosis of OHCs, IHCs, and nonsensory cells of the organ of Corti correlates well with the findings of previous reports (Alam *et al*, 2000; Wang *et al*, 2003; Huang *et al*, 2000).

Although vestibular function has rarely been objectively monitored during cisplatin therapy, there are several reports of the absence of symptoms of vestibular toxicity (Aguilar-Markulis *et al*., 1981; Reddel *et al.,* 1982; Strauss *et al*., 1983), suggesting that this is an uncommon complication of cisplatin therapy.

1.7.3. Conclusion

Of all these agents the aminoglycosides are the only agents documented to carry a recognised (as opposed to anecdotal) risk of irreversible ototoxicity.

There is good evidence that out of all drugs used in the treatment of patients with haematological malignancies aminoglycoside antibiotics are by far the most ototoxic (ie: affects hearing and balance). There is a possibility of both separate and/or synergistic interaction between AGs and the other drugs mentioned.

The possibility of independent ototoxic risk with these other agents was explored by literature review summarised above.

Table 1.4 summarises the different drugs used in the treatment of haematological malignancies with their ototoxocity.

Table 1.4

Drug	Ototox clinical evidence	Ototox experimental evidence	Evidence of synergistic action between drug and AG	Mechanisms of ototoxicity
Gentamicin	+	+	+	Free radicals with excitotoxicity, possible Ca++/phospholipid effects
Vancomycin	-	-	+	
Erythromycin	+reversible	-	?	?
Alkylating agents	possible	possible	possible	?
Vinca Alkaloids	+	-	likely	neurotoxicity
Methotrexate	-	-	possible	Demyelination
Cisplatin	+	+	+	Free radicals, cytokines, DNA damage

1.8. AIM OF THE STUDY

The aim of this study was to establish the relative risk of ototoxicity of aminoglycoside use in treating patients with haematological malignancy.

1.9. HYPOTHESES

The working hypotheses of this thesis are centred about the assumption that AG exposure, together with other potential cochlear and vestibular toxins may result in both recognised and unrecognized neurotoxic damage to the two

systems. The hypotheses can be summarised as follow: Does exposure to drug combination with or without aminoglycosides lead to:

1. Damage over the speech frequency range and detected by standard PTA (0.25 to 8 kHz).

2. Subclinical damage to the higher frequency range detected by the HFPTA (10-16 kHz).

3. Damage over 2-6 kHz in DPOAE generation.

4. Balance deficit detected by the customised dynamic posturography.

The primary testing of these main hypotheses formed the experimental basis of Chapters 4-7. This was done by investigating the above hypotheses based on the measures of auditory and vestibular function.

CHAPTER 2: PATIENTS AND METHODS

2.1. PATIENTS

2.1.1. Ethical approval

Ethical permission was obtained from the Research and Ethics committee of the Leicestershire Health Authority. Before taking part in the study, the patients were seen by their haematology consultant and judged well enough to take part in the study. The patients were recruited from the haematology outpatient department at the Leicester Royal Infirmary. Recruitment was carried out randomly, without prior knowledge of treatment history.

2.1.2 Patient recruitment

A total number of 50 patients participated in this study. This number was limited by the patients being treated within the haematology unit.

The patients were recruited from the haematology outpatient department when they went for their routine follow up. They were first given an information sheet to explain why the study was being carried out, the nature of the tests and how they would be performed. All patients gave their written and informed consent and were told that they were free to withdraw from the study at any time. Prior to the study, patients were asked to complete a questionnaire on hearing loss/balance disorders prior to their blood malignancy. The questionnaire specifically asked about a past history of familial deafness, birth

trauma, meningitis, chronic middle ear disease or surgery, use of ototoxic agents and noise exposure.

The above information was taken into account when analyzing the results. Only patients who took potentially ototoxic medications prior to their diagnosis of blood malignancy were excluded from the study.

2.1.3 Patient group

The patient group is described in chapter 3 profiling patients demographics and disease as well as hearing and balance disturbances.

2.1.4 Patient Medical History Review

The patients' files were retrospectively and comprehensively reviewed for therapeutic history. This included recording: Gentamicin blood levels and renal function; Brain CT scans to exclude any brain metastasis. Drug charts for each drug; duration, number of courses of treatment and total dose of each drug. Individual doses were entered into spreadsheets, then added up for each drug, to obtain a total dose. The route of administration of the drugs was also noted.

2.2. OTOTOXIC RISK

A categorical risk was calculated based on the literature review of potentially ototoxic drugs (In Goodman and Gilman, 1975). Using these references to develop criteria, the categorical risk for antibiotics and chemotherapeutic agents was drawn up as in table 2.1.

Table 2.1

		Risk		Risk
Chemotherapeutic agents 1				
-Vinblastine	Up to 80 mg	1	>80 mg	2
-Vincristine	Up to 10 mg	1	>10 mg	2
Chemotherapeutic agents 2				
Melphalan	<200 mg	1	>200 mg	2
Cyclophospamide	<15 g	1	>15 g	2
Cisplatine				2
Aminoglycosides	<5 g	1	>5g	2
Reversible ototox				
Erythromycin				1
Vancomycin				1
Teicoplanin				1

2.3. AUDIOMETRIC METHODS

All patients underwent standard otoscopic examination to establish a patent ear canal and evidence of disease or trauma prior to performing audiometric testing. Tympanometry was then performed to establish a normal middle ear function, as determined by the presence of a normal type A tympanogram. Patients with abnormal findings on otoscopy or tympanometry were not excluded from the study, but a note was made of the results and these were taken into consideration when analysing their data.

2.3.1 Standard Pure Tone Audiometry

All fifty patients underwent standard pure tone audiometry (PTA) testing. Standard PTA testing was performed at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz using a KC50 audiometer and TDH49 headphones. Both the audiometer and the headphones were calibrated before and after the study within the

Department of Medical Physics in the Leicester Royal Infirmary. Care in headphone placement was taken to reduce the effects of suboptimal positioning on subject estimation of higher frequency thresholds (4-8 kHz) in the standard PTA (Flottorp, 1995).

All tests were performed in a standard soundproofed audiology booth in the Department of Medical Physics at the Leicester Royal Infirmary. The standard PTA test procedure was carried out according to BSA recommended protocol (Anonymous, 1981; Shipton, 1988, British Journal of Audiology, 1981 and 1985) with thresholds determined with a 5 dB resolution. In cases of marked unilateral hearing loss, bone conduction with narrow band masking noise was delivered to the contralateral ear when the threshold at test frequency was >40dB above the threshold in the contralateral ear (British Journal of Audiology, 1986).

Criteria for determining hearing loss

Criteria employed for establishing a standard pure tone audiometry hearing loss were based on a study done by Lutman and Davis (1994) and Davis (1983).

The criteria applied for evidence of pure tone hearing loss from the standard PTA in the patients with haematological malignancy up to the age of 30 were

based on the criteria of thresholds consistently measured at 25 dB HL or above at two or more frequencies. Over the pure tone frequency range of 0.25 to 8 kHz this was generally equivalent to the 90th percentile value obtained in the study carried out by Lutman and Davis (1994) where they had examined the hearing thresholds in 300 subjects. Over the age of 30, age matched 90th percentile values derived from a separate study from Davis (1983) were used for criteria in the UK and in Ireland. Therefore, patients who had thresholds above the 90th percentile either in the below or above age 30 group were considered having a threshold elevation most likely due to drug exposure whilst in hospital receiving treatment for their neutropenia and/or disease. Patients below the 90th percentile were considered having normal hearing unaffected by the treatment. The age matched percentiles were used to allow group analysis. The 90th percentile was used to remain conservative when considering hearing loss, therefore comparing patients' thresholds with the worst possible unscreened population's thresholds.

Male and female thresholds were then plotted and the percentile distribution relative to the 50th percentile was calculated. This percentile estimate of threshold was then plotted against categorical risk. The statistical significance of the distribution was done using a one-tailed Poisson test. The correlation between the categorical risk and the percentile threshold was then calculated by Spearman's rank correlation.

2.3.2. High Frequency Pure Tone Audiometry

All fifty patients underwent high frequency pure tone audiometry testing. High frequency pure tone audiometry (HFPTA) was used to measure thresholds at

10, 12, 14 and 16 kHz, using ER-2 insert earphones. When driven with a 1-V root mean square (rms) signal, the ER-2 generated 100 + or-2 dB sound pressure level (SPL) over a range of 10 to 16 kHz within the coupler. Signals over the range of 10 to 16 kHz, to a maximum of 1-V rms, were generated separately by a function generator (TG 230) and fed into the external input of the KC 50. The KC 50 was then used as an attenuator for these signals, as in standard PTA (Mulheran *et al*, 2001).

Use of Exponential fitting regime to calculate the 50th percentile

In the absence of clinically accepted standards for HFPTA thresholds, the criteria for establishing normal HFPTA thresholds in patients with haematological malignancy were based on a group of control subjects (N=53). These subjects were recruited from Leicester University and the Leicester Royal Infirmary. The median for each frequency (10-16 kHz) was calculated using an exponential fitting regime as the method used to calculate the percentiles in Lutman and Davis study (1994). The patients' thresholds were plotted for each frequency. The distribution of thresholds on either side of the 50th percentile was analysed using the statistical tests described in section 2.5.

2.3.3. Otoacoustic emissions

Distortion product otoacoustic emissions (DPOAE) recordings were made using commercially available equipment (Otodynamics ILO 92, Hatfield, UK). Measurements were performed using the growth function paradigm. This measures the distortion products generated by the cochlea as a function of the intensity level of the two primary stimulating tones f_1 and f_2. For the

purposes of this study analysis of the $2f_1 - f_2$ distortion product alone was made. Stimulus parameters were: f_1dB = f_2dB; f_2/f_1= 1.22; stimulus range 35-70 dB SPL in 1.5 dB increments.

Distortion product OAEs were obtained for f_2 = 2, 4 and 6kHz. These values of f_2 were chosen to enable a reasonable resolution of distortion product emission generation to be made over the mid to higher frequency range particularly as animal models of gentamicin ototoxicity show that ototoxic damage progresses from high to low frequency along the cochlea (eg Aran, 1981).

No higher frequencies were utilised as 6 kHz was the upper frequency that could be generated by the ILO 92. The 2 $f_1 - f_2$ distortion product OAEs at each frequency were analysed off-line. For each plot the stimulus intensity required to generate an emission with an amplitude of –10 dB SPL was measured. This value was chosen as it was sufficiently above the noise floor of the system to enable measurement of the emission to be made with confidence.

Each point of the plot was obtained by averaging at least 16 samples. The noise floor during measurement was typically kept below –15 dB SPL. Where necessary, further averaging was carried out to reduce the noise floor, especially about the –10 dB SPL iso-dP to ensure accuracy of measurement. In some cases the DPOAE plot was extrapolated to the point where its locus would intersect with the –10 dB value.

Calibration of the ILO 92 system was performed as recommended by the manufacturer (Otodynamics Ltd. Hertfordshire). Operation of the ILO 92 system electronics with its host computer was carried out by running an internal self diagnostic routine. The electrical output of the stimulus transducers was found to be within +/- 1% of specification. The probe microphone was calibrated using an Etymotic ER2 earphone as a signal source coupled to the probe via a B&K2 cc coupler. The ER2 was driven to produce a 60 dB SPL swept pure tone signal over 500 Hz to 6 kHz, the microphone output was then measured by ILO92. Stimulus calibration was carried out by coupling the ear probe housing the drive units to a B&K 4134 microphone by a B&K 2 cc coupler. The sound pressure levels generated were measured by a B&K 2608 amplifier. The output from the probe drivers and the sensitivity of the microphones remained stable (within +/-1dB) over the period the study was carried out (Kemp *et al*, 1990).

2.4. INTEGRATED BALANCE ASSESSMENT

Prior to balance testing by computerised posturography, standard neuro-otological examination was carried out. This consisted of the following tests.

Romberg test. The patient stands still with his eyes shut. If there is loss of balance, this indicates incompletely compensated unilateral lesion or bilateral vestibular damage.

Unterberger test. The patient is asked to march on the spot with the eyes shut. The amount of rotation to the right or to the left greater than 30 degrees implies asymmetrical labyrinthine function, with the patient rotating usually to the weaker side.

Head thrust test. The patient looks at a distant object and the examiner suddenly rotates the head on one side. The head is then rotated in the other direction. If repeated saccadic corrections of the eyes are needed to return to the target, this is suggestive of VOR gain on that side.

Head shake test. The patient is asked to actively shake his head for 15-20 seconds. After he stops, the examiner immediately looks at the eyes of the patient. If a nystagmus is observed and there was no nystagmus prior to head shaking, this is suggestive of statically compensated peripheral lesion.

Assessment of cranial nerves integrity. The twelve cranial nerves are tested

Assessment of reflexes. The knee jerk and ankle reflexes are tested.

2.4.1 Computerised Dynamic Posturography

Computerised dynamic posturography was chosen as the least invasive balance function testing procedure. Although the guidelines of the American Society of otolaryngology/head and neck surgery recommend the use of ENG to detect vestibulotoxicity, posturography was used, in agreement with the haematologists, in patients with blood malignancies as the amount of tests

had to be kept to a minimum and because some patients needed customized vestibular rehabilitation. Posturography was also used because a significant amount of patients in the present study took vinca alkaloids. These drugs are known to be neurotoxic. There is a vast amount of literature stating that patients taking these drugs have balance problems, but of motor origin (Wright *et al*, 2005). Posturography has the advantage of being able to assess both motor and sensory components of the balance system. In clinical, experimental and patient welfare respects it has significant advantages over caloric testing and electronystagmography. In particular, caloric testing would very likely result in unacceptable discomfort in this patient group.

To limit the time spent by patients undergoing testing, we chose posturography that is of value in detecting which part of the balance system is affected. It is also extremely useful in planning the treatment in terms of vestibular rehabilitation.

An equitest posturography machine was used and was calibrated according to the manufacturer's recommendations. The test was carried out in a dedicated room of the Audiology Department at the Leicester Royal Infirmary. The patient's height was first measured and this, along with age, was then entered into the computer. The patient was asked to take his shoes off, then, secured in a harness, positioned on the equitest platform. The patient was then attached to straps on the upper frame of the equitest machine. The test was then started when the patient stated he was ready and could be temporarily interrupted or abandoned at the patient's request.

Description of the equitest

The test was divided into two parts: the first part evaluates the Sensory Organisation Testing (SOT), whilst the second part evaluates the Motor Control Testing (MCT). The SOT contains six conditions and for each condition the patient performed three trials, with each trial given a score out of 100. The computer then takes the mean of the three trials and gives a score out of 100 for each condition. A score of 100 means that no sway is detected, whilst a score of 0 indicates severe instability. The outcomes of both sets of tests were age and height corrected by an internal program within the equitest computer. Unfortunately, after direct request to the manufacturer explicit plots of the corrective data were not made available for direct inspection.

The Motor Control Testing is made of small, medium and large backward and forward translations. The results of the tests were age and height corrected by an internal program within the equitest computer. Again, explicit plots of the corrective data were not available from the manufacturer for direct inspection.

It is well recognised that at rest in an upright position, normal subjects exhibit considerable sway (Kollegger *et al.*, 1992). Within the equitest system, normal sway patterns at rest, or when challenged, are recorded. However, movements within a cone of +/- 12.5° are recorded by the equitest system as equivalent to “100”. Degrees of movement outside this cone (age and height corrected) are assigned decreasing values of postural stability down to “0”.

The six conditions of the SOT testing are as follows:

Condition 1: Eyes open, horizon stable, platform stable: measures the normal sway, the “internal system noise”

Condition 2: Eyes closed, horizon stable, platform stable: measures the normal sway when the visual inputs are removed. The subject has to rely on his somatosensory and vestibular inputs only.

Condition 3: Eyes open, horizon swayed, platform stable: the visual informations are altered by the horizon sway and the subject has to rely on his somatosensory and vestibular inputs.

Condition 4: Eyes open, horizon stable, platform swayed: the somatosensory informations are altered by the platform sway and the subject has to rely on his vestibular and visual inputs.

Condition 5: Eyes closed, horizon stable, platform swayed: the visual informations are absent and the somatosensory informations are altered by the platform sway. The subject only has his vestibular inputs to rely on.

Condition 6: Eyes open, horizon swayed, platform swayed: the visual and proprioceptive informations are altered and the subject has to rely on his vestibular inputs.

The age-matched limits of normality of function were taken from the Handbook of Balance Function Testing. They are shown in table 2.2. The results of patients with blood malignancy were compared with those data.

Table 2.2

SOT CONDITION	20-59 (n=112)	60-69 (n=54)	70-79 (n=29)
1	94 (90)	94 (90)	89 (70)
2	92 (85)	92 (85)	86 (63)
3	91 (86)	89 (80)	88 (82)
4	82 (70)	82 (77)	78 (69)
5	69 (52)	69 (51)	62 (45)
6	67 (48)	67 (49)	53 (27)

Table 2.2: Means with 95th percentiles in brackets. SOT scores taken from Jacobson GP, Newman CW, Kartush JM, Handbook of Balance Function Testing (eds), (1993) St Louis, Mosby-Year Book, Inc. A sway within a cone of 12.5 degrees is regarded as normal and on CDP, the subject is given a score of 100. If the subject sways more than 12.5 degrees, the score is a function of the amount of sway. If the sway is to the extent that the subject falls, a score of 0 is then assigned. Scores between 0 and 100 represent the amount of sway between a fall (score=0) and the normal limit of sway (score=100).

Figure 2.1

Condition	Vision	Support	Patient Instructions
1 	Normal	Fixed	Stand quietly with your eyes OPEN
2 	Absent	Fixed	Stand quietly with your eyes CLOSED
3 	SwayRef	Fixed	Stand quietly with your eyes OPEN
4 	Normal	SwayRef	Stand quietly with your eyes OPEN
5 	Absent	SwayRef	Stand quietly with your eyes CLOSED
6 	SwayRef	SwayRef	Stand quietly with your eyes OPEN

Figure 2.1: The 6 sensory organisation tests conditions showing which sensory input cues are available or accurate for each condition. Adapted from the Equitest manual, Neurocom sensory Impairment Assessments, Neurocom protocols, Neurocom International. Inc.

The SOT sensory ratios were also taken into account for the results analysis.

These ratios give information about the relative performance of each sensory component in determining postural control.

The somatosensory ratio

The somatosensory ratio is derived from Condition 2/Condition 1 and normally returns a value greater to or about 1. A low ratio means relative sway increases with the eyes closed and the lower the ratio is taken to indicate that the subject makes poor use of the remaining somatosensory references.

The visual ratio

This derives from the ratio of Condition 4/Condition 1 and is based on assessing whether sway increases when somatosensory cues are inaccurate. A low ratio would mean relative sway increases further when the somatosensory cues are nullified and this low score is taken as indicating that the subject is making poor use of visual references.

The vestibular ratio

This derives from the ratio of Condition 5/Condition 1 and measures whether sway increases when somatosensory cues are inaccurate. A low ratio would mean relative sway increases further again when visual cues are removed and somatosensory cues are nullified. A low score is taken as indicating that the subject makes poor use of vestibular cues, or that vestibular cues are unavailable.

2.5. STATISTICAL ANALYSIS

Statistical analysis was preceded by review of each of individual patient's data set. The following overview revealed that the distribution of variables such as drug exposure were not normally distributed and also showed an absence of clear ranking effects with exposure to drugs. This in part reflected the very heterogeneous make up of the patient population. This necessitated that much of the data set was then considered on an individual basis.

Categorical analysis was then carried out using predefined criteria based on previous studies (PTA and DPOAEs and posturography). For HFPTA comparison of the patients' data sets was carried out against control gained from within the LRI and with other comparable studies.

Where analysed, the significance of pass/fail outcome frequency was tested by chi squared test. Following application of normality tests for the data sets, the sub-groups of pass/fail balance data in both the non aminoglycoside and aminoglycoside treated were compared by t-test. Correlation of effect on summated aminoglycoside therapy on severity of SOT scores was carried out using Spearman's rank correlation.

The Mann-Whitney or Wilcoxon two-sample test was also used throughout the thesis for non parametric testing to establish whether two independent samples come from the same distribution.

The Z-test was used to examine whether the distribution of samples could be approximated to either a normal or a symmetrical distribution.

The one-tailed Poisson test was used when thresholds were explicitly expected to be worse following treatment. If not, then a two-tailed test was used.

A significance level of $p<0.05$ was adopted throughout the thesis.

CHAPTER 3: THE PATIENT GROUPS: DEMOGRAPHICS AND DISEASE

3.1. PATIENTS

A total of fifty patients were recruited for this study. these were aged between 19 to 78 years with a median age of 46.5 years with 29 males:21 females. Although acute lymphoblastic leukaemias and chronic myeloid leukaemias are slightly more common in males, the gender distribution here was not significantly different from the 50:50 ratio. (binomial test). Interestingly, four patients out of the fifty who took part in the study were Asians. The Leicester population is about 40% Asian (Leicester City council, website:leic.gov.uk/council). If haematological malignancies were of equal incidence in the Asian race and in the Caucasian population, one would expect to see 20 Asian patients in this study of an overall 50 patients.

Incidence of haematological malignancies in Asians

Leukaemias

Pang *et al* (2002) concluded that the incidence of leukaemia, either considered as a whole or as individual types, did not appreciably vary between US-born and foreign-born Asian-Americans. Irrespective of their birthplace, Asian Americans possess one or more characteristics which make their risk for leukaemias less than that of US whites.

Multiple Myeloma

According to the Centers for Disease Control and Prevention, Division of Cancer, it was shown that black people had the highest incidence rate for myeloma. Hispanic people had the second highest incidence of getting myeloma, followed by white and

Asian/Pacific Islander people.

Non Hodgkin's lymphoma

Lisa *et al* (1996) concluded that the risk of diffuse lymphoma was similar in Chinese-and Japanese-Americans and US-born Whites. With the exception of follicular lymphoma, the basis for the relatively low incidence of NHL in Asian-Americans does not lie in exposures or characteristics that differ between the migrants themselves and their descendants.

Hodgkin's disease

Glaser and Hsu (2002) found that, given environmental and lifestyle differences between the US and Asia, the consistently low rates of HD in Asians suggest genetic resistance to disease development, possibly associated with HLA type. International and inter-ethnic differences, and risk factor patterns in case–control data, implicate environmental influences in the etiology of HD.

Although it appears from the above reviews that the incidence of all haematological malignancies seems overall lower in the Asian population when compared to the Caucasian race, the number of Asian patients in the present study is only four, assuming a 50% lower incidence than in Caucasians we would have expected to see 10. A binomial test was used to establish the significance. P was equal to 0.025. The reason for this low number of Asian patients in this study is unclear.

Patients and malignancies

The number of patients for each malignancy, together with the age range and the Male/Female ratio are presented in table 3.1

Table 3.1

Malignancy	Number	M/F ratio	Age range
AML	11	6/5	27-72
APML	3	1/2	40-49
CML	4	3/1	35-67
ALL	8	4/4	19-54
NHL	7	2/5	20-78
HD	7	5/2	21-43
MM	8	6/2	43-71
Waldenstrom	1	1/0	70
AA	1	1/0	23

These patients had at least one episode of neutropenia/sepsis and were treated with antibiotics with or without gentamicin.

Patients had finished their treatment no less than three months and no more than five years prior to the study. Patients exhibiting the following conditions were excluded from the study: any known neurological disease; chronic active otitis media; Meniere's disease; autoimmune disease; rheumatological disorders; previous joint replacement surgery; brain metastasis; history of central or peripheral balance disorder prior to diagnosis of haematological malignancy.

The above details were entered into master spreadsheet

Although there was an intention to perform all the tests on all patients, a proportion was unable to tolerate the full battery of tests due to their health status.

3.2. CATEGORICAL RISK

A categorical risk score was calculated for each patient as explained in chapter 2. The categorical risk was plotted against age and sex, shown in figures 3.1 and 3.2 respectively. The categorical risk was not found to be correlated to age and sex as the Spearman's rank correlation returned no significance. The mean categorical risk was calculated for each type of malignancy and is shown in Figure 3.3. Again, there is no trend to indicate that a malignancy has a higher categorical risk than another. Although all APML patients received aminoglycosides, the other malignancies are almost equally divided among patients who received AG as part of their treatment and those who did not get AG treated.

Figure 3.1. Categorical risk vs age

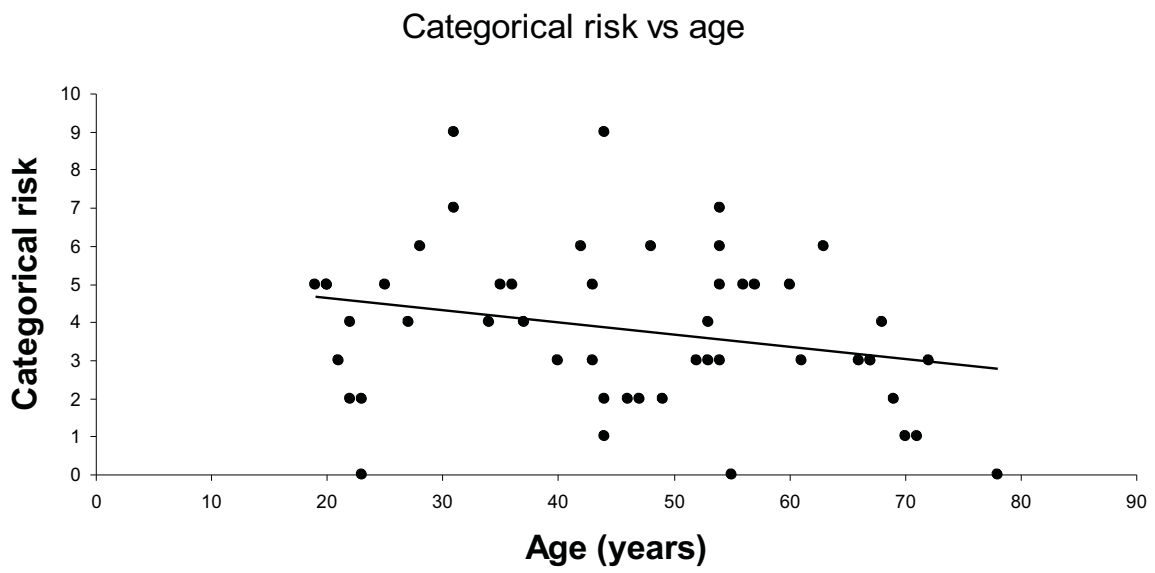


Figure 3.2. Categorical risk vs sex where Male=1 and Female=2

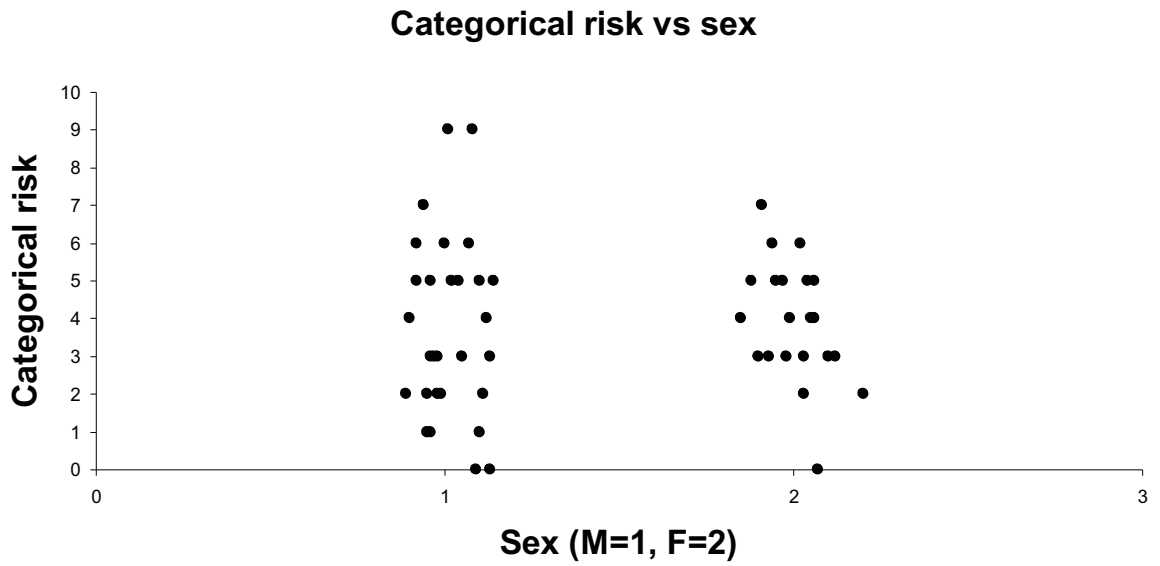
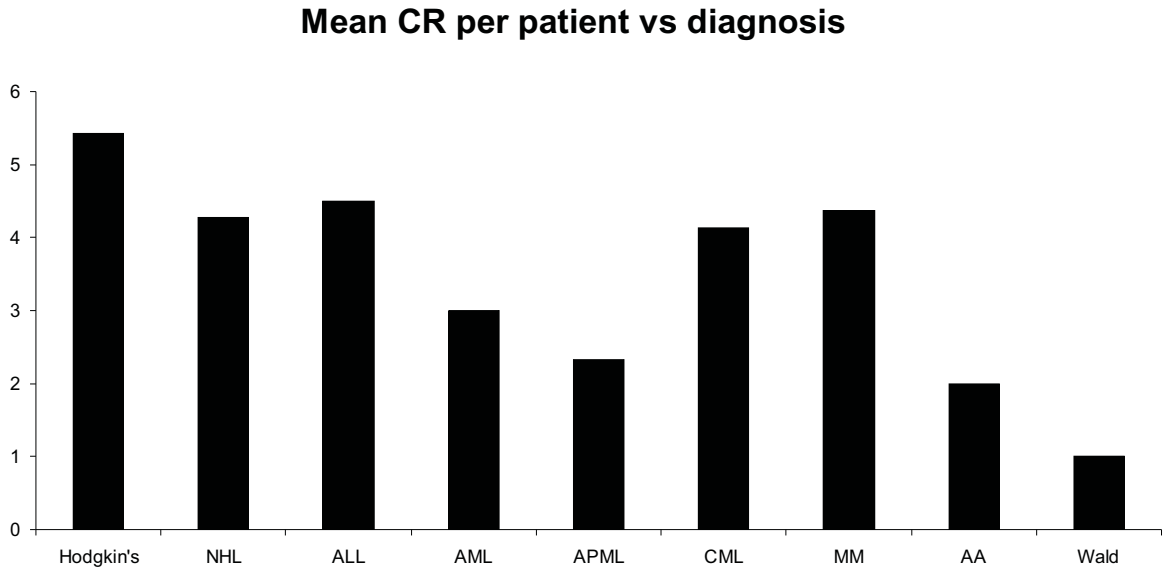


Figure 3.3: Categorical risk vs diagnosis



CHAPTER 4 : OCCURRENCE AND RISK OF COCHLEAR PERFORMANCE DEFICIT IN PATIENTS WITH BLOOD MALIGNANCY DETERMINED BY PURE TONE AUDIOMETRY.

4.1. INTRODUCTION: THE ROLE OF THE PURE TONE AUDIOGRAM IN CLINICAL DETERMINATION OF HEARING LOSS.

This chapter deals with results gained from the Pure Tone Audiometry or PTA, in determining the occurrence and relative risk of hearing loss in patients with heamatological malignancy. PTA in audiology provides the clinician with the information necessary to determine whether there is a hearing deficit most likely to cause difficulties in speech perception. These frank losses would usually be in excess of 30 dB HL over those frequencies important to speech perception ie from 100 Hz up to about 6 kHz (Moore, 1998).

In determining whether this loss was iatrogenic, particular care was taken to take into account the effects of other major factors with a recognised effect on hearing thresholds. The two major potential factors affecting the patients in this study were aging and prior noise exposure. These are discussed in sections 4.4.1 and 4.4.2. With aging, the availability of age-matched data assists making the judgement and the 90^{th} percentile values of Lutman and Davis (1994) and Davis (1983) for the UK population were generally used here to assist in decision making. However, it was not always assumed that elevated thresholds below the 90^{th} percentile were not partly due to drug therapy and the patients' history was taken into account when doing this. Noise exposure was not possible to quantify and the patients own description of severity and duration were used as qualitative guides.

The PTA results in this chapter form the basis of clinical judgement to the presence of frank hearing loss. The following chapter on High Frequency can only be used as a guide to likely iatrogenic damage to the cochlea This has more mechanistic relevance but can also be as a potential 'early warning' of cochlear damage above 8 kHz. Similarly, the results from the DPOAE chapter do not supersede the use of the PTA in making clinical judgement on degree of hearing loss but are used in determining site and mechanism of action of oto trauma.

4.2 STANDARD PURE TONE AUDIOMETRY

PTA is the "gold standard" clinical test in detecting hearing loss affecting speech frequencies. It is used universally to measure the subject's hearing thresholds (the minimum sound level at which a specific response can be obtained) at the frequencies ranging from 0.25 Hz to 8 kHz. This is the clinical norm (Browning, 1986).

To measure hearing, a logarithmic scale is necessary because the ear responds equally to equal multiples of sound intensity. This means that intensity is exponentially related to loudness perception. The bel is the logarithm (log) to the base 10 of the ratio of the sound intensity being measured to a reference intensity that is constant and is measured in W/m^2 (Pickles, 1991). When the magnitude of a sound is specified in decibels, the word "level" is used to refer to its magnitude. A given number of dB represents an intensity or power ratio, not an absolute intensity. In order to specify the absolute intensity of a sound it is necessary to state the intensity of a sound I1, that is some dB above or below a reference intensity. The reference intensity most commonly used is 10^{-12} W/m^2, which is equivalent to a pressure

of $2X10^{-5}N/m^2$ or 20 micro Pascals. A sound level specified using this reference level is referred to as a sound pressure level (SPL) (Pickles, 1988). The tympanic membrane has a surface area of more or less 1 cm^2, which would mean that at 0 dB SPL, the energy level would be 10^{-16} Watts.

Sound intensity in $dB=20\log_{10}$ sound pressure level (Pickles, 1991). Each dB increase represents a ten-fold increase in the intensity of sound ($\log_{10}10=1$), a 3.3 fold increase in sound pressure. The perception of intensity follows a logarithmic scale and for example an increase in 3 dB would double the loudness.

The auditory system is less efficient at detecting sounds at the upper and lower ends of the frequency spectrum than in the middle regions. The detection of sounds in decibels of sound pressure level (dB SPL) produces a pure tone audiogram that, in a normally hearing subject, would not be flat. This would make abnormalities very difficult to be identified in daily practice. A decibel scale of human hearing was designed so that 0 dB hearing level (HL) would be the expected threshold of detection of a pure tone irrespective of its frequency. The amount of energy at 0 dB HL at each frequency is not the same. It represents the threshold at each test frequency for a group of presumed otologically normal young adults (Moore, 1997).

In the dB HL scale, normal hearing subjects would be expected to have a flat audiogram at 0 dB HL. Pure tones of several frequencies are tested, usually 250, 500, 1000, 2000, 4000 and 8000 Hz for air conduction and 500, 1000 and 2000 Hz for bone conduction.

Masking is used to raise the threshold in the non-test ear using air-conducted sound. This overcomes any cross-hearing and allows an accurate and reliable assessment of the test ear for either air or bone conduction. Masking is essential and must be applied in all bone conduction measurements (Gray and Hawthorne, 1992).

4.3. OTOSCOPY AND TYMPANOMETRY

Before any investigations, a thorough otoscopic examination of the ear is mandatory as it may reveal some obstruction of the ear canal, abnormality of the ear canal, the tympanic membrane and the middle ear or signs of previous surgery of the tympanic membrane or middle ear. These can be important factors that confound threshold estimation.

Tympanometry is also useful as it measures volume compliance and middle ear pressure. Tympanometry varies the external auditory canal air pressure through a rubber seal that occludes the external auditory canal to create an airtight seal. Varying the applied canal air pressure from +200 to –400 mm daPa (1 daPa equals 1.02 mm H_2O) alters the stiffness and the position of the tympanic membrane. Tympanic membrane compliance is maximal when air pressure on both sides of the eardrum is equal. The tympanogram is the graphic display obtained by the use of tympanometry. The abscissa of the tympanogram records air pressure in daPa and the ordinate of the tympanogram records tympanic membrane compliance. The normal tympanogram usually peaks at 0 daPa. If the pressure within the middle ear is negative, the peak of the graph will be in the negative pressure zone of the tympanogram. Thus, the peak of the tracing along the longitudinal axis is an indirect indication of the middle ear pressure. The height of the tympanogram

is also evaluated because decreased tympanic membrane compliance (increased stiffness) will be manifest as a lower or absent peak (Bluestone and Klein, 1995).

The tympanogram also gives indication about the canal volume. The normal range is between 2 and 4 ml. If this value is elevated, this indicates a tympanic membrane perforation or a flaccid tympanic membrane (Lee, 1995).

4.4. USE OF PTA IN THE DIAGNOSIS OF DIFFERENT AUDITORY PATHOLOGIES

The shape of the PTA curve can sometimes suggest and/or confirm a clinical diagnosis. Typical curve examples are given below for the commonest pathologies encountered in “cochlear” hearing loss, which means a sensorineural hearing loss, with damage to the cochlea. In this study two primary confounding factors were presbyacusis and noise exposure.

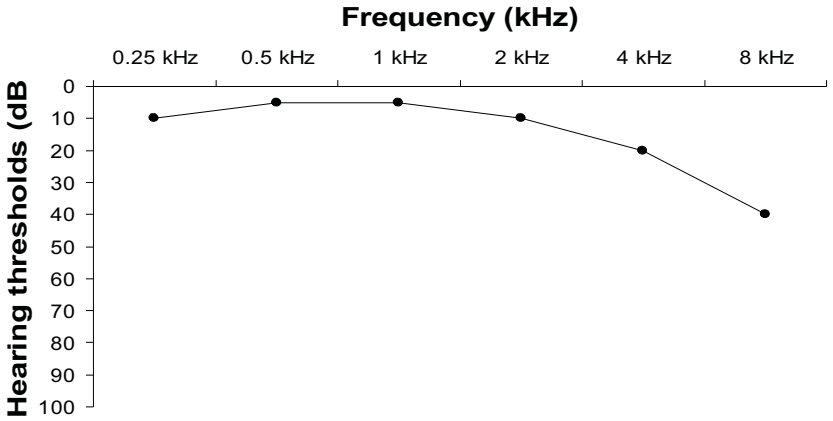
4.4.1.Presbycusis

Age-related hearing impairment or presbycusis is the most common sensory deficit in the elderly. Approximately 35% of people between 60 and 70 reports having hearing deficit (Huang, 2007), particularly when they try to converse with several people at a time, or when the background noise is too high. When a pure tone audiogram is performed in that aging population, it shows thresholds of 25 dB or more (average of the thresholds at the frequencies of 0.5, 1, 2 and 4 kHz). In its most typical form, age-related hearing loss is symmetrical, sensorineural and more pronounced in the high frequencies. Males are generally more severely affected than females (Huang, 2007). An

example of audiogram showing signs of age-related hearing loss is shown in figure 4.1.

Although there is a constant decline in hearing acuity with aging, age of onset, progression and severity of presbycusis show great variation, larger in the high frequencies and that increases with age. Age-related hearing loss is correlated with degeneration of hair cells, cochlear neurons and stria vascularis (Huang, 2007).

Figure 4.1 Typical audiogram of presbycusis

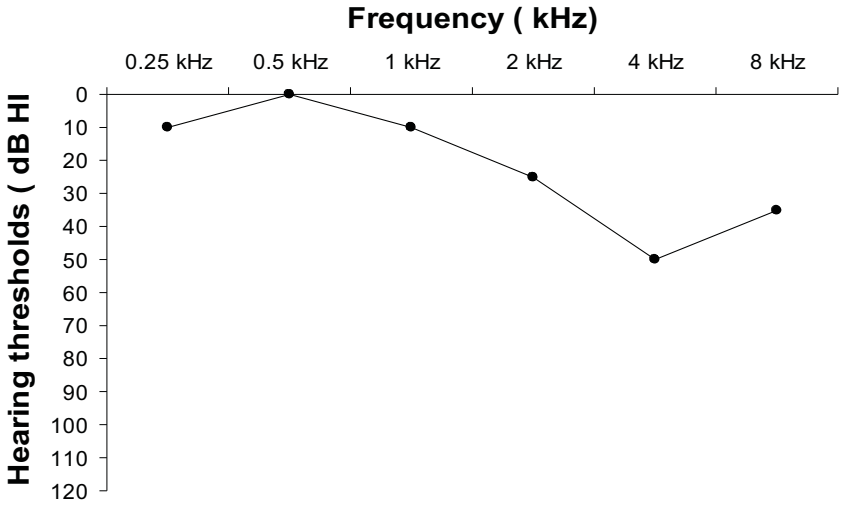


A previous exposure to noise is likely to have negative effects on the hearing but the interaction between NIHL and age-related hearing loss is difficult to determine (Rosenhall, 1993). The most commonly accepted assumption is a simple cumulative effect of noise and aging on the hearing (Gorai and Pal, 2006).

Recently, an interaction between NIHL and age-related hearing loss has been reported (Gates *et al.*, 2000). NIHL before old age reduces the effects of

aging at noise-associated frequencies, but accelerates the deterioration of hearing at adjacent frequencies (Rosenhall, 1993). Knowing that age-related hearing loss affects higher frequencies first and that NIHL very often affects the frequency of 4 kHz, the likely progression of the pattern of the PTA curve would therefore be sloping down from 4 to 8 kHz. An example of audiogram of a 70 year-old patient with previous noise exposure is shown in figure 4.2.

Figure 4.2: Typical audiogram of presbycusis with previous noise exposure. There is threshold elevation from the frequency of 4 kHz



4.4.2. Noise induced hearing loss

According to Arslan and Orzan (1998), noise-induced hearing loss (NIHL) has no specific or exclusive audiological signs. The disorder is identified on the strength of a high probability and as a result of an exclusion process. However, in daily practice, the threshold elevations are mainly observed at and around the frequency of 4kHz. Although the histopathologic correlate to chronic NIHL is injury to the cells of the inner ear, the pathogenesis involves interactions between all three divisions of the auditory system: the external,

middle and inner ears. The broadband noise seen in industry is converted by the fundamental resonance of the EAC to a 3 kHz noise. This leads to the characteristic 4 kHz notch seen on the audiogram in noise-exposed individuals (Dobie, 1993).

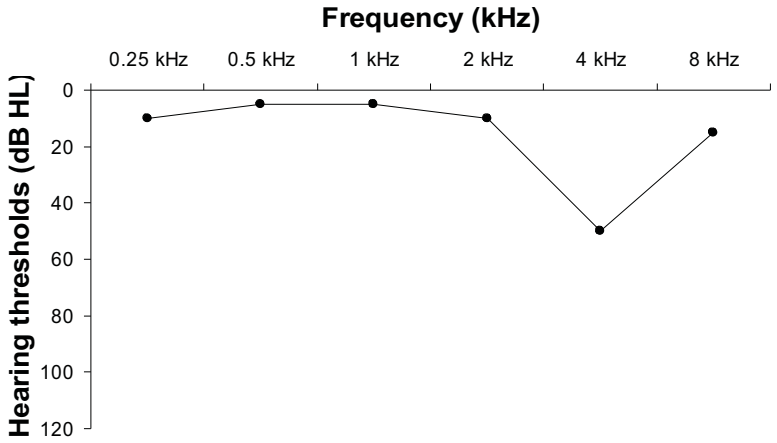
A typical audiogram of NIHL is shown in figure 4.3.

4.4.3 Relationship between noise exposure and ototoxins

Gratton *et al* (1990) explored the potentiation of cisplatin ototoxicity by noise in the chinchilla. The effects of exposure to cisplatin alone, noise alone or concurrent exposure to both agents were compared in terms of the threshold shift of the auditory evoked potential and the amount of hair cell loss. The study showed that the combination of cisplatin plus noise produced significantly more hair cell loss and hearing loss at the high frequencies than did either the noise or cisplatin alone when the noise level was 85 dB SPL or higher; there was no interaction when the noise level was 70 dB SPL. The amount of the interaction, when present, was constant regardless of the noise level. These results indicated that moderate to high levels of noise can exacerbate cisplatin ototoxicity.

Henderson *et al* (1999) showed that in spite of the differences in the nature of the insult, the hearing loss from ototoxic drugs and noise exposure share a number of similarities in cochlear pathology. They explored the common factors between noise-induced hearing loss and ototoxicity Their results suggested the role of reactive oxygen species in creating hearing loss and the potential protective role of glutathione.

Figure 4.3: Typical audiogram of a person with noise-induced hearing loss



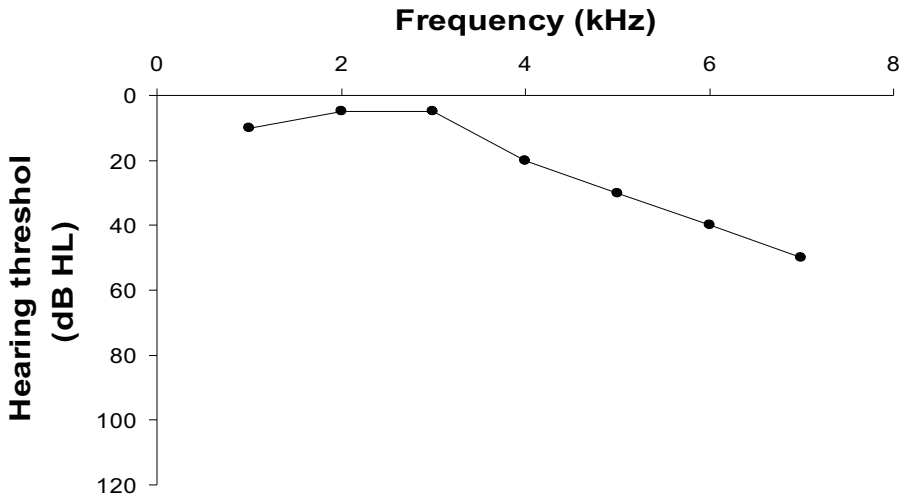
4.4.4. Ototoxicity

Ototoxic drugs can affect the ear in different ways. Only the drugs with a.documented ototoxicity and used in the present study will be mentioned here

(i) Aminoglycoside antibiotics: the threshold elevation will typically affect the high frequencies, with a steep slope, as discussed in length in the main introduction. An example of audiogram of a patient with aminoglycosides ototoxicity is shown in figure 4.4.

(ii) Cisplatin: Ototoxicity is associated with irreversible high frequency hearing loss and tinnitus (Schaefer *et al*., 1985) and the audiogram is similar to the one encountered in patients with aminoglycosides ototoxicity. The onset of hearing loss is usually delayed until after accumulation of multiple doses. One patient from this study had cisplatin therapy.

Figure 4.4. Typical audiogram of a patient with AG ototoxicity



4.5. PREVIOUS REPORTS OF HEARING LOSS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCY

There are very few studies relating the incidence of ototoxicity in patients with heamatological malignancy.

A study performed at Southampton hospital (El-Bakry, 1998) recruited twenty eight patients with blood malignancies over a two-year period. Those patients received gentamicin, often in association with beta-lactams when they developed febrile neutropenia. The standard treatment dose was 7 mg/kg, once daily. This dose is also the one used in the patient group tested in this study. The Southampton study measured cochleotoxicity by ways of pure tone audiogram, showing a threshold elevation in the high frequency range up to 8 kHz. The vestibulotoxicity was measured in one patient by caloric testing. There is an obvious lack of consensus in the study, with no mention of the risk or occurrence of ototoxicity in their neutropenic patient group. High frequency

pure tone audiometry was not performed, although it is known that those frequencies are the first ones to be affected by gentamicin. In one patient only, caloric testing was performed and was said to be abnormal, but there was no explanation of this findings. No other ototoxic drugs were explicitly reported as having been used.

This study by El Bakry (1998) was important as it raised the question of susceptibility of patients with haematological malignancy to ototoxicity. Apart from this study, little or no attention has been given to hearing loss and/or balance disorder in that patient group. In the Leicester Royal Infirmary, some awareness of potential ototoxicity in the patients with haematological malignancies was raised by Dr Andrew Swann, consultant microbiologist.

4.6. RESULTS

External and middle ear function

In the present study, the otoscopic examination revealed the presence of ear wax in ten patients. The wax was removed prior to testing. It also revealed a bilateral tympanic membrane perforation unknown to the patient. Bone conduction was performed twice in the study

The group of patients with blood malignancies was divided into two groups: one group who did not receive AGs or group 1 (N=17) and one group who received AGs (or group 2) as part of their treatment during their neutropenic state (N=33).

GROUP 1: Patients not receiving aminoglycosides n=17

Three patients have a strong history of noise exposure:

Case 1

A 44 year-old man used to serve in the British forces for 10 years with no ear protectors. He then worked in a timber factory, again with no ear protectors.

Case 2

A 55 year-old man worked on a building site since the age of 16 and never wore ear protectors, and already complained of subjective hearing loss since his thirties.

Case 3

A 71 year-old man worked as a maintenance engineer in a factory. He also used to go shooting. He never wore ear protectors neither at work nor during his leisure activities.

Patient with middle ear surgery

Case 4

A patient had bilateral mastoidectomy for cholesteatomas in childhood and was, since the operation, left with some degree of hearing deficit.

Patient with congenital hearing loss

Case 5

A patient suffered from congenital hearing loss, documented in the same hospital records. This patient was already fitted with bilateral hearing aids prior to her malignancy.

The thresholds of patients who did not receive gentamicin as part of their treatment are represented on a graph for each frequency, together with the age and sex corrected percentiles. The right and left ears are represented on the same graph, but males and females are on different graphs as males and females have different values for their percentiles.

Male thresholds vs age for the frequencies of 2, 4 and 8 kHz are represented in figures 4.5-4.7 respectively.

Female thresholds vs age for the frequencies of 2, 4 and 8 kHz are represented in figures 4.8-4.10 respectively.

Figure 4.5: Males, 2 kHz

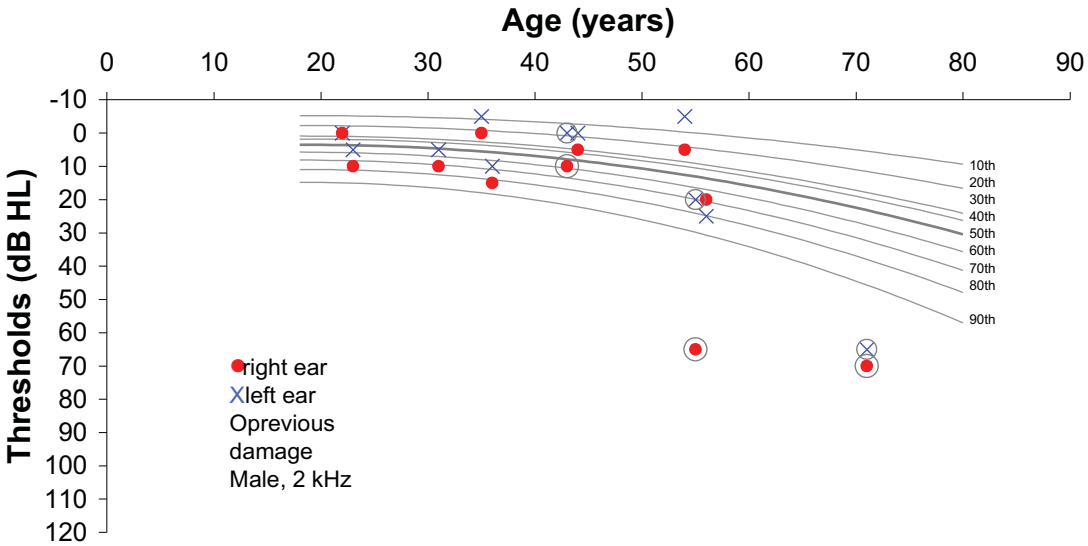


Figure 4.6: Males, 4 kHz

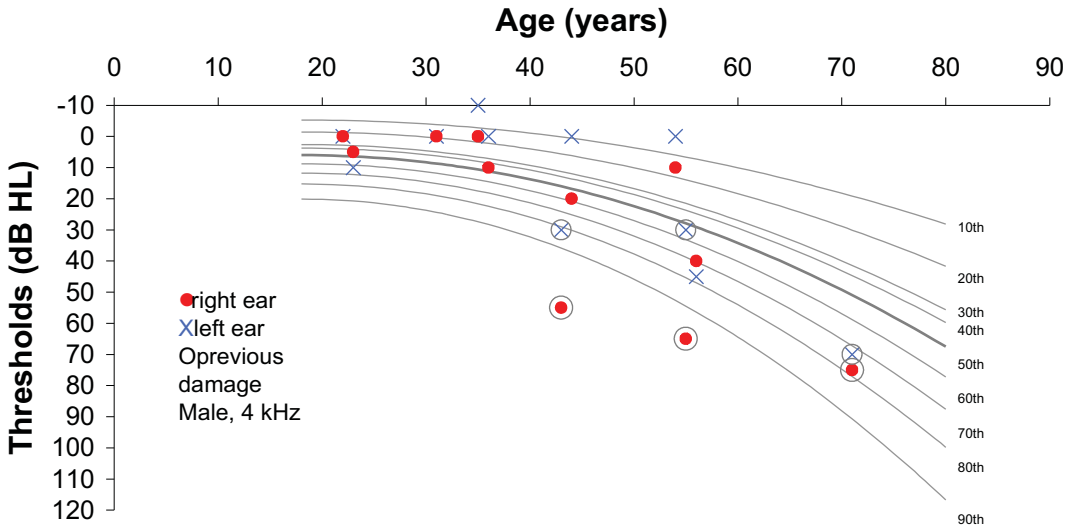


Figure 4.7: Males, 8 kHz

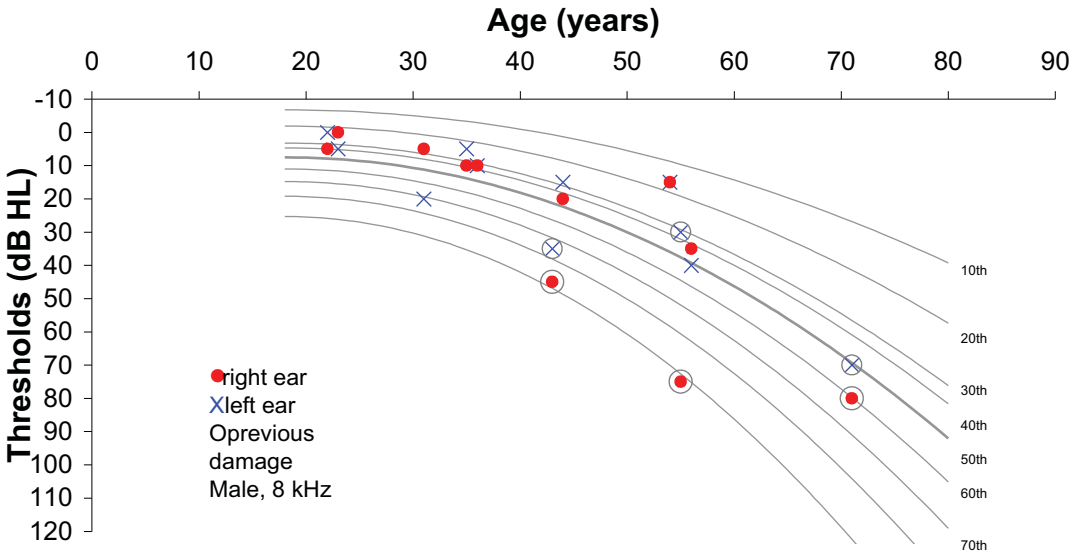


Figure 4.8: Females, 2 kHz

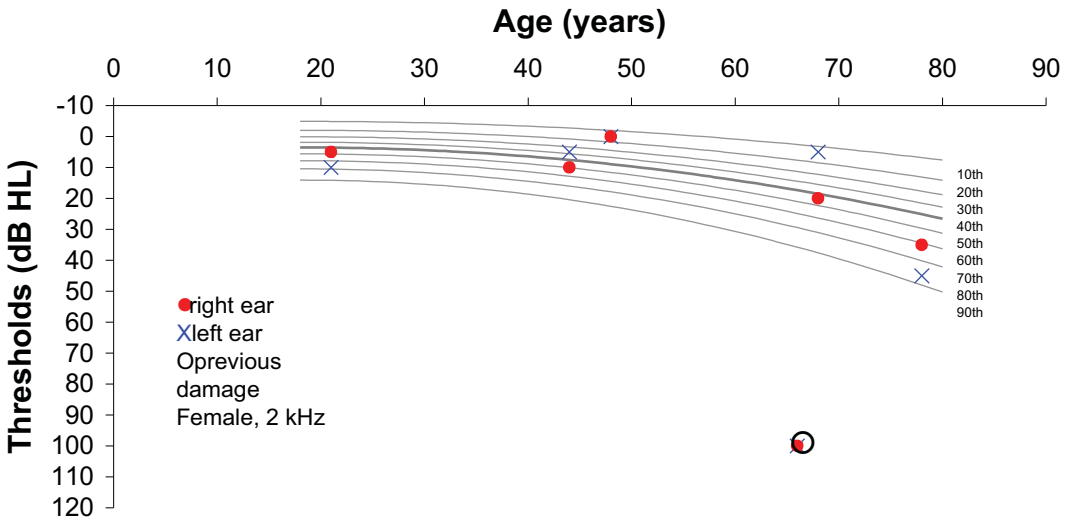


Figure 4.9: Females 4 kHz

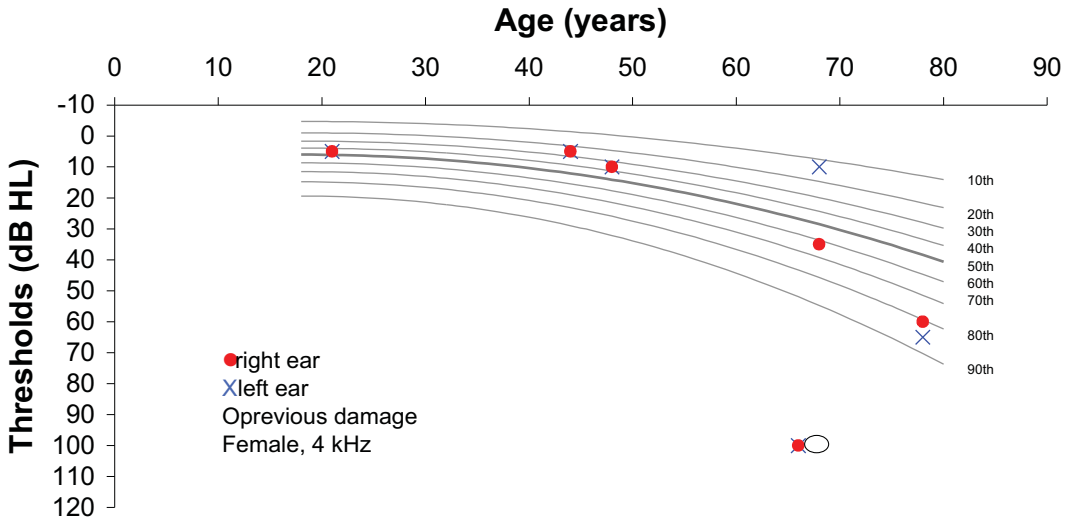
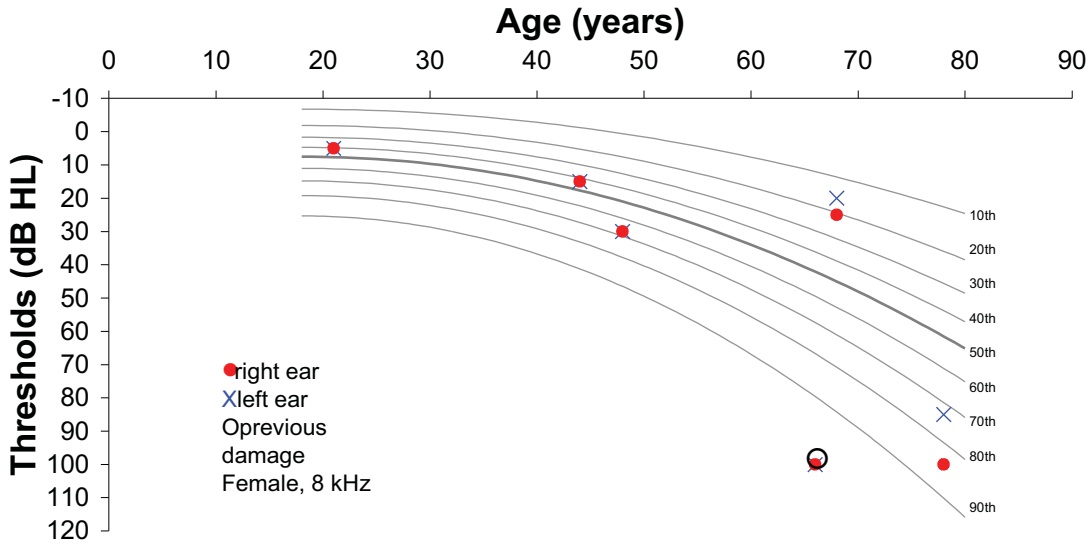


Figure 4.10: Females 8 kHz



In figures 4.5-4.10, the threshold of each patient fell into an age related percentile band. For each patient, the age related percentile band value (eg 30th-40th) for both sexes was then plotted against the categorical risk and represented in figures 4.11-4.13 for the frequencies of 2-8 kHz respectively.

Figure 4.11: 2 kHz

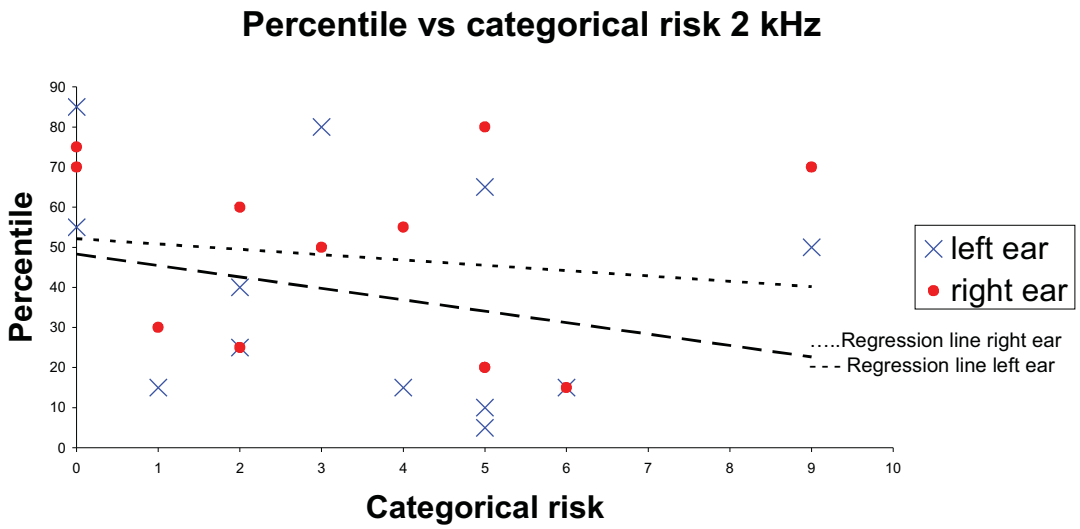


Figure 4.11. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 2 kHz

Figure 4.12: 4kHz

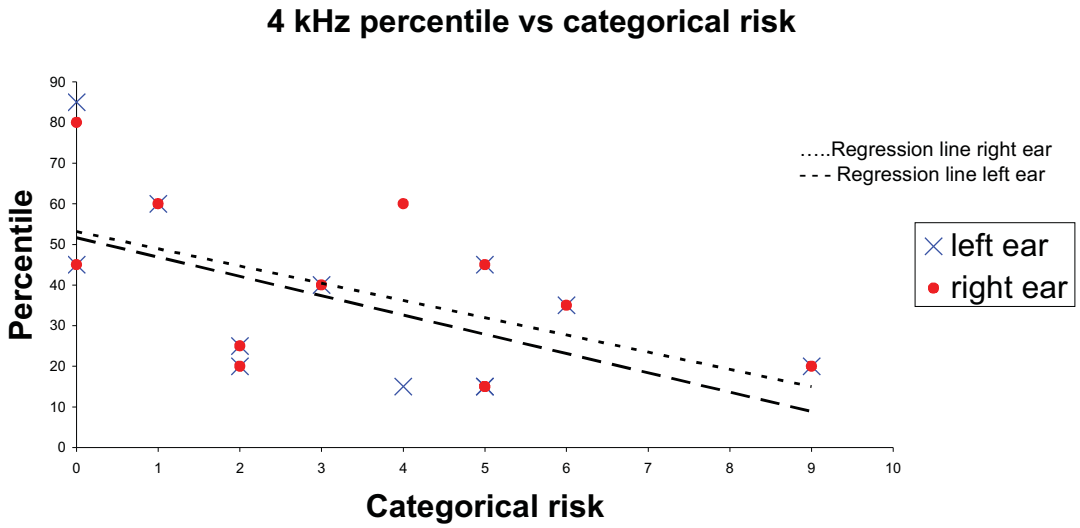


Figure 4.12. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 4 kHz.

Figure 4.13: 8kHz

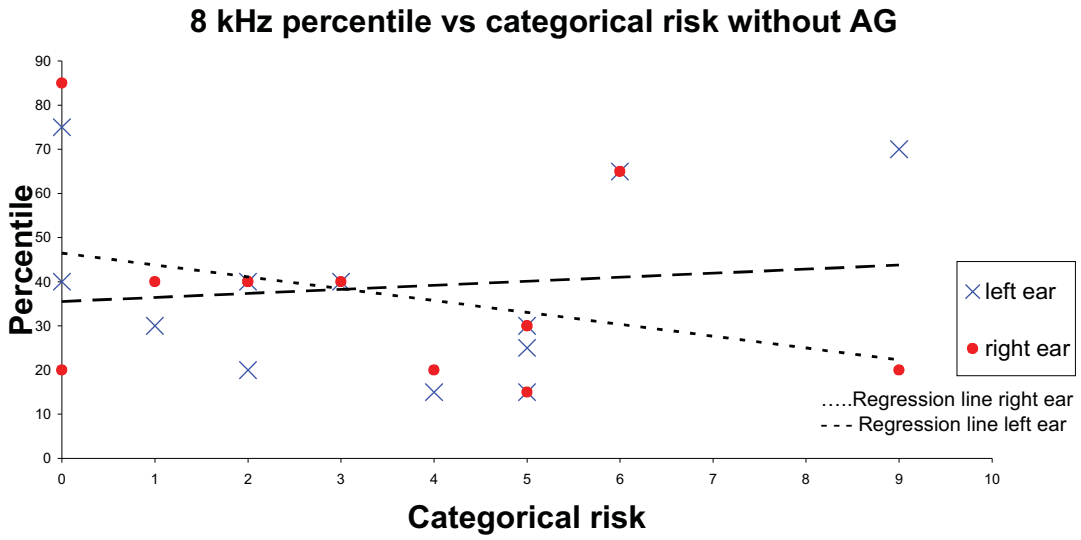


Figure 4.13. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 6 kHz.

Table 4.1 shows the correlation coefficients of percentile vs categorical risk for all the patients who did not receive AGs as part of their treatment (N=17). The values in brackets represent the correlation coefficients once the patients who had a loss prior to the study have been removed (N=12)

Table 4.1

	Right ear	Left ear
	Correlation coefficient	Correlation coefficient
2 kHz	-0.312 (NS) (-0.147 NS)	-0.294 (NS) (-0.270 NS)
4 kHz	-0.483 (NS) (-0.548 NS)	-0.5 (NS) (-0.590 NS)
8 kHz	-0.403 (NS) (-0.353 NS)	0.0819 (NS) (0.117 NS)

To see whether the patients of the non gentamicin treated group distribute equally on either side of the 50th percentile, patients above and below the 50th percentile were counted. Patients with a known hearing loss prior to the study were excluded to remain as conservative as possible in the interpretation of the results. This brought the number of patients down to 12. The significance of their distribution was calculated using the one-tailed binomial test. Table 4.2 shows the thresholds above and below the 50th percentile for the right and left ears as well as their p value. A p value inferior to 0.05 was considered to be significant.

Table 4.2: Distribution of patients above and below the 50th percentile and its significance

	Right ear			Left ear		
	>50th%ile	<50th%ile	P value	>50th%ile	<50th%ile	P value
2 kHz	7	5	0.387	4	8	0.193
4 kHz	3	9	0.072	2	10	0.01
8 kHz	2	10	0.01	3	9	0.072

This table would suggest that taken overall, non AG treated patients in this study had better than average PTA thresholds, particularly over 4-8 kHz. This was after taking into account the effects of both age and gender. A $p<0.05$ is significant.

GROUP 2: Patients receiving aminoglycosides therapy n=33

In this group, four patients had a strong history of noise exposure:

Case1

A 54 year-old man worked in the British army for 15 years and then worked in the motorbike trading. Years before the diagnosis of his malignancy, he consulted an ENT surgeon who told him that he had a noise-induced hearing loss affecting the frequency of 4 kHz.

Case 2

A 54 year-old carpenter worked with no ear protectors for 30 years, but was not aware of any hearing deficit prior to treatment. He noticed difficulties in following a conversation, mainly in a noisy environment immediately after treatment.

Case 3

A 61 year-old man served in the British army for 30 years with no ear protectors.

Case 4

A 42 year-old man worked in a hammer factory for 20 years with no ear protectors. This patient did not receive gentamicin, but was given streptomycin for suspected tuberculosis in his neutropenic stage. He had 2 courses of streptomycin for a total of 8 days. The medication was given once daily. The total dose was 6 g.

Case 5: A patient had perforated tympanic membranes

Otoscopic examination revealed bilateral perforated tympanic membranes. The perforation was dry, central, and involved about 30% of the surface of the tympanic membrane.

The thresholds are plotted against age for both males and females at the frequencies of 2-8 kHz in figures 4.14-4.19

Figure 4.14: Males 2 kHz

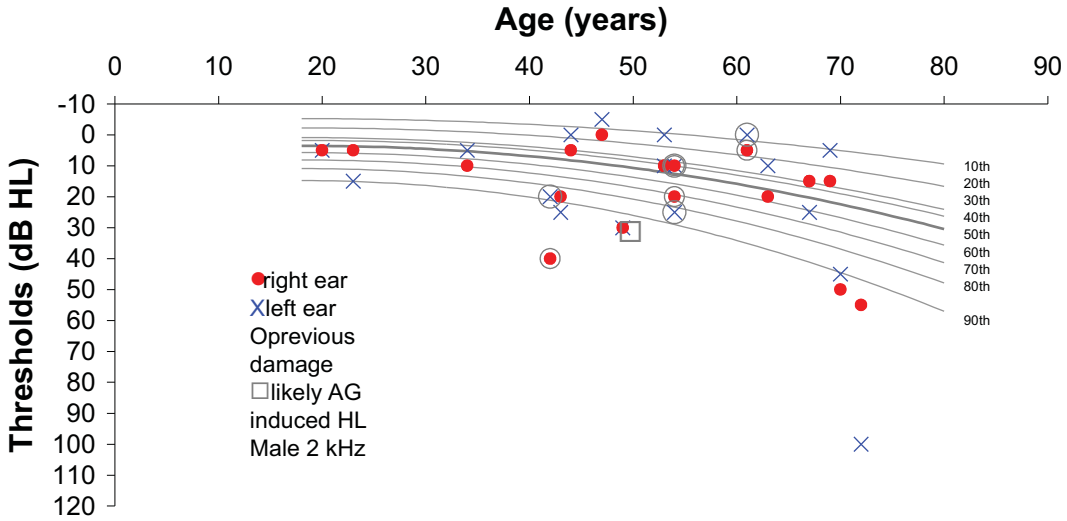


Figure 4.15: Males 4 kHz

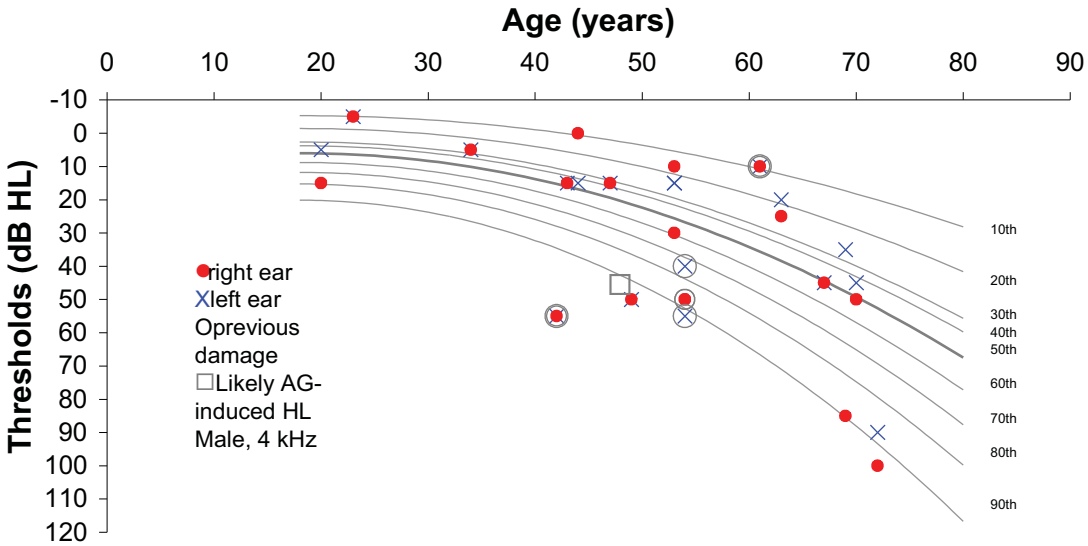


Figure 4.16: Males 8 kHz

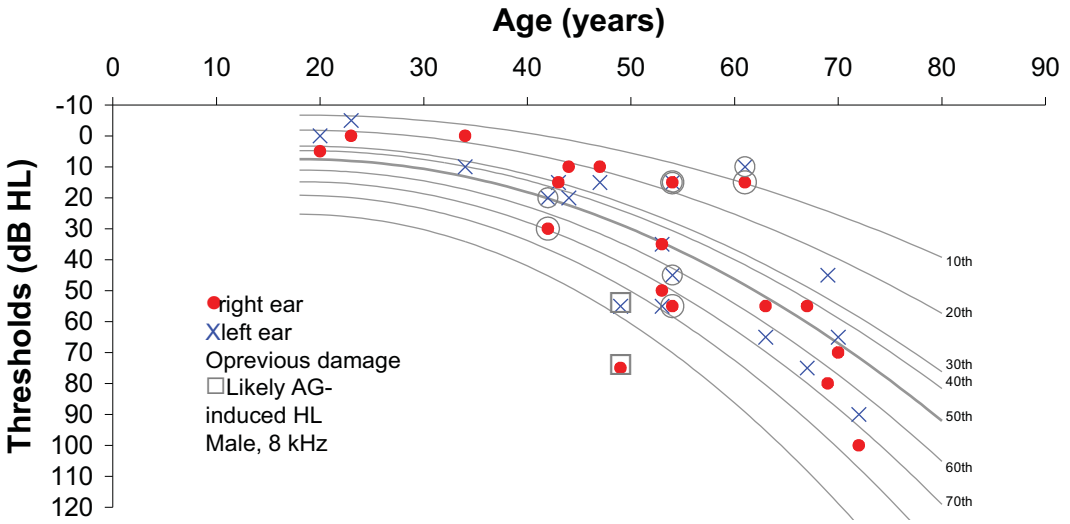


Figure 4.17: Females 2 kHz

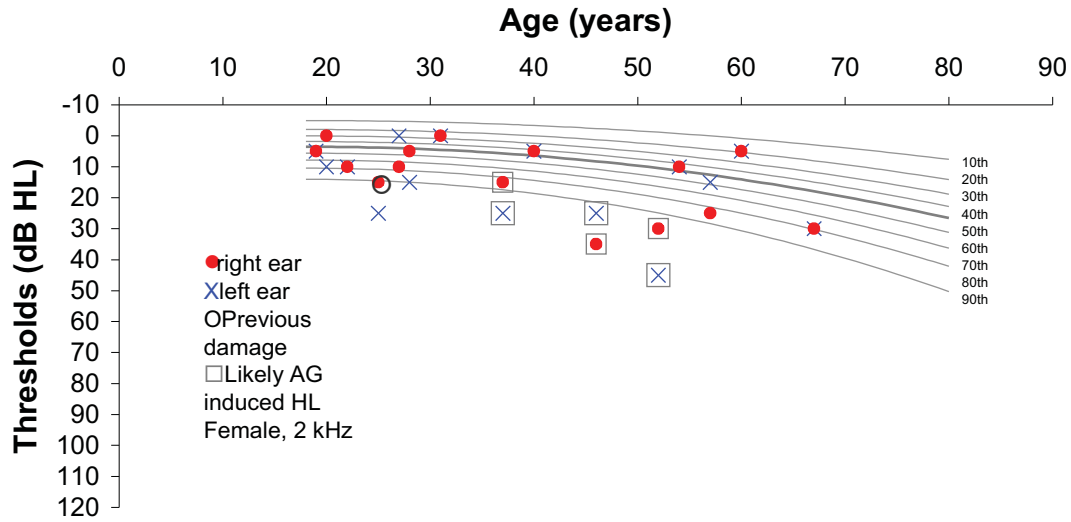


Figure 4.18: Females 4 kHz

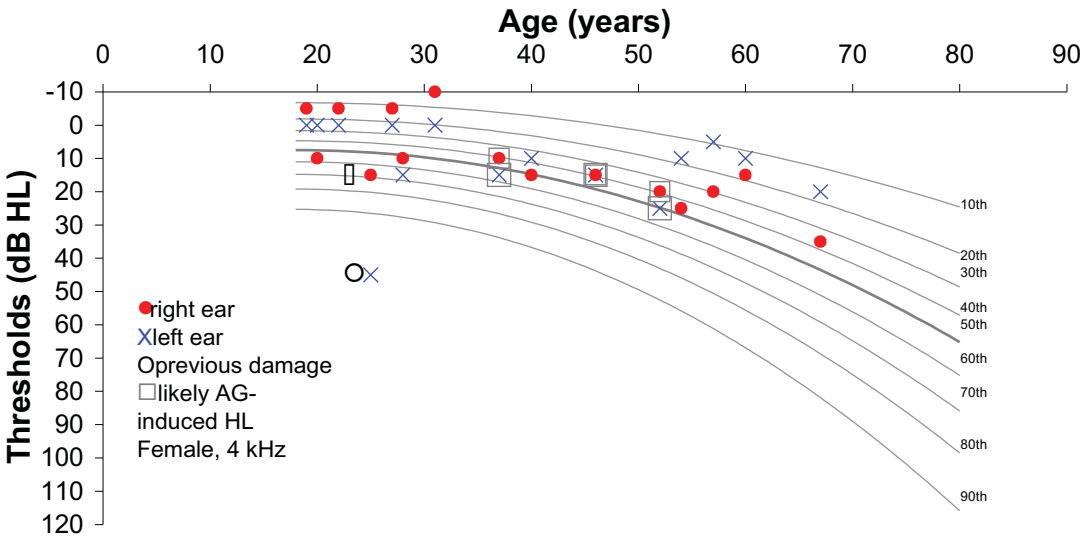
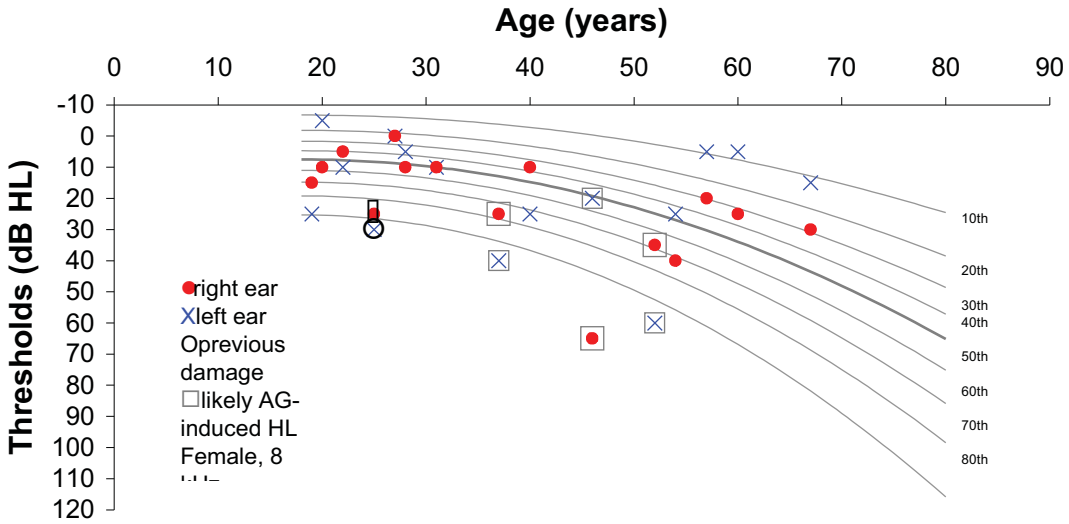


Figure 4.19: Females 8 kHz



In figures 4.14-4.19, the threshold of each patient fell into an age related percentile band. For each patient, the age related percentile band value (eg 30th-40th) for both sexes was then plotted against the categorical risk and represented in figures 4.20-4.22 for the frequencies of 2-8 kHz respectively.

Figure 4.20: 2 kHz

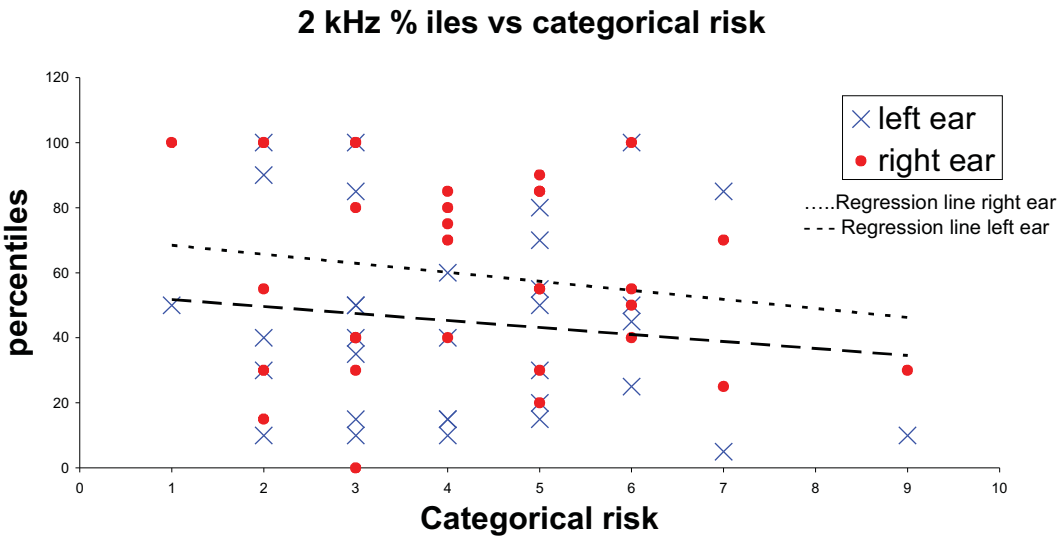


Figure 4.20. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 2 kHz.

Figure 4.21: 4 kHz

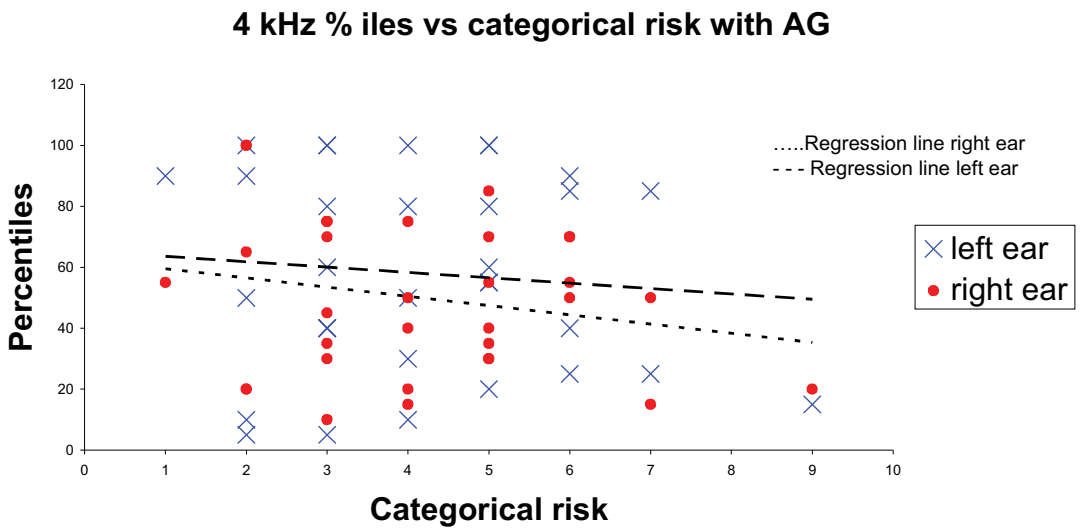


Figure 4.21. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 4 kHz.

Figure 4.22: 8 kHz

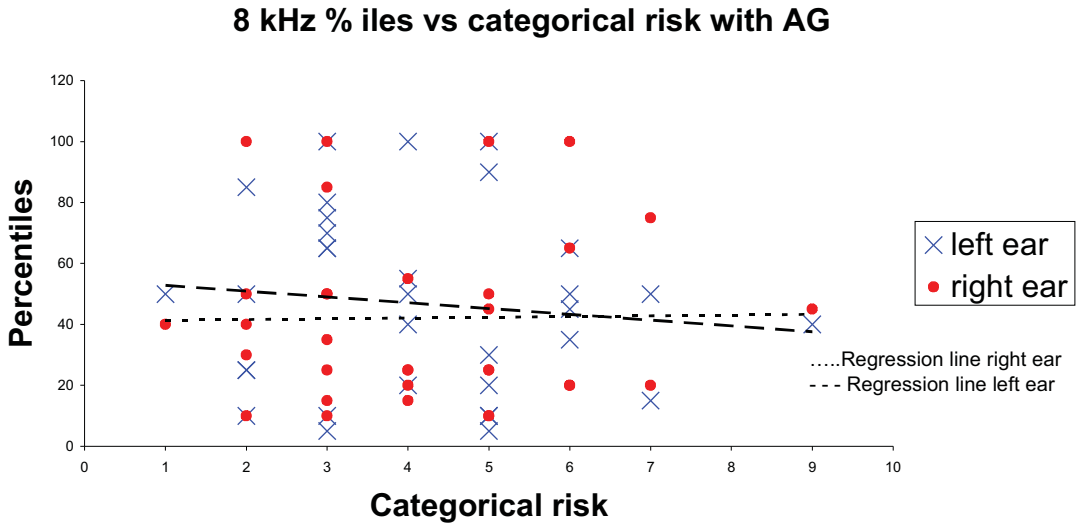


Figure 4.22. The percentiles are plotted against the categorical risk. The regression lines show that there is no linear relation between the percentiles and the CR at 6 kHz.

Table 4.3 shows the correlation coefficient between the percentile and CR and its significance for AG treated patients (N=33). The values in brackets represent the correlation coefficients once the patients who had a known loss prior to the study have been removed.

Table 4.3

	Right ear	Left ear
	Correlation coefficient	Correlation coefficient
2 kHz	-0.168 (NS) (-0.232 NS)	-0.129 (NS) (-0.248 NS)
4 kHz	-0.215 (NS) (-0.234 NS)	-0.094 (NS) (-0.169 NS)
8 kHz	0.014 (NS) (-0.043 NS)	-0.114 (NS) (-0.092 NS)

Additionally, to see whether the patients of the gentamicin treated group distribute equally on either side of the 50th percentile, patients above and below the 50th percentile were counted. Patients with a known hearing loss prior to the study were excluded to remain as conservative as possible in the interpretation of the results. This brought the number of patients down to 28. The significance of their distribution was calculated using the one-tailed binomial test.

Table 4.4 shows the numbers above and below the 50th percentile for the right and left ears as well as their p value.

Table 4.4: Distribution of patients above and below the 50th percentile and its significance

	Right ear			Left ear		
	>50th%ile	<50th%ile	P value	>50th%ile	<50th%ile	P value
2 kHz	16	12	0.285	8	20	0.018
4 kHz	13	15	0.425	15	13	0.425
8 kHz	6	22	0.001	11	17	0.172

From a clinical point of view, it is however also useful to try and determine which subgroup of malignancy is more at risk to develop a HL affecting the speech frequencies. Table 4.5 summarises the haematological malignancies and their associated hearing loss as detected by standard pure tone audiometry. If we do not take into account patients who had a hearing loss prior to the study, 2 patients from the AML group and 2 patients from the APML group present threshold elevation affecting the speech frequency range. These four patients with threshold elevation on PTA received gentamicin as part of their treatment. The criteria to determine HL was that the patients were above the 90th percentile for at least one frequency. The median total gentamicin received in by the four patients with hearing loss was 7 g (over 12.5 days) against 3.2 g (over 7 days) against the rest of the patients in this group. Whilst greater in those with hearing loss ascribed to the gentamicin, the median total dose and days were not significantly different to the lower values of the rest of the group (Mann Whitney test). The one-tail Mann-Whitney test returned a p value of 0.254.

Table 4.5

Malignancy	HL on PTA
AML N=11	
AG N=8	2 (25%)
No AG N=3	1 NIHL
APML (all AG)N=3	**2 (66%)**
CML (all AG) N=4	**1 NIHL**
ALL N=8	
AG N=6	1 NIHL
No AG N=2	0 (0%)
NHL N=7	
AG N=3	0
No AG N=4	1 cong HL
HD N=7	
AG N=2	0 (0%)
No AG N=5	1 NIHL
MM N=8	
AG N=5	1 NIHL
No AG N=3	1 NIHL+1 ear op
WALD N=1 (AG)	**0 (0%)**
AA N=1 (AG)	**0 (0%)**

AML=acute myeloid leukaemia, APML=acute promyelocytic leukaemia, CML=chronic myeloid leukaemia, ALL=acute lymphoblastic leukaemia, NHL=non Hodgkin's lymphoma, HD=Hodgkin's disease, MM=multiple myeloma, WALD=Waldenstrom macroglobulinaemia, AA=aplastic anaemia, NIHL=noise induced hearing loss, cong HL=congenital hearing loss.

4.7. DISCUSSION

The use of the standard PTA here provides the most appropriate measure of clinically recognised auditory dysfunction. In this study it was also the only data set that was gained for all patients. This was used in conjunction with careful application of filtering criteria of the other main recognised causes of hearing loss. These included: UK population age matched thresholds to account for any general aging effects; history of other known ototrauma;

especially exposure to noise, other ototoxic agents and surgical intervention. The results for both groups and their relevance to generating approximate estimates of risk are now considered below.

4.7.1. Group 1 PTAs. Patient receiving no aminoglycoside therapy

After consideration of each individual in this group of 17 patients, 5 patients or nearly 30% presented hearing loss due to mixed cause, with three having noise exposure one having had middle ear surgery and one presenting with congenital hearing loss. The remaining twelve patients had apparently normal hearing. Over the standard PTA range this would support the conjecture that the haematological malignancy condition and the treatments for this condition did not significantly contribute to any marked and clinically defined hearing loss. This is further confirmed by the correlation coefficients between thresholds and categorical risk as shown in table 4.1 as these coefficients returned no significance.

Table 4.2 shows that the thresholds are distributed more obviously below the 50^{th} percentile than above. This goes against the hypothesis that drug combination with the absence of aminoglycosides would increase hearing thresholds in the speech frequency range. The p value inferior to 0.05 means that there are more patients below the 50^{th} percentile than the expected 50/50 distribution on either side of the 50^{th} percentile. This is not interpreted as meaning the treatments or malignancy improve thresholds rather that the patients entering the study on the whole probably had better than average hearing over the standard audiometric frequencies.

4.7.2. Group 2 PTAs. Patient receiving aminoglycoside therapy

Nine patients out of thirty three or 27% of patients show a hearing loss when compared to the age matched 90th percentile values. Of these, four (12%) had hearing losses that could possibly be accounted for by noise exposure, either alone or potentiated by aminoglycosides. One further patient had bilateral perforations of the tympanum that could have accounted for their loss.

Interestingly of the noise exposed patients, one was a patient who had received streptomycin for suspected TB, which is known to be the most ototoxic of the aminoglycosides (Prazic and Salai, 1975). This patient had therefore been placed in the aminoglycoside treated group but had not received gentamicin therapy. This patient's hearing loss was very marked with a peak loss at 4-6 kHz of 60-70 dB HL. This loss could be explained in terms of his unprotected exposure to impulsive noise over 20 years, although a contribution by the streptomycin to this loss cannot be fully discounted.

However, because of the possibility of mixed loss this patient is not included in with those patients considered to have hearing loss attributable to aminoglycoside therapy. This is probably also appropriate due to the difference in the reported difference in ototoxic ranking between these two aminoglycosides (Rybak, 1986).

It was of interest that noise exposure was a surprisingly common factor in this study. Qualitatively it was of such an estimated level and duration that it could have accounted for the threshold elevation. This exposure is relevant, as

prior noise exposure can be a factor in 'weakening' the cochlea's ability to deal with subsequent ototrauma (Prasher, 1999).

After having carefully screened all patients for hearing loss due to aging and noise exposure, the diagnosis of the four patients is also almost the same: two patients had AML and two patients had APML. It is not considered likely that their hearing loss was due to acute myeloid leukaemia itself as AML has never been reported to be associated with hearing loss, unless localized in the cerebello-pontine angle, which was not the case in these four patients. One of these four patients, the 37 year-old lady with AML had a high WBC (110) at presentation. The 49 year-old man with APML had no record data available. The 52 year-old lady with AML and the 46 year-old lady with APML had normal WBC at presentation.

From the data collected on exposure to other drugs in this study there were also no drugs used exclusive to those four with a likely aminolgycoside induced loss. None of them took any other known potentially ototoxic drugs (vinca-alkaloids, nitrogen mustards or cisplatin). The only drug these patients have in common is Ara-C (cytarabine), together with gentamicin. However, 17 of the other patients in this group also had Ara-C therapy and it is not known to be ototoxic. It has however been shown to have some effect on NMDA receptors *in vitro* and may have some possibly modulatory effect on aminoglycoside toxicity at this site (Ahlemeyer *et al*., 2002). There is no documented description of the mechanism of action of cytarabine on the NMDA receptors. It is known that AGs produce an excessive stimulation of the N-methyl-D-aspartate (NMDA) receptors (Darlington and Smith, 2003).

The four patients with hearing loss likely to be due to AG therapy did not appear to have any other drugs in common. Notwithstanding the small numbers of patients, there was no evidence of any correlation with the amount of aminoglycoside received and the degree of hearing loss. Although one may argue that the loss in those four patients was only marginal when compared to the thresholds of the control group, it is important to remember that the controls from Lutman and Davis and Davis are done on an *unscreened* UK population of different age groups, so that the data of patients with blood malignancies are being compared with the worst possible data (90^{th} percentile) of an *unscreened* population.

4.7.3 Estimates of ototoxic risk in patients with haematological malignancy receiving aminoglycoside therapy –comparison with other reports of risk

Based on this incidence reported here, the occurrence of ototoxicity in these patients is about 12%. This translates as about a 1 in 10 risk for an overall median aminoglycoside exposure of 4g given over a median of 8 days.

It is interesting to compare these results with previous studies utilising the PTA as a measure of hearing loss. In clinical studies carried out in non neutropenic patient groups, the typical dose in those patients would be 3 to 8 mg/kg/day of gentamicin, given 3 times a day for a period of 7 to 10 days. Cochleotoxicity was determined by PTA, before and after a course of aminoglycosides. The criteria for ototoxicity in these studies was an increase by 15 or 20 dB of 2 or more thresholds in one or both ears after treatment (Cone, 1982; Govaerts *et al.*, 1990; Kahlmeter and Dahlager, 1982). The incidence of ototoxicity ranged from 0 to 16%, with a median incidence of

about 7.5% (Cone, 1982; Govaerts *et al*., 1990; Kahlmeter and Dahlager, 1982).

The median number of total days of gentamicin exposure that the patients from this study received is comparable to that of other reports falling within the 7-10 day range. In our study, the dose of gentamicin was 7 mg/kg/day, usually given in a once daily dose, proved to be efficacious for febrile, immunocompromised patients (Hatala *et al*., 1997). This again falls within the 3-8 mg/kg/day range quoted above although the once daily dosing contrasts with the more typical thrice daily dosing routine used in the past.

Gentamicin blood levels and renal function were checked on a daily basis. Gentamicin would have been stopped should the gentamicin blood level or renal functions were above limits, but in all patients these were within normal limits (peak levels < 12 µg/ml; trough levels < 2 µg/ml).

With the relatively small sample of patients in this study it would appear that the incidence and risk of ototoxicity as measured by standard PTA in the haematological malignancy patient group would appear to be generally comparable with that reported in other studies. Unfortunately, previous studies have often failed to explicitly state the total aminoglycoside exposure. Neither have there been estimates of the severity of losses sustained by patients. Consequently, this limits the ability to more properly compare the overall risk of hearing loss due to aminoglycoside use.

4.7.4. Relevance of PTA findings to sites and mechanisms of aminoglycoside cochleototoxicity in humans

The absence of linear relationship between the dose and the severity of threshold elevation see here is likely to reflect the multifactorial contribution to the onset and progression of AG-induced hearing loss. (Aran *et al*.,1995; Dulon *et al*., 1993; Crann *et al*., 1996).

Apart from this general observation, the PTA results do not contribute further to the elucidation of site and mechanism and would be explainable by a combination of the schemes proposed by the groups of Schacht (1998) and Basile (1996) discussed in Chapter 1 section 1.6.2.

As shown in table 4.3, there is no correlation between the thresholds and the categorical risk in the gentamicin treated patients.

After excluding the patients who were known to have a hearing loss prior to taking part in the study, table 4.4 shows that the distribution of patients on either side of the 50th percentile is not statistically significant when compared to the expected equal distribution. One p value is significant, but this is for patients below the 50th percentile (greater than expected) for the right ear at the frequency of 8 kHz. This means that patients in the present study have better thresholds than what may be expected in a normal population. This, goes against the original hypothesis that drug combination associated with aminoglycoside therapy would lead to threshold elevation in the speech frequency range.

In summary, this chapter shows that non AG treated patients had no deficit in the standard PTA following therapy. In AG treated patients, about 12% of patients showed elevation of thresholds likely to be due to AG therapy. This appears to be generally in line with reports in other patient groups. Interestingly, there did not appear to be any relationship between exposure to aminoglycosides and risk of hearing loss.

CHAPTER 5: OCCURRENCE AND RISK OF COCHLEOTOXICITY IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCY DETECTED BY HIGH FREQUENCY PURE TONE AUDIOMETRY.

5.1. INTRODUCTION

High Frequency Pure Tone Audiometry (HFPTA) in patients exposed to aminoglycosides is of considerable use as a clinical tool. This is because in both animal and human studies the usual pattern of basal to apical cochlear degeneration that occurs with most ototoxic agents has been shown to result in an initial high frequency hearing loss (Brummet *et al*., 1972; Brummet, 1980; Huizing and DeGroot, 1987). With prolonged exposure, this progresses to lower frequencies as measured by standard PTA. The primary advantage of using HFPTA is that changes above the frequency of 8 kHz usually occur before hearing loss in the conventional frequency range. The utility of detection in the high frequency range therefore resides in the detection of early losses due to the particular vulnerability of high frequency (basal) cells in the cochlea (Brummet, 1980 and Aran *et al.*, 1995). In this study HFPTA testing was therefore employed for detection of cochleotoxic effects at higher frequency.

A number of research groups have developed techniques to measure sound perception at high frequencies (eg. Dreschler, Van der Hulst and Tange, 1984 and Fausti *et al.*, 1979). HFPTA reproducibility workers has been previously shown to be consistent (Stelmachowicz *et al*., 1989; Green *et al*., 1987). HFPTA allows detection of incipient hearing loss before reaching the speech

frequencies that start around 6 kHz (Moore, 1998). The patient is normally unaware of this loss. HFPTA is no more difficult to perform than conventional audiometry and usually employs the same threshold decision procedure.

5.1.1. General review of previous HFPTA studies

The HFPTA testing can be done in three different ways according to the position of the sound source in relation to the eardrum:

(i) Free field:

(ii) Closed field using circumaural earphones

(iii) Closed field using insert earphones.

The advantages and disadvantages of each of these techniques are briefly compared below.

(i) Open field: examples and results

This technique has the advantage of best calibration but there can be large variation in threshold due to positional effects (Osterhammel and Osterhammel, 1979).

(ii) Closed field-headphones: examples and results

This technique allows less variation due to positional effects and is therefore more reliable than the free field, but presents problems with acoustic shadow if the sound source is not directly opposite the external meatal opening. There are also interferences from standing waves over the distance to drum of 1.8 to 3.3 cm. These coincide with distances of about a quarter of the effective wavelength of air conducted frequencies. This means

cancellation/augmentation of the wave may occur, leading to increases in variance (eg, Feghali and Bernstein, 1991).

At these higher frequencies, sound pressure at the tympanic membrane then critically depends on the placement of the sound source and on the size and shape of each individual external ear (Frank, 1990; Green *et al*., 1987). Thus, presentation of the same sound may yield substantially different sound pressures in different ears. Moreover, only a slight change in the position of an earphone may yield large changes in sound pressure at the tympanic membrane.

Dreschler *et al.* (1985) and Dreschler and Hultz (1987 cited in Stelmachowicz, 1989) found that in spite of the possible inaccuracies of headphone positioning, which may influence especially the high-frequency thresholds, the standard deviations obtained for frequencies above 8 kHz were nearly as small as for audiometry up to 8 kHz. This supports the testing of high frequencies giving a good and reliable indication of cochlear damage in the base of the cochlea. Dreschler and Hultz (1987) also returned age related mean threshold values similar to those of Mulheran *et al*. (2001).

(iii) Closed field using insert earphones.

This technique again offers a lower variation due to positional effects. Insert earphones can be calibrated using an artificial ear (Stelmachowicz *et al*., 1989) and also cut down positional problems with standing waves as the source is typically about 1.5 cm from drum in a typical canal.

Some comparison of the two closed field techniques yielded the following mean threshold values (see Table 5.1). Because of the absence of internationally accepted HL values above 8 kHz the values are all given as dB SPL. All dB SPL thresholds increased with increasing frequency as the sensitivity of the cochlea decreases.

For closed field-headphones, Frank (1990) tested 200 ears of subjects ranging in age from 18 to 28 years old (mean age=21.6 years) using circumaural earphones. He used an audiometer whose maximal output was 120 dB SPL. These provided the most sensitive estimates of High Frequency threshold to within one dB resolution. These subjects had normal standard PTA thresholds. Schechter *et al*. (1986) found thresholds using circumaural earphones in patients aged 21 to 26 years old although with higher estimates by about 10 dB at 12 and 14 kHz.

Green *et al*., (1987) used a laboratory design insert tube earphones with a distal sound source. This study returned broadly similar values over 10-16 kHz as Mulheran *et al* (2001) found using commercially available ER2 insert earphones. Mean thresholds increased from 20 to 65 dB SPL over this frequency range.

The means from these two studies differ with those obtained by Stelmachowicz *et al* (1989), using a modified insert earphone tube. This study

reported the change in the high frequency thresholds in different age groups and appears to be the most extensive study to date on HFPTA thresholds.

Table 5.1: Comparison of HFPTA mean thresholds from a range of studies

Freq(kHz) Mean dB SPL threshold				***Age range***	***Type***	***Researcher***
10	***12***	***14***	***16***			
18	24	31	61	*18-28*	Circumaural	Frank (1990)
25	30	42	62	*?*	Circumaural	Schechter (1986)
20	25	40	65	*20-30*	Circumaural	Dreschler et al (1987)
25	30	35	65	*20-26*	Insert	Green (1987)
20	30	45	65	*20-29*	Insert	Mulheran (2001)
30	38	52	75	*20-29*	Insert	Stelmachowicz (1989)
32	42	58	90	*30-39*	‘’	‘’
40	50	74	105	*40-49*	‘’	‘’
70	88	88	120	*50-59*	‘’	‘’

5.2. USE OF HFPTA IN CLINICAL STUDIES

HFPTA has been used in a number of previous clinical studies to detect early onset hearing loss due to a range of ototraumas. These are briefly reviewed

below and show that substantial and previously unrecognised damage occurs at the higher frequency part of the cochlea.

(i) Aminoglycosides

Fausti *et al* (1992) conducted a study on patients receiving AGs. Serial conventional (0.25-8 kHz) and high-frequency (9-20 kHz) hearing threshold monitoring was done prospectively in 53 hospitalized patients. Hearing loss occurred in 47% of the ears studied, with hearing loss first appearing in the high-frequency range in 71% of ears showing change.

In this study, the criteria for ototoxicity were operationally defined as >20 dB change at any one test frequency, >10 dB change at any two consecutive frequencies, or loss of response at three consecutive frequencies where responses were previously obtained. The criteria chosen for distinguishing ototoxic hearing loss were based partly on test-retest data obtained in ill patients. Threshold changes meeting these criteria were categorised as ototoxic if changes were repeated for a minimum of two consecutive test treatments or if they occurred at the final test session. The last hearing test was done six months after the final dose of AGs.

(ii) Noise

Fausti *et al* (1981) conducted a study to determine whether previous noise exposure would affect the thresholds in the high frequency range. High-frequency (8 to 20 kHz) hearing sensitivity was compared in thirty-six military men with histories of steady-state or impulsive noise exposure. The age range was 20 to 29 years old. Threshold shifts were prominent for the steady-state

noise subjects from 13 to 20 kHz. Mean thresholds from 8 through 12 kHz were maximally 20 dB poorer than a sample of normal young adults. Audiometric configurations for this group were generally symmetrical above 8000 Hz. For the impulsive noise group, substantial shifts in sensitivity were seen from 2 to 20 kHz and the high-frequency audiometric configurations were often asymmetrical. The variability of subjects in this group was greater than that seen in the steady-state noise exposed sample.

(iii) HFPTA was also used to detect high frequency loss on patients who received a treatment with cisplatinum (Fausti *et al* (1994)) and on post bacterial meningitic patients (Mulheran *et al* (2004)).

5.3. RESULTS

In this current study, a group of control subjects was recruited from the audiology and ENT departments of the Leicester Royal Infirmary and from Leicester University staff. All subjects had normal thresholds on standard PTA and none had a history of ear pathology or noise exposure.

The limit of the HFPTA intensity in this study using the ER2 insert was 100dB SPL, which is above any physiologically useful signal level. When HFPTA values were above this limit, data was plotted on the threshold Y axis as ‘110 dB SPL’ as a number was needed for graph purposes.

In order to maximize the use of the data, it was decided to use an exponential fitting regime provided in excel, to the one employed by Lutman and Davis (1994) This regime was performed on the control subject data for each

frequency. The patients' thresholds (Y axis) were plotted against age (X axis) for the frequencies of 10, 12, 14 and 16 kHz for both the non gentamicin treated group and the gentamicin treated group.

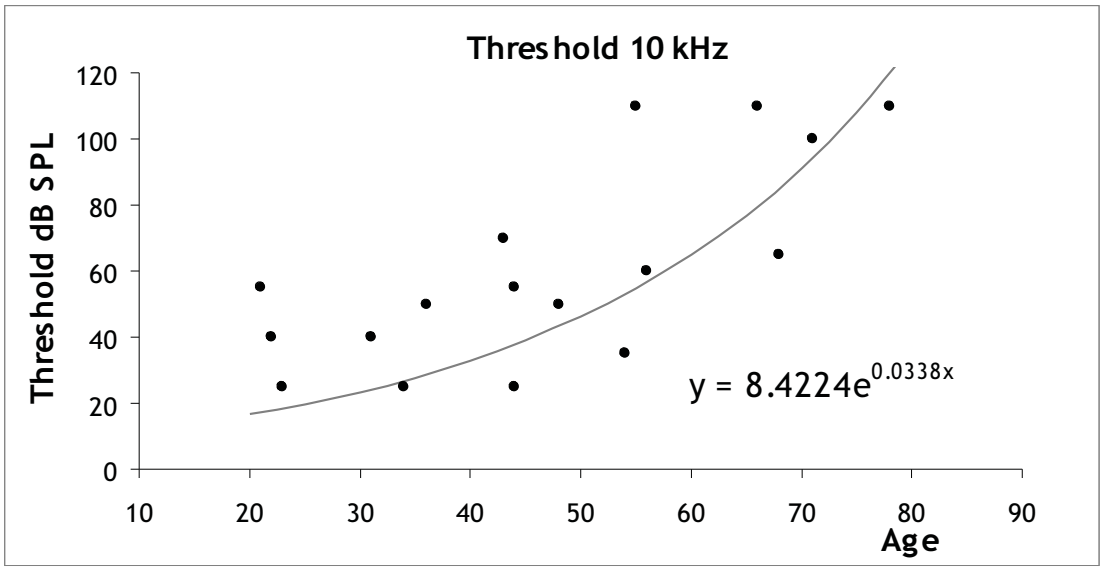
5.3.1. GROUP 1 Non-gentamicin group

Out of those 17 patients who did not receive gentamicin as part of their treatment, three were known to have noise induced hearing loss, one had congenital hearing loss and one had bilateral middle ear surgery. These five patients who had a loss prior to the study have been included in the graphs, but the analysis of the results has been done once excluding those patients and once including them.

Patients' thresholds are plotted against age with the 50th percentile being calculated with a method similar to that employed by Lutman and Davis (1994) who use the exponential modelling for calculating the median threshold value. The thresholds for each ear of all patients who did not receive gentamicin as part of their treatment are shown on the plots for the frequencies of 10-16 kHz in figures 5.1-5.4 respectively.

Figure 5.1. Thresholds 10 kHz

Right ear 10 kHz



Left ear 10 kHz

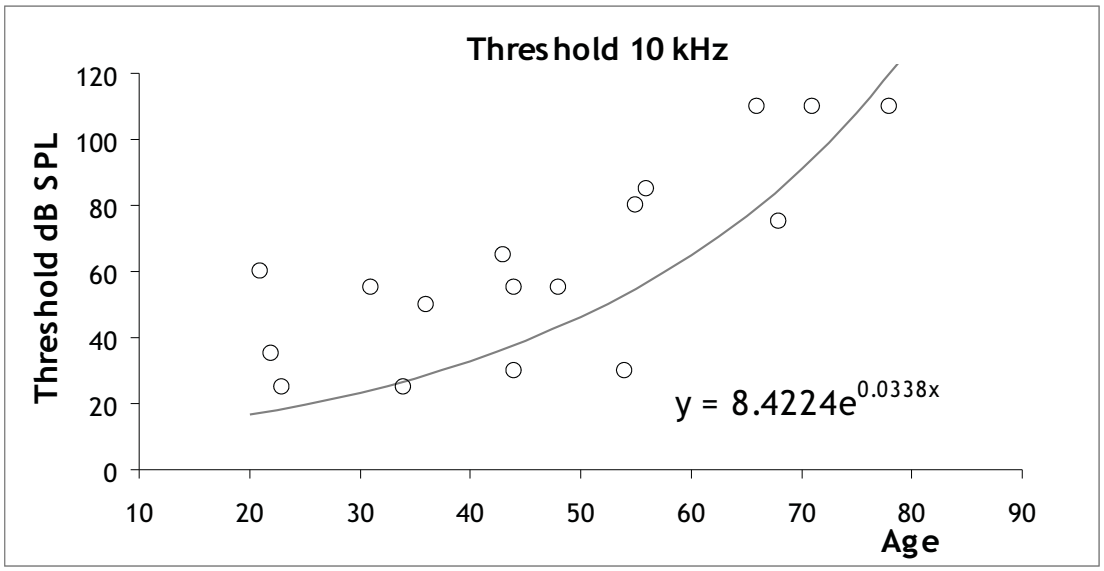
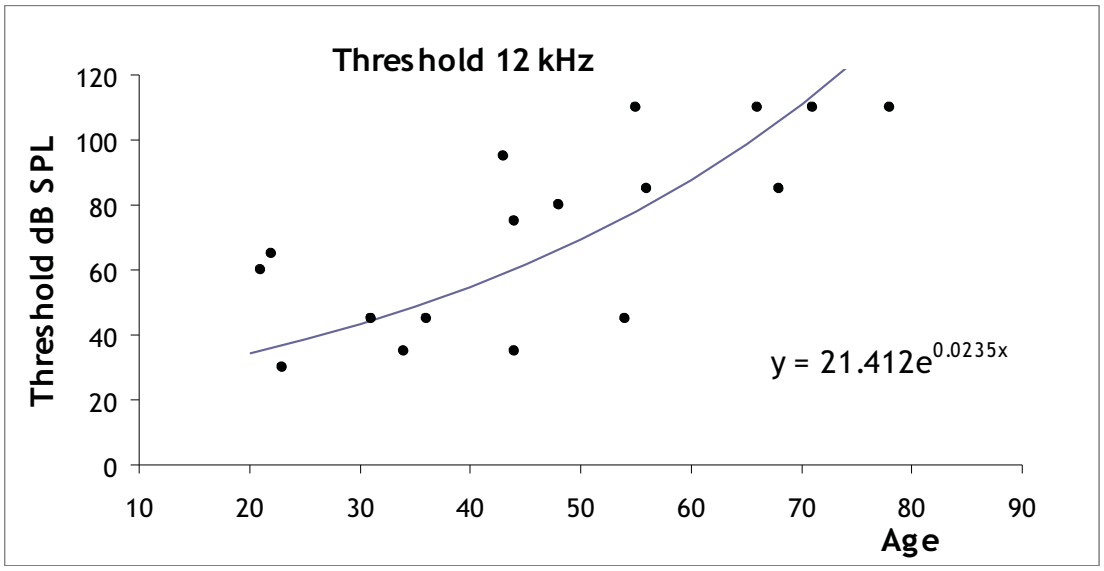


Figure 5.1.Thresholds in dB SPL are plotted against age for the frequency of 10 kHz. Their distribution on either side of the 50th %ile of controls is shown.

Figure 5.2: Thresholds 12 kHz

Right ear 12 kHz



Left ear 12 kHz

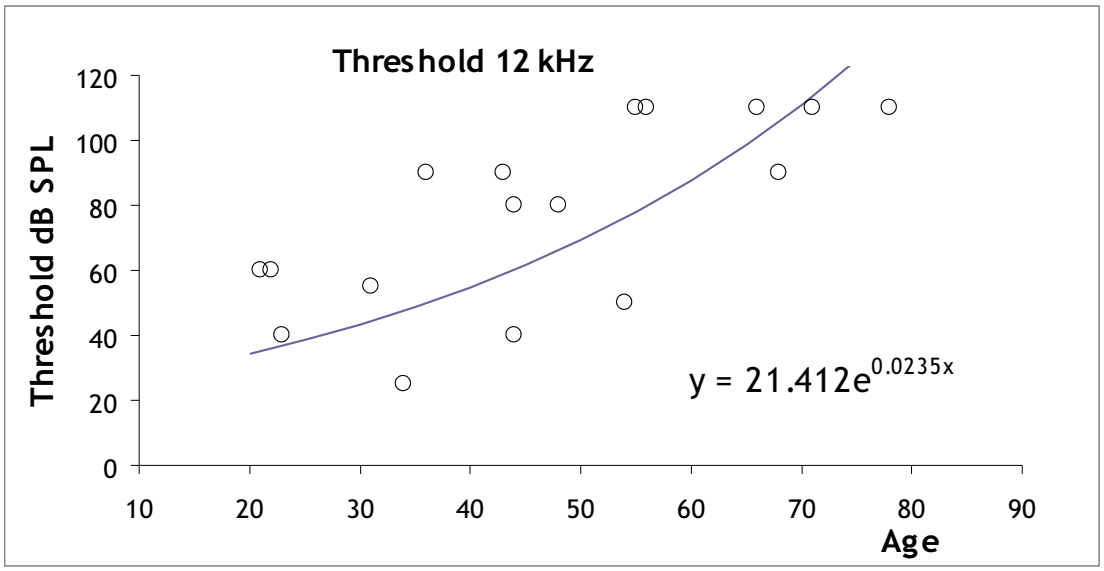
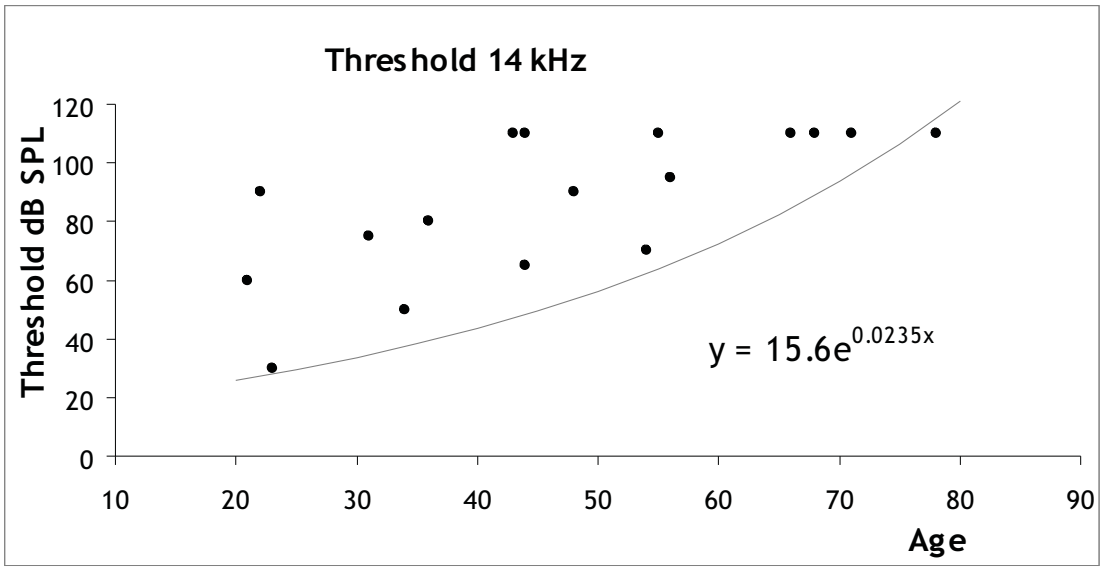


Figure 5.2.Thresholds in dB SPL are plotted against age for the frequency of 12 kHz. Their distribution on either side of the 50th %ile of controls is shown.

Figure 5.3: Thresholds 14 kHz

Right ear



Left ear

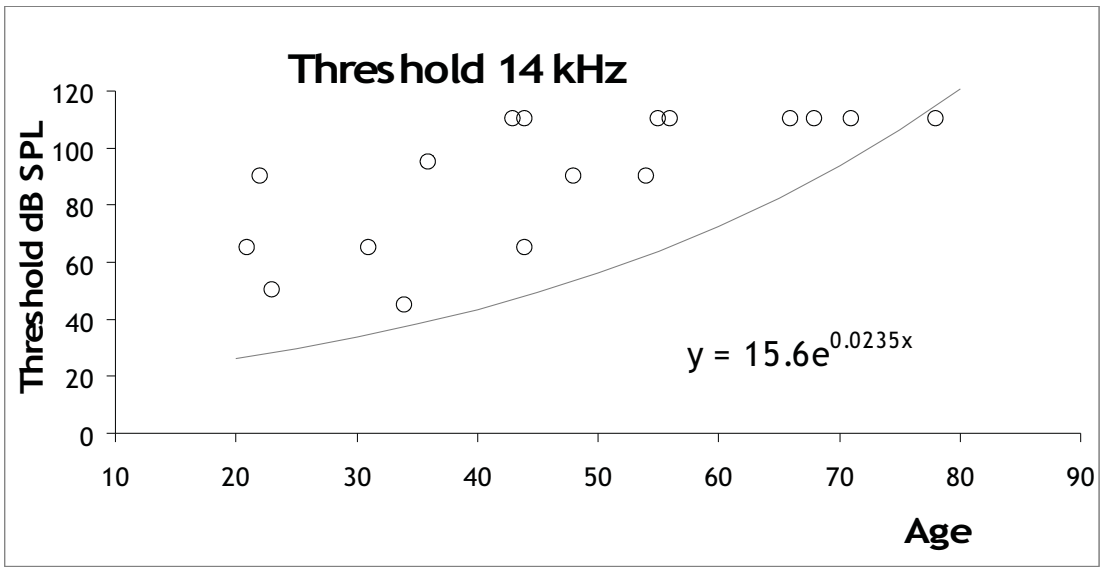
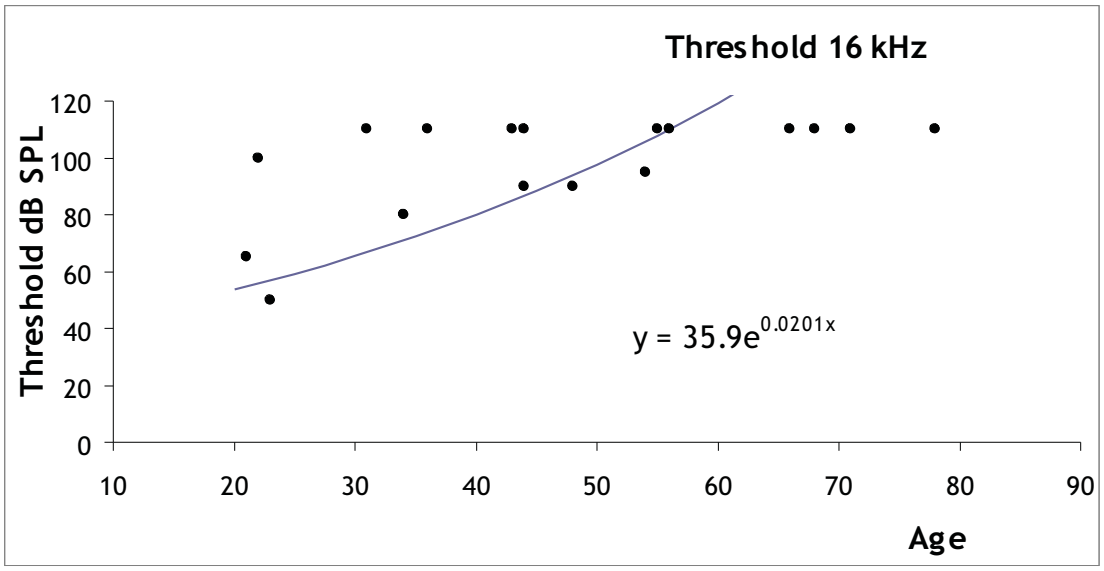


Figure 5.3.Thresholds in dB SPL are plotted against age for the frequency of 14 kHz. Their distribution on either side of the 50th %ile of controls is shown. At this frequency, all thresholds are above the 50th %ile.

Figure 5.4: Thresholds 16 kHz

Right ear



Left ear

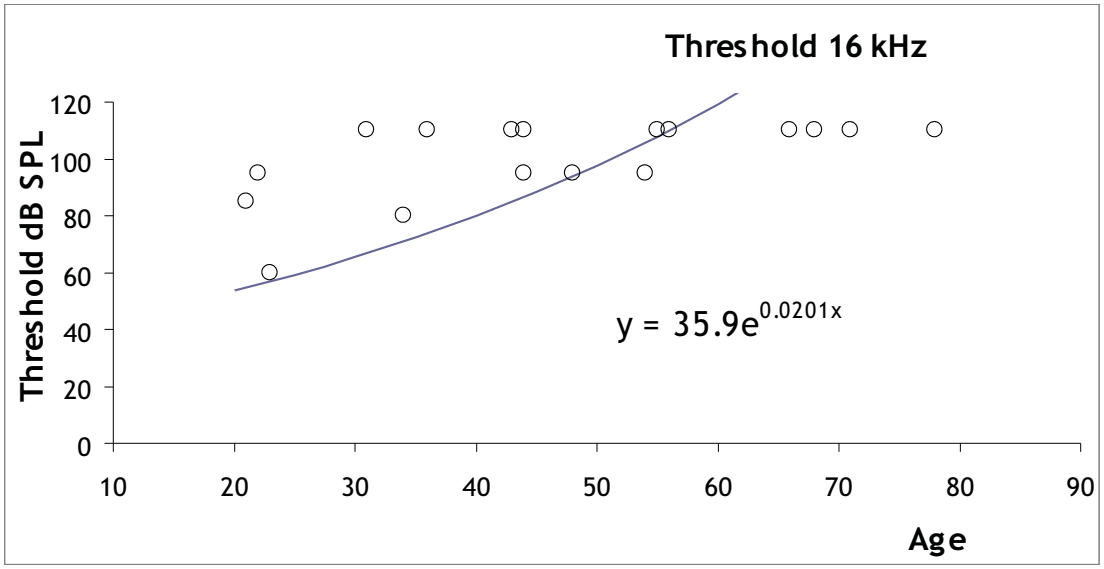


Figure 5.4.Thresholds in dB SPL are plotted against age for the frequency of 16 kHz. Their distribution on either side of the 50th %ile of controls is shown. At this frequency, most thresholds are above the 50th %ile.

Table 5.2 shows the p values when comparing the number of patients whose thresholds are above and below the 50th percentile for the frequencies of 10-16 kHz using the one-tailed binomial test. This table includes the patients with a known HL prior to the study (N=17)

Table 5.2

	Right ear			Left ear		
	>50th%	<50th%	P value	>50th%	<50th%	P value
10 kHz	12	5	0.0721	12	5	0.0721
12 kHz	9	8	0.5	11	6	0.166
14 kHz	16	1	0.0003	16	1	0.0003
16 kHz	10	7	0.314	12	5	0.0721

Table 5.3 shows the p values when comparing the number of patients whose thresholds are above and below the 50th percentile for the frequencies of 10, 12, 14 and 16 kHz using the one-tailed binomial test. This table excludes the patients with a known HL prior to the study (N=12) for the frequencies of 10, 12 and 14 kHz, as well as the patients above 60 years of age for the frequency of 16 kHz as, at that frequency, the median crosses the nominal line at 60 years of age (N=10)

Table 5.3

	Right ear			Left ear		
	>50th%	<50th%	P value	>50th%	<50th%	P value
10 kHz	7	5	0.387	7	5	0.387
12 kHz	5	7	0.387	7	5	0.387
14 kHz	11	1	0.0031	11	1	0.0031
16 kHz	7	3	0.171	9	1	0.0135

Taken together tables 5.2 and 5.3 show clear evidence of hearing loss at 14 kHz. There is also evidence at 16 kHz in the left ear. This suggests that there might be some high frequency ototoxicity of the non gentamicin treated group.

Analysis of categorical risk vs thresholds

Additionally to establish whether the thresholds above the 50th percentile were associated with a higher categorical risk, the median categorical risk was calculated for patients above and below the 50th percentile. This is shown in table 5.4.

Table 5.4; Median Categorical Risk (CR) for thresholds above and below the 50th percentile for the right and left ears at the frequencies of 10, 12, 14 and 16 kHz

	Right ear			Left ear		
	<50th%	>50th%	P value	<50th%	>50th%	P value
10 kHz	4.5	3	0.376	4	3	0.479
12 kHz	3	3	0.24	3	3	0.33
14 kHz	-	3	0.11	-	3	0.11
16 kHz	5	3	0.29	5	3	0.235

Looking at the median categorical risk for thresholds above and below the 50th percentile, the median categorical risk was not found to be significant using the Mann-Whitney test. For the frequency of 14 kHz, there are no thresholds below the 50th percentile. In no case for either ear was there any statistical significance between the medians.

The thresholds were also plotted against the categorical risk for the frequencies of 10-16 kHz in figures 5.5-5.8

Figure 5.5: 10 kHz

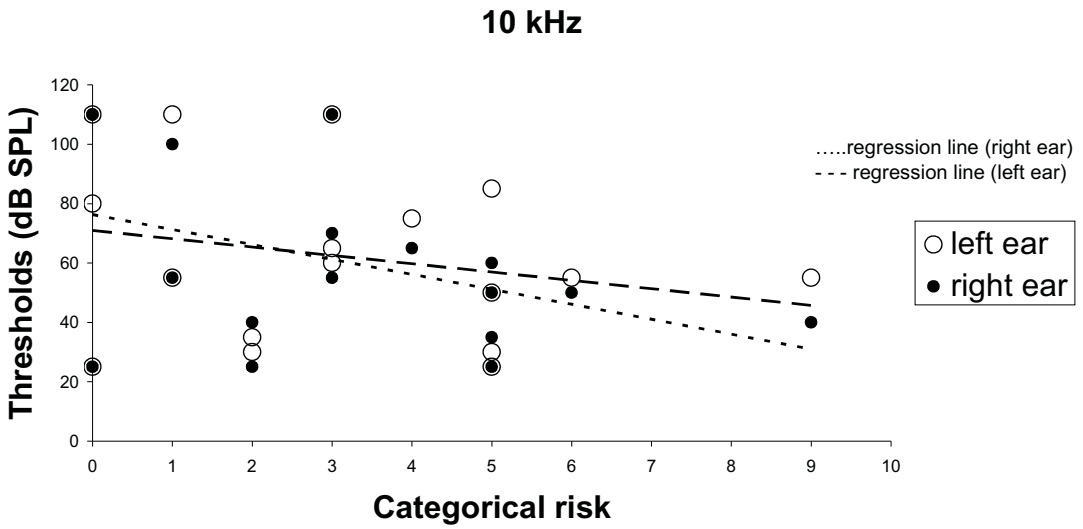


Figure 5.5. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 10 kHz.

Figure 5.6: 12 kHz

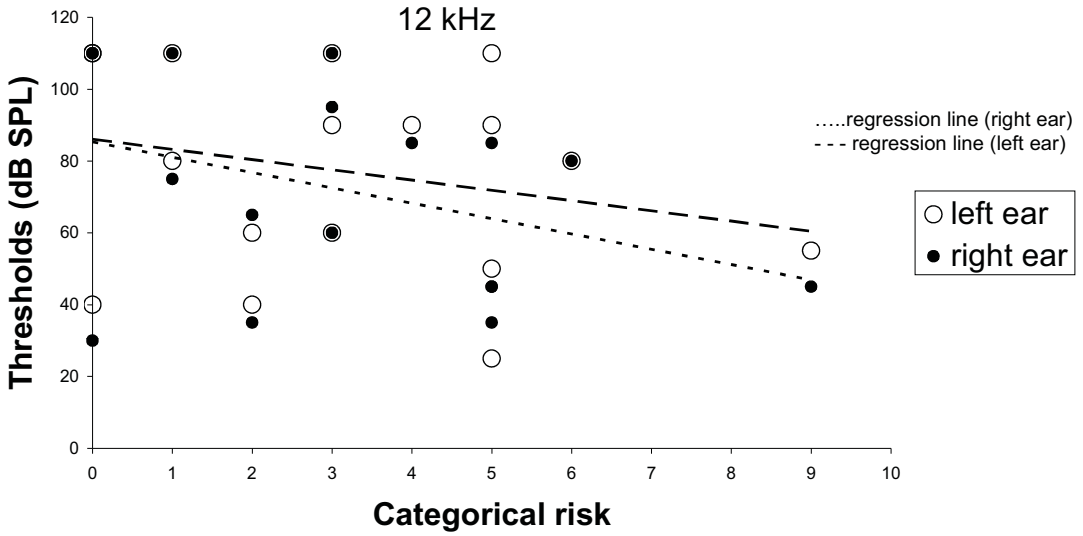


Figure 5.6. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 12 kHz.

Figure 5.7: 14 kHz

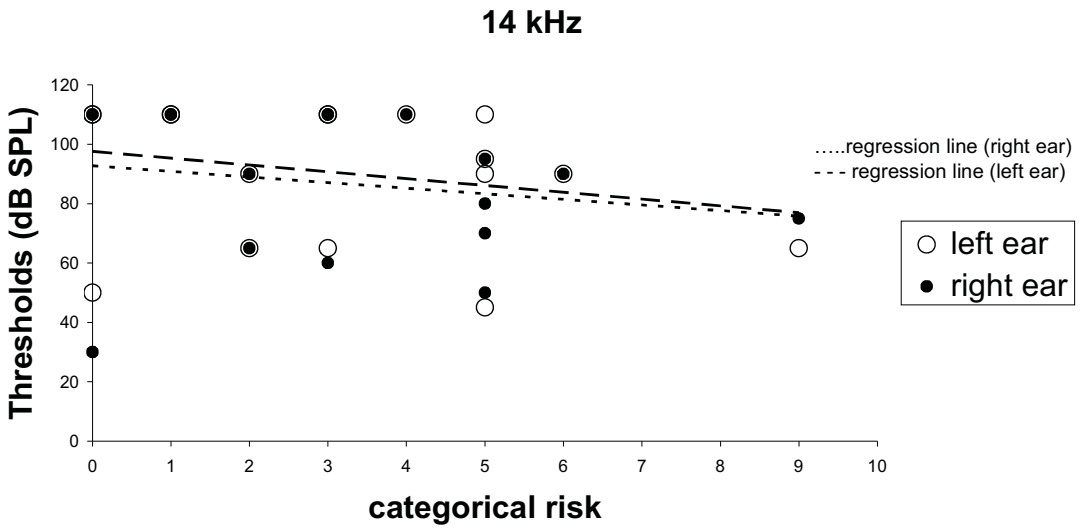


Figure 5.7. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 14 kHz.

Figure 5.8: 16 kHz

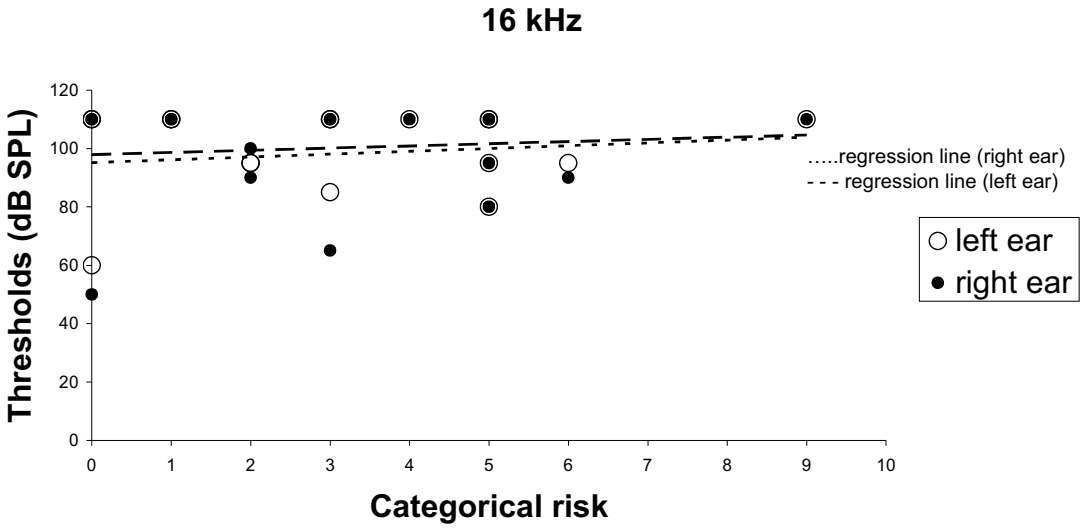


Figure 5.8. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 16 kHz.

Table 5.5 shows the Spearman's rank correlation coefficients between the thresholds and the categorical risk. In no case did the correlation coefficient reach significance.

Table 5.5

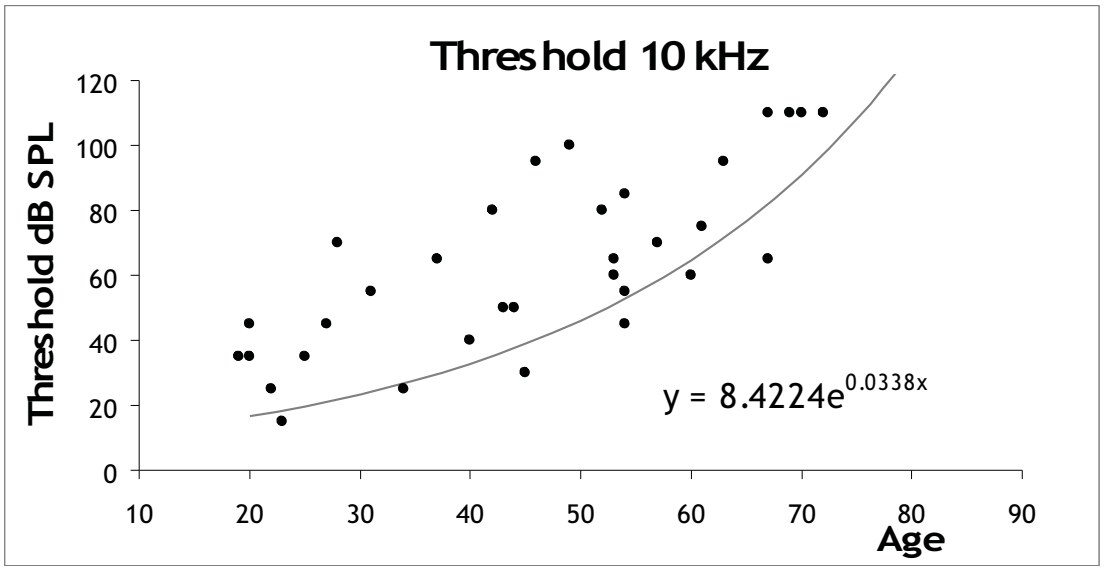
	Right ear	Left ear
	Correlation coef	Correlation coef
10 kHz	-0.414 (NS)	-0.237 (NS)
12 kHz	-0.363 (NS)	-0.247 (NS)
14 kHz	-0.186 (NS)	-0.244 (NS)
16 kHz	0.132 (NS)	0.13 (NS)

5.3.2. GROUP 2 Gentamicin treated group

As was done for the non gentamicin treated group, the distribution of the patients in the present study around the median value from the controls fitted by the exponential plots in excel at frequencies 10-16 kHz. This is shown in figures 5.9-5.12 respectively.

Figure 5.9: Thresholds 10 kHz

Right ear



Left ear

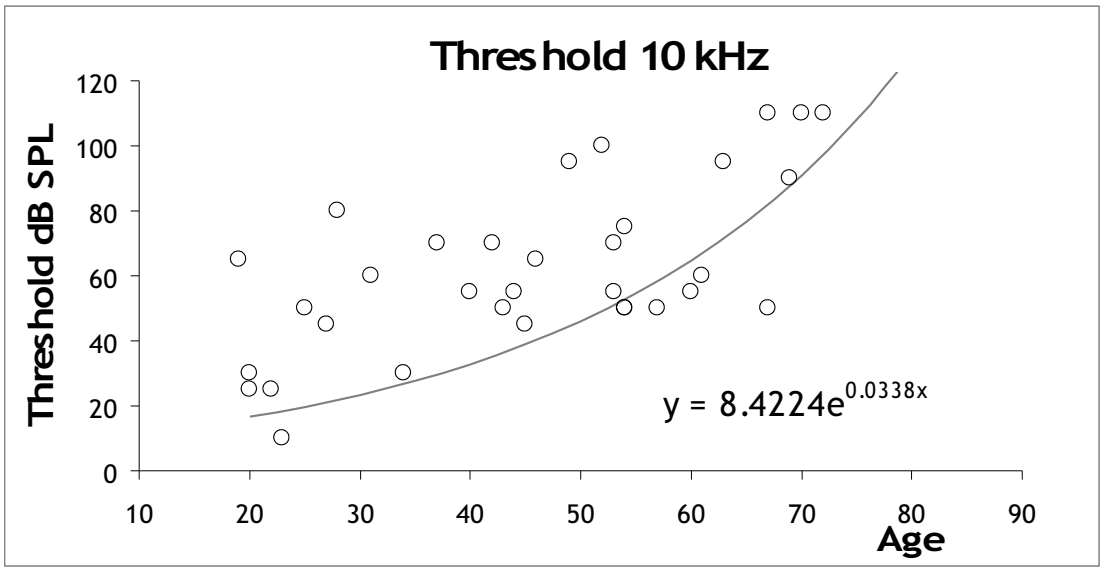
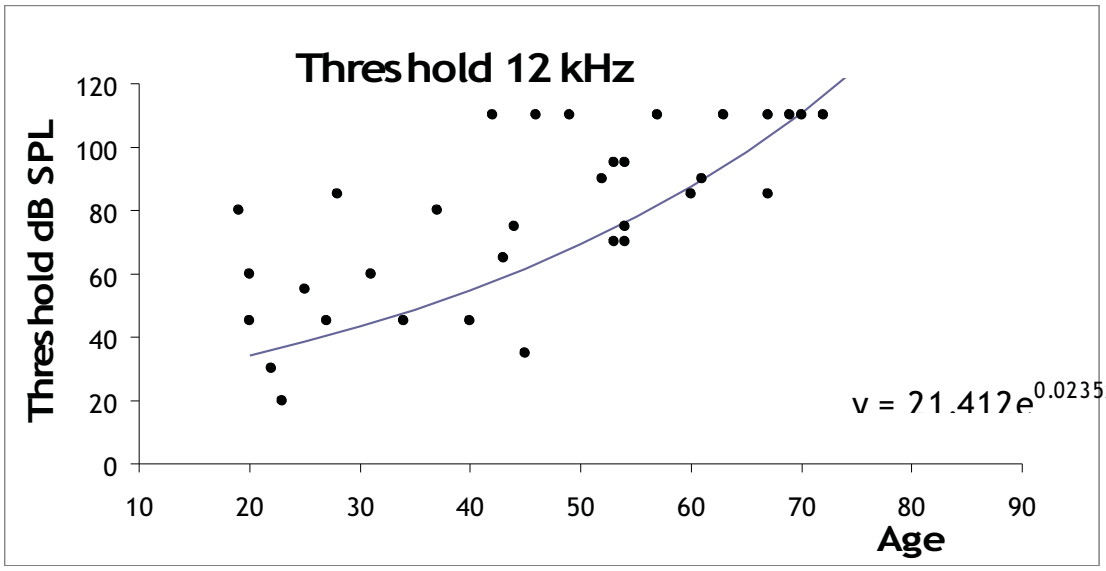


Figure 5.9.Thresholds in dB SPL are plotted against age for the frequency of 10 kHz and their distribution on either side of the 50th %ile of controls is shown.

Figure 5.10: Thresholds 12 kHz

Right ear



Left ear

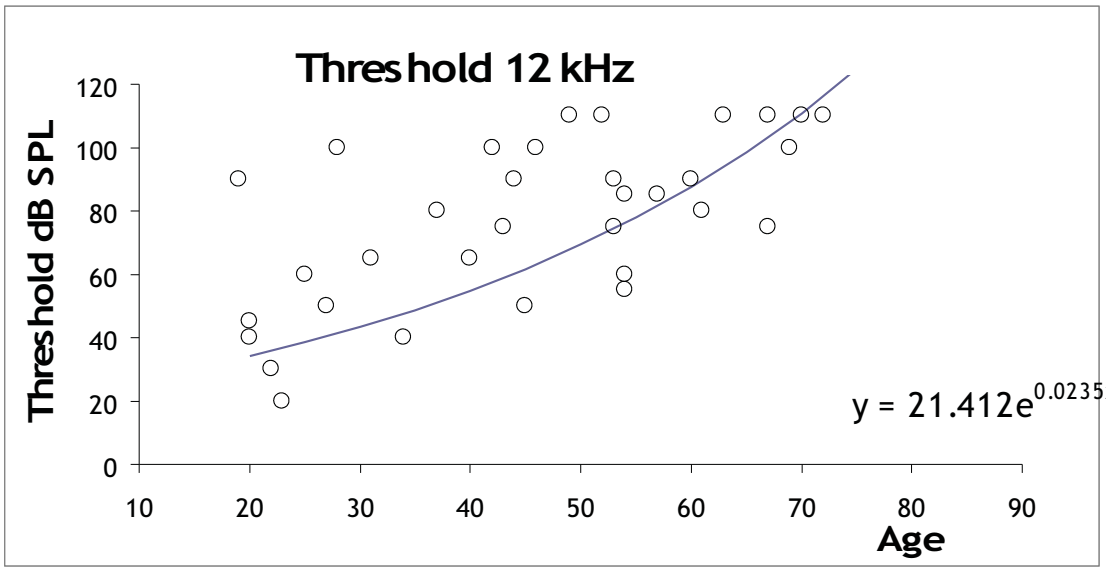
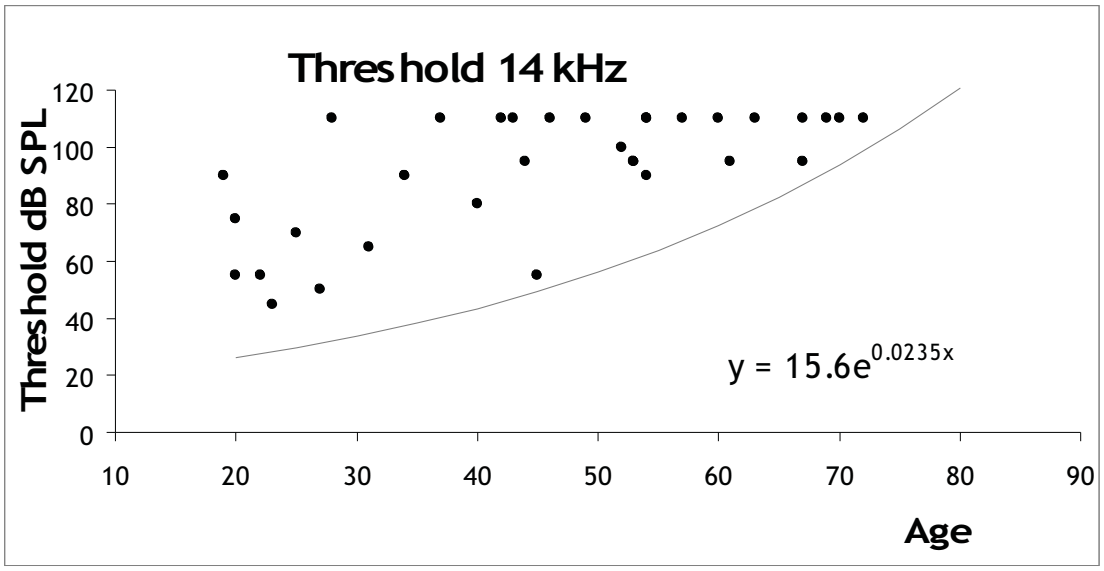


Figure 5.10. Thresholds in dB SPL are plotted against age for the frequency of 12 kHz and their distribution on either side of the 50th %ile of controls is shown.

Figure 5.11: Thresholds 14 kHz

Right ear



Left ear

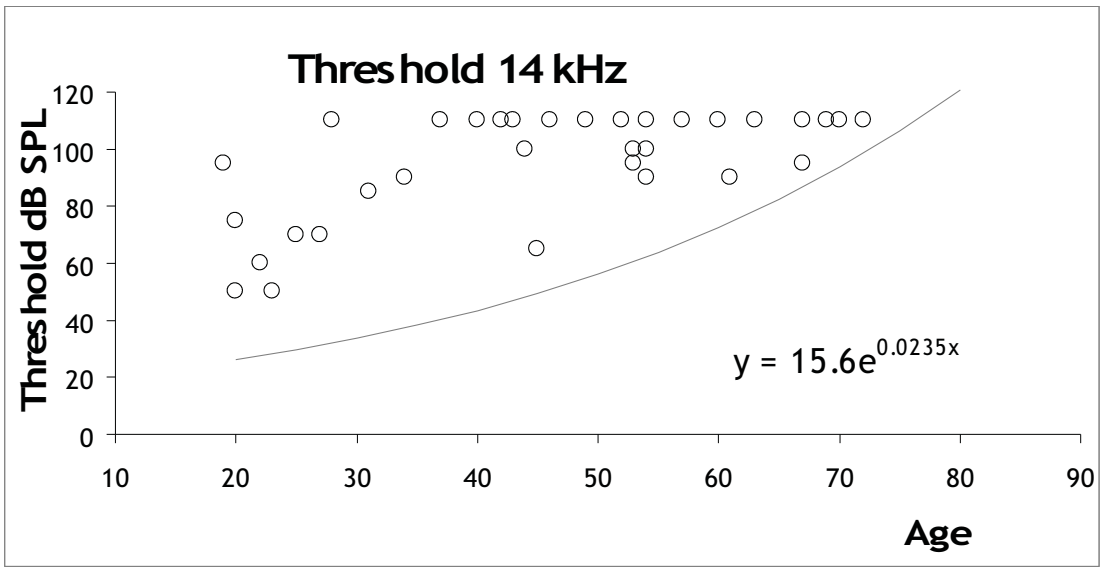
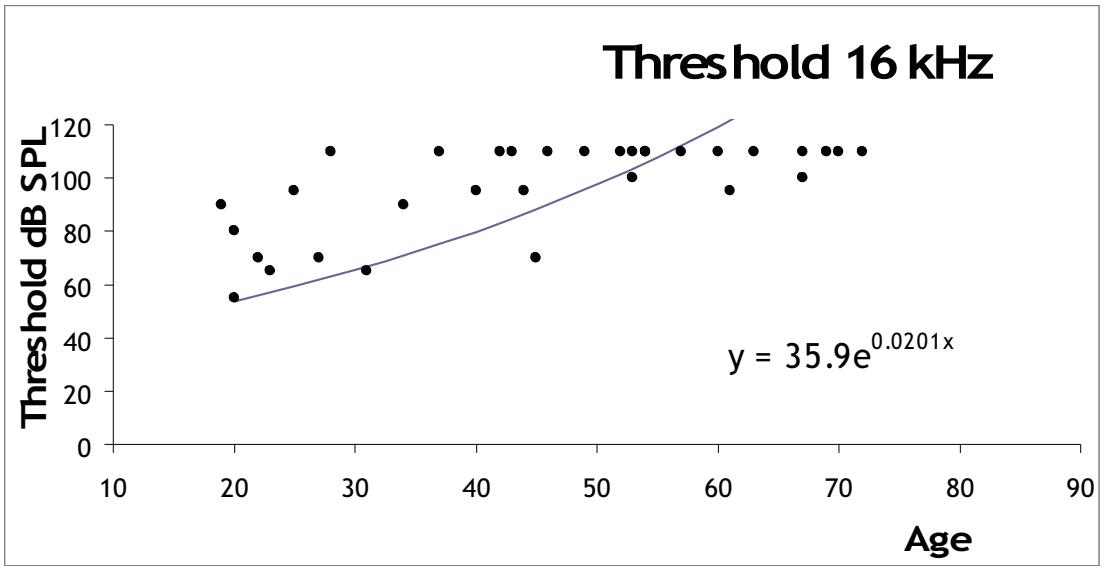


Figure 5.11.Thresholds in dB SPL are plotted against age for the frequency of 14 kHz and their distribution on either side of the 50th %ile of controls is shown. At that frequency, all thresholds are above the 50th %ile.

Figure 5.12: Thresholds 16 kHz

Right ear



Left ear

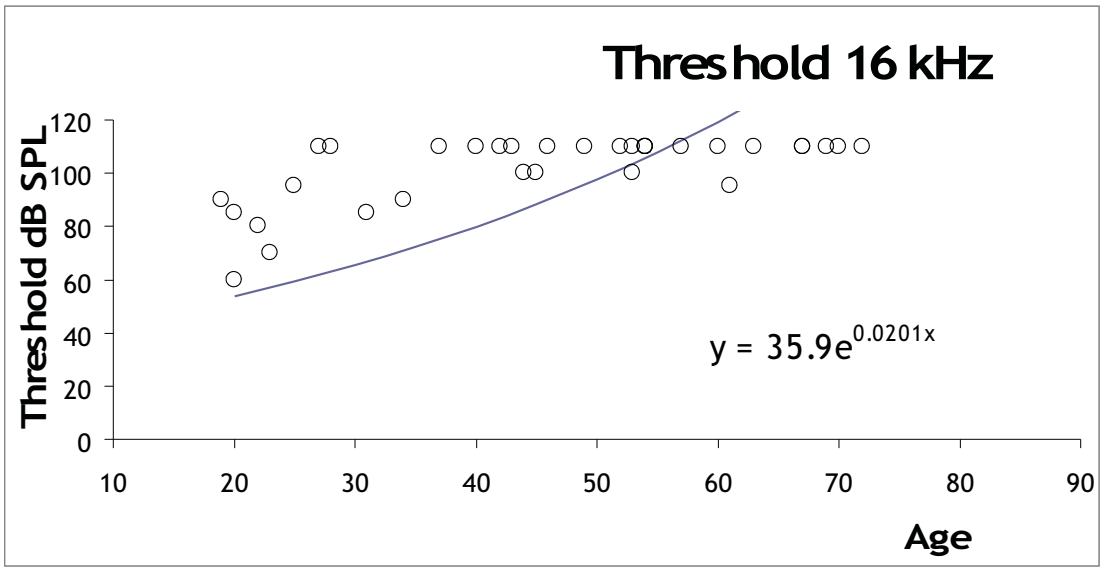


Figure 5.12.Thresholds in dB SPL are plotted against age for the frequency of 16 kHz and their distribution on either side of the 50th %ile of controls is shown. At that frequency, all thresholds are above the 50th %ile.

Table 5.6 shows the p values for patients whose thresholds are above and below the 50th percentile for the frequencies of 10-16 kHz using the one-tailed binomial test. This table includes the patients with a known hearing loss prior to the study (N=33).

Table 5.6

	Right ear			Left ear		
	>50th%	<50th%	P value	>50th%	<50th%	P value
10 kHz	27	6	0.00016	27	6	0.00016
12 kHz	21	12	0.0813	22	11	0.04
14 kHz	33	0	<0.00001	33	0	<0.00001
16 kHz	21	12	0.0813	24	9	0.006

Table 5.7 shows the p values for patients whose thresholds are above and below the 50th percentile for the frequencies of 10-16 kHz using the one-tailed binomial test. This table excludes the patients with a known hearing loss prior to the study (N=28) for the frequencies of 10, 12 and 14 kHz, as well as the patients above 60 years of age for the frequency of 16 kHz as, at that frequency, the median crosses the nominal line at 60 years of age (N=22).

Table 5.7

	Right ear			Left ear		
	>50th%	<50th%	P value	>50th%	<50th%	P value
10 kHz	23	5	0.0004	23	5	0.0004
12 kHz	17	11	0.172	19	9	0.043
14 kHz	28	0	<0.00001	28	0	<0.00001
16 kHz	17	5	0.008	19	3	0.004

Taken together these tables show there is a greater degree of threshold elevation for patients exposed to AG across all frequencies except for 12 kHz in the right ear.

Table 5.8 represents the mean categorical risk for thresholds above and below the 50th percentile.

Table 5.8

	Right ear		Left ear	
	<50th%	>50th%	<50th%	>50th%
10 kHz	3.5	4	3	4
12 kHz	3	3	3	4
14 kHz	0	4	0	4
16 kHz	4	4	4	4

The thresholds are plotted against the categorical risk for the frequencies of 10- 16 kHz in figures 5.13, 5.14, 5.15 and 5.16 respectively.

Figure 5.13

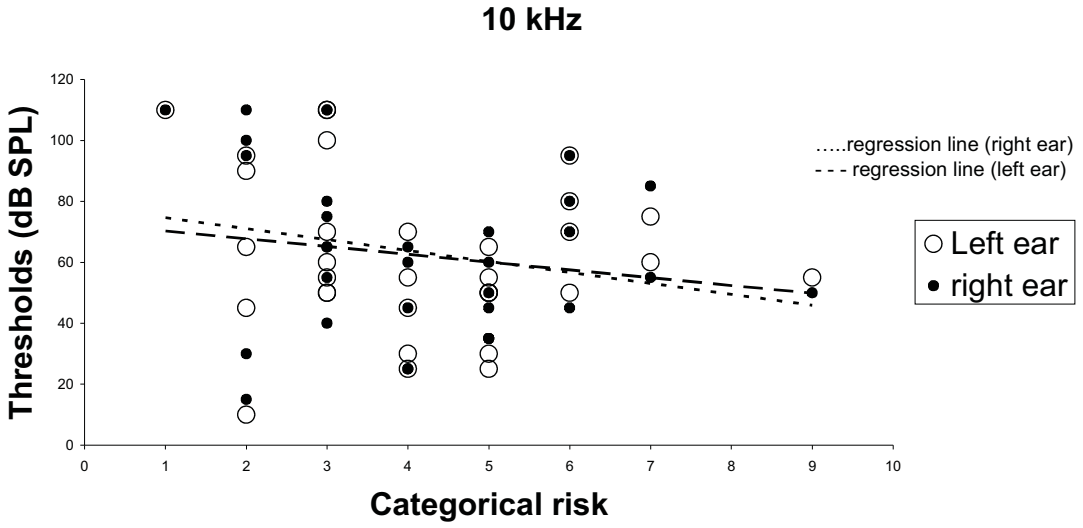


Figure 5.13. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 10 kHz.

Figure 5.14

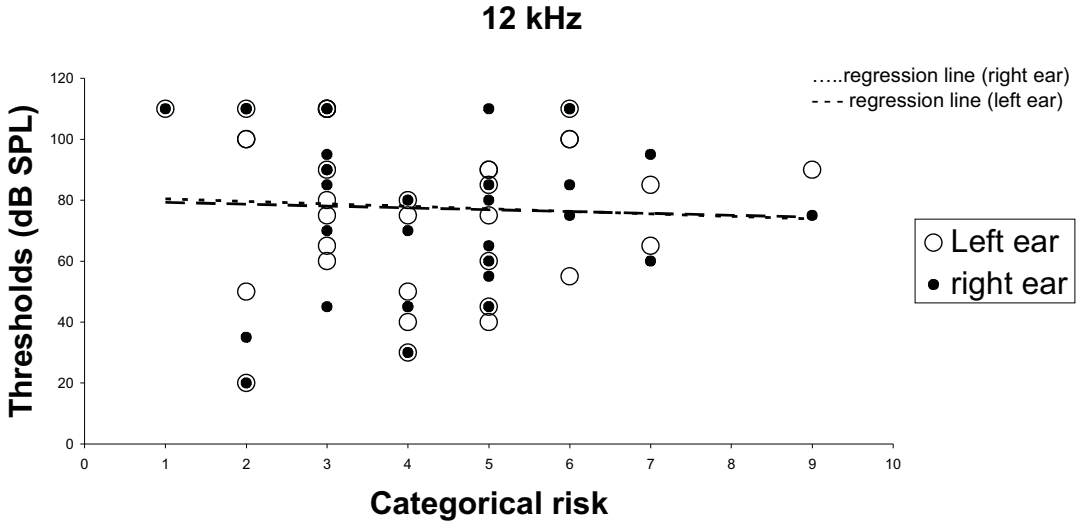


Figure 5.14. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 12 kHz.

Figure 5.15

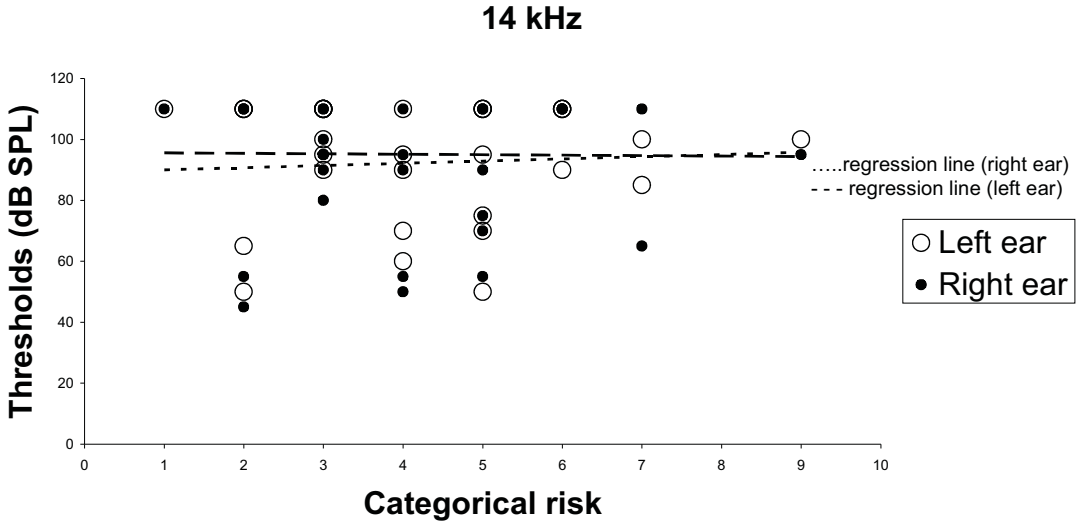


Figure 5.15. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 14 kHz.

Figure 5.16

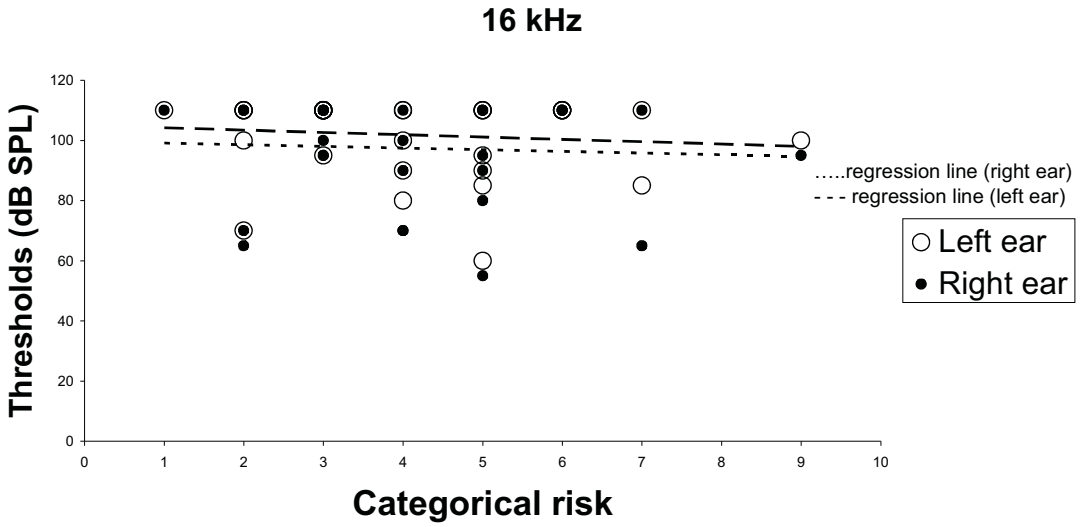


Figure 5.16. The thresholds are plotted against the categorical risk. The regression lines show that there is no linear relation between the thresholds and CR at 16 kHz.

To see whether there is a significant relationship between the thresholds and the categorical risk, the correlation function was calculated, giving a Spearman's rank correlation coefficient. The p value was obtained using Spearman rank correlation tables. The results are shown in table 5.9.

Table 5.9.

	Right ear	Left ear
	Correlation coefficient	Correlation coefficient
10 kHz	-0.233 (NS)	-0.177 (NS)
12 kHz	-0.054 (NS)	-0.041 (NS)
14 kHz	0.06 (NS)	-0.0144 (NS)
16 kHz	-0.058 (NS)	-0.1046 (NS)

The median categorical risk was measured for thresholds above and below the 50th percentile for each frequency and did not show any statistical significance by the Mann-Whitney test.

The aminoglycosides exposure between the thresholds above and below the 50th percentile was also measured to try and identify if patients with raised thresholds would have received a higher dose of aminoglycosides and whether this was statistically significant using the Mann-Whitney test. Table 5.10 shows the p values for each frequency (except for 14 kHz where all thresholds were above the 50th percentile and therefore no relation was possible to determine).

Table 5.10: Thresholds vs AG dose

	10 kHz		12 kHz		14 kHz		16 kHz	
	Right	left	Right	left	Right	left	Right	left
P value	0.239	0.254	0.191	0.109	-	-	0.267	0.41
Mean AG dose (g)	**<50th**3.9 **>50th** 4.5	3.52 4.86	3.59 4.83	3.3 4.96	- 4.36	- 4.36	3.7 5.1	3.89 4.4

5.4. RESULTS FOR DIFFERENT GROUPS OF HAEMATOLOGICAL MALIGNANCIES

From a clinical point of view, it is also meaningful to detect which haematological malignancy puts the patient at risk to develop cochlear damage evidenced by threshold elevation on HFPTA.

1. AML(N=11)/APML (N=3)

The three patients who had APML received AG. They all show threshold elevation on HFPTA. Two of those three patients already had threshold elevation on standard PTA. These three patients with APML have in common

the following drugs: AG, mitoxanthrone, etoposide and cytarabine. The incidence of threshold elevation in patients with APML is therefore 100%, keeping in mind that the sample here is very small.

In the patients suffering from AML, five (3 received AG, 2 did not) show threshold elevation on HFPTA. One patient had a history of NIHL. We can then consider that four patients out of eleven with AML (=36%) have evidence of HL on HFPTA. There is no common drug to these four patients.

2. CML (N=4)

All patients of this group have normal thresholds on HFPTA

3. ALL (N=8)

Five of these eight patients (=62.5%) have threshold elevation on HFPTA. Four patients with elevated thresholds received AG, one did not. The patients with raised thresholds have in common the following medications: vancomycin, etoposide, cytarabine (one patient received it intrathecally as well as intravenously, one received it intrathecally only and the others received it intravenously).

4. Non Hodgkin's lymphoma (NHL) (N=7)

Two patients only show threshold elevation on HFPTA. One of these patients had congenital HL. There is therefore one patient left (14%), who received AG, with HL on HFPTA.

5. Hodgkin's lymphoma (HD) (N=7)

Six of the seven patients have threshold elevation on HFPTA. One of them had a history of NIHL. This leaves us with five patients out of seven (=71%) having HL on HFPTA. Two of those patients received AG and three did not. The drug common to those five patients is teicoplenin. The other drugs common to all patients except one is cytarabine, etoposide and melphalan.

6. Multiple myeloma (MM) (N=8)

Five patients have threshold elevation on HFPTA. One has a history of mastoidectomy in childhood, and another one has a history of NIHL. It can then be considered that three patients have a HL on HFPTA (=37.5%). The drugs common to those three patients are AG and cyclophopsphamide.

7. Aplastic anaemia (N=1)

This patient has normal HFPTA thresholds.

8. Waldenstrom macroglobulineamia (N=1)

This patient has normal HFPTA thresholds.

Figure 5.17 summarises the different types of haematological malignancies and their risk (in terms of percentage) for each malignancy to develop threshold elevation determined by HFPTA.

Figure 5.17

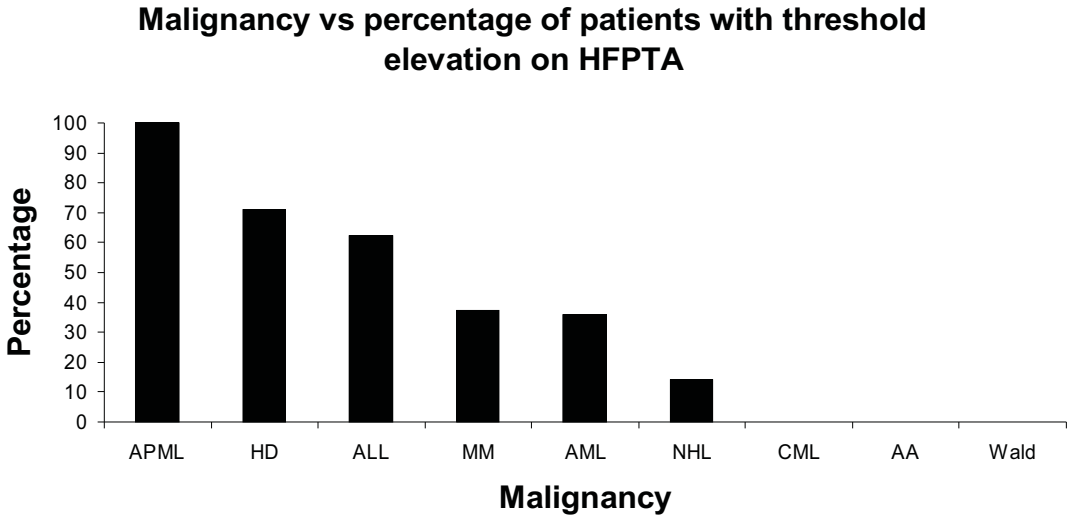


Table 5.11 shows the total categorical risk for each malignancy as well as the categorical risk per patient for each haematological malignancy

Table 5.11

Diagnosis	N	Total CR	CR/patient
AML	11	33	3
APML	3	7	2.3
CML	4	12	4.1
ALL	8	36	4.5
NHL	7	30	4.3
HD	7	38	5.4
MM	8	35	4.4
AA	1	2	2
WALD	1	1	1

5.5. DISCUSSION

The use of the HFPTA provides a further secondary measure of early subclinical auditory dysfunction. As with the standard PTA, its use in conjunction with careful filtering criteria of other recognised causes of hearing loss, enabled new estimates to be made of likely iatrogenic hearing loss in this patient group. This could therefore provide early warning of cochlear damage to clinicians treating this patient group.

5.5.1. Group 1 HFPTAs. Patient receiving no aminoglycoside therapy

The HFPTA results suggest that medical interventions or possibly the haematological malignancy itself may have led to subclinical deficit over the 10-16 kHz range. Looking at figures 5.1 to 5.4, it appears clear that as the frequency increases, the proportion of patients' thresholds above the exponential median increases. This is certainly obvious at 14 kHz where all thresholds were above the age-related median.

Looking at figures 5.1 to 5.4, the formula to calculate the median thresholds as a function of age is also of interest; at 10 kHz, the median threshold starts at 8.4 dB SPL to increase exponentially as 0.034 times the age. At 12 kHz, the median threshold starts at 21 dB SPL and increases exponentially as 0.0235 times the age. Surprisingly, at 14 kHz, the starting median threshold is lower than for 12 kHz as it starts at 15 dB SPL, but the exponential growth is similar to the one noted for 12 kHz. At 16 kHz, the starting median threshold is much higher (35.9 dB SPL) but the exponential growth with age is slightly lower than for the frequencies of 12 and 14 kHz. This flattening or reduction in the exponent may be caused by the upper limit of 100 dB SPL for threshold estimates. Given the fact that high frequencies are considered to be more sensitive, one would have expected the exponential value to increase as frequency increases. This may be due to limited data set or it may reflect a greater sensitivity to damage at 10 kHz.

In table 5.2, the p value is statistically significant at 14 kHz. This suggests that patients with haematological malignancies may be prone to develop subclinical hearing losses at high frequencies without AG therapy. The p values are comparable when all patients are included and when patients with a known hearing loss prior to the study are excluded as shown in table 5.3. Taken together, this is evidence that the patients with haematological malignancies who have had medical interventions are more susceptible to high frequency loss. This still stands when patients with preexisting hearing loss have been excluded. This has not been reported previously.

Table 5.4 shows that there is no statistical difference in the median categorical risk for patients whose thresholds are above and below the 50th percentile. The p value is not significant for any of the frequencies. Therefore, whilst it is possible that medical intervention presents risk, the analysis showed that the relationship between drug exposure and thresholds is not a linear one.

Similarly, table 5.5 summarises the correlation between thresholds and categorical risk and was not significant for each frequency in either ear. To test the hypothesis that potentially ototoxic drug exposure would increase the risk of threshold elevation on HFPTA, the median categorical risk was calculated for patients whose thresholds are above the 50th percentile and was compared with the median categorical risk of patients whose thresholds were below the 50th percentile. The median CR for patients' thresholds above the 50th percentile was found to be the same or lower than the patients'

thresholds below the 50th percentile. This suggests that drug exposure does not influence threshold elevation, which partly goes against the hypothesis expressed above (ie that drugs and/or malignancies affect thresholds). However, in this study the categorical risk was calculated from the potential ototoxicity of certain drugs reported in the literature in the absence of explicit risk estimates. The author considers that the results above may also mean that the effects of drug combination are not linear and that their administration could still be responsible for these threshold elevations. It may also mean that other drugs have an ototoxicity that has not been reported. Alternatively, the multiple applications of drugs as discussed here may affect either blood supply or cochlear metabolism in some non linear way.

Alternatively, the malignancy itself may have been responsible for these thresholds elevations but this hypothesis could not be directly tested here.

5.5.2. Group 2 HFPTAs. Patient receiving aminoglycoside therapy

The HFPTA results shown in figures 5.9 to 5.12 suggest that medical interventions or possibly the haematological malignancy itself may have led to threshold elevation over the 10-16 kHz range. This again was most clearly observed at 14 kHz where the thresholds of all patients fall above the 50th percentile.

In constrast to what was observed for the non gentamicin group, the p value in the AG group is statistically significant when comparing the amount of

patients whose thresholds are above and below the 50th percentile for most frequencies (Tables 5.6 and 5.7). This suggests that the patients who took AG as part of their treatment are at even higher risk of developing threshold elevation on HFPTA.

Taking the results of table 5.6 and table 5.7 together, there is very clear evidence that the p values are highly significant threshold elevation over 10-16 kHz. This finding would be most easily interpreted in terms of the known tonotopically selective cochleotoxicity of the aminoglycosides and would be in broad agreement with the reports of Fausti *et al* (1992) and other workers.

However, as with the non AG treated group, figures 5.13 to 5.16 do not show any obvious correlation between HFPTA thresholds and categorical risk. This is further confirmed in table 5.8 where the summary correlation coefficients were not significant.

Similarly, table 5.9, where the mean categorical risk is also represented for patients whose thresholds are above and below the median, did not show any statistical significance by the Mann-Whitney test. Again, these results suggest that the categorisation used here did not reflect the true risk presented. These results suggest that the categorisation may not reflect the true risk presented.

To identify whether the AG exposure was higher for patients whose thresholds were above the 50th percentile, the AG doses for patients whose

thresholds were above and below the 50th percentile were compared using the Mann-Whitney test. The p values were found not to be significant. This basically means that the total dose that the patients received was not correlated to their level of hearing loss.

5.5.3. Possible contribution by other drugs and disease to elevated HFPTA thresholds

Although a great deal of effort was made in reviewing the drugs with a known or potential ototoxic risk according to the literature, there does not seem to be any obvious pattern of drug administration that renders the patients more likely to develop hearing loss determined by HFPTA. There may be some drugs potentiating the ototoxic effects of others by a mechanism so far undescribed. The disease itself and/or the treatment may make the body more susceptible to high frequency hearing loss.

In the non AG treated group variable doses of Teicoplanin was administered to all patients with elevated HFPTA and its use was less prevalent (3/10) in those with 'normal' HFPTAs. No prevalence of differential Teicoplanin use was apparent within the AG treated group.

In the present study, there is therefore evidence of possible mixed drug effect by considering elevation related to AG drug exposure alone and then explainable by categorical risk.

5.5.4.Susceptibility to threshold elevation and malignancy

It seems that some malignancies render the patients more susceptible to develop cochleotoxicity at high frequencies determined by HFPTA. Keeping in mind the small sample of patients for each pathology, it appears that patients with CML (N=4) may have been protected against the cochleotoxic effects as all the patients in this group have normal thresholds on HFPTA. On the other extreme, all patients with APML have evidence of thresholds elevation on HFPTA. However, the number of patients for each subgroup of malignancy is too low for extrapolation.

From table 5.11, it appears that the categorical risk per patient is not related to threshold elevation on HFPTA; for instance, despite the small sample of patients for APML (N=3), the CR/patient is lower when compared with others malignancies. Once more, there does not appear to be a correlation between CR and threshold elevation.

5.5.5. Hyperviscosity

The potential for developing hyperviscosity syndrome is mainly in the AML/APML, CML and MM subgroups of malignancies. As said above, patients with CML all have normal HFPTA thresholds. In the APML group, the WBCs were within the normal range for 2 of the 3 patients and were not available at the time of diagnosis for the 3rd patient. In the AML group, only one patient had an elevated WBC (110) at presentation. This patient received AG as part of her treatment and had elevated HFPTA thresholds bilaterally. In

the MM group, two patients have no presentation data available, but all the other patients had normal WBC and PV at presentation.

It seems that hyperviscosity does not play a major role in thresholds elevation in patients with haematological malignancies in this study.

5.5.6. Pathology, metabolism, toxicity: possible mechanisms

It is possible that at the higher frequencies over 10-16 kHz, the general combined use of a range of therapeutics known to carry some risk of oto and neurotoxicity, could contribute to a generalised metabolic stressing of the cochlea which led to elevation of threshold. This along with the considerable stress haematological malignancy places on the whole body may have contributed to the elevated thresholds seen here in both patient groups.

This general hypothesis would need further exploration in a much larger study of patients with haematological malignancies. The fact that the cochlea at higher frequencies is generally more susceptible to ototrauma does lend some support to a mixed stressor hypothesis (Aran, 1995; Brummet, 1980; Prasher and Canlon, 1999).

5.5.7. Relevance of HFPTA findings to sites and mechanisms of aminoglycoside and iatrogenic cochleototoxicity in humans

As with the earlier PTA findings, the absence of linear relationship between AG dose and severity of threshold elevation further reflects a multifactorial contribution to the onset and progression of AG-induced hearing loss. (Aran

et al., 1995; Dulon *et al*., 1993; Crann *et al*., 1996). The finding of mixed non AG and AG associated risk of sub clinical cochleotoxicity as indicated by HFPTA thresholds in this study appears to be novel and may also be further evidence of broader multifactorial cochlear vulnerability in patients with compromised systemic function.

CHAPTER 6: MEASUREMENT OF DISTORTION PRODUCT OTOACOUSTIC EMISSIONS (DPOAEs) AND COMPARISON WITH PTA RESULTS.

6.1. INTRODUCTION

Otoacoustic emissions are signals generated by the ear spontaneously or in response to acoustic stimulation. Otoacoustic emissions are believed to be generated in the cochlea by the outer hair cells (Kemp, 1978; Norton, 1992; Probst *et al*, 1991).

Numerous experimental and theoretical studies indicate that OAEs are normal products of the hearing process (Kemp, 1986). However, direct measurements of basilar membrane motion support the notion that an active, vulnerable, biomechanical process within the organ of Corti uses metabolic energy to enhance the sensitivity and frequency tuning of basilar membrane vibration (Ruggero & Rich, 1991). Thus, the most popular theory is that the stimulus processing performed by the cochlea reflects the operation of active mechanisms that sharpen and amplify the basilar membrane response to low-level sound. Collectively, these enhancement processes have become known as the “cochlear amplifier” (Davis, 1983).

In this view, the organ of Corti is assumed to include a series of cochlear amplifiers distributed along the basilar membrane. Recent findings demonstrate that the cochlea’s most common and most vulnerable receptor cell, the outer hair cell, exhibits cycle-by-cycle motile responses *in vitro* to

voltage commands presented at acoustic frequencies (Santos-Sacchi, 1990). Based on that evidence, it is now believed that the OHCs are the physiologic basis of the cochlear amplifier. In this interpretation, the cochlear amplifier is thought to consist of a positive feedback loop between basilar membrane vibration and the motile responses of OHCs triggered by their receptor potentials in response to basilar membrane movement. It is thought that in response to acoustic stimuli, and sometimes spontaneously, some of the energy of the cochlear amplifier propagates from distant locations along the cochlea toward the oval window at the base and through the middle ear, to the outer ear canal where it is detected as OAE.

Changes in the electromotile activity of the OHCs can be measured as OAEs (Kemp, 1978). OAEs can therefore be used as a monitor to the sharp tuning of the sound transduction. Measurement of OAEs provide an objective method of cochlear function as they require no voluntary response from the patient but cannot be fully used as a measure of cochlear sensitivity over and above the PTA (Kemp, 1990).

The primary clinical value of OAEs is that their presence indicates that the preneural cochlear receptor mechanism is able to respond to a sound in a normal way (Kemp, 1990). OAEs are frequency selective and frequency specific so that it is possible to gain information from different parts of the cochlea simultaneously. OAEs measurement does not directly translate into PTA threshold measurements and they do not replace the PTA as the ‘gold standard’ of audiometric measurement.

6.1.1. Use of OAEs in discrimination of retrocochlear lesions

Review of the literature appears to support the contention that when PTA thresholds fall below about 30 dB HL in most ears, then OAEs are often not seen or easily measured (Harris and Probst, 1997; Kimberley *et al*, 1997; Robinette and Durrant, 1997). Only normal ears with PTA thresholds better than at least 30 dB HL will produce emissions with a moderate amplitude above the noise floor (Kimberley *et al*, 1997; Gaskill and Brown, 1993). This reduction in OAE with PTA is also in part related to reduction in amplitude with age and often increase in PTA thresholds as described by Kimberley *et al.* (1997).

However, if otoacoustic emissions are present when PTA thresholds are above 25-30 dB HL then this has been taken as being indicative of a retrocochlear lesion that underlies performance deficit. This use in differential diagnosis for more central lesions from the IHC/afferent cochlear nerve synapse onwards for threshold elevation is discussed in detail by Harris and Probst (1997). In this chapter this formed the primary use of OAEs used in this current study.

Conversely, it is recognised that otherwise normal ears may not produce measurable emissions due to mainly passive biomechanical factors outlined by Kemp (1997). These include the impedance offered to passage of the emission via the ossicular chain and eardrum itself.

In clinical applications, independent features of true OAEs can be tested. In the case of DPOAEs, these features are the latency, amplitude and frequency selectivity of the OAE (Brown and Kemp, 1984; Kemp and Brown, 1984). Although they are not routinely used in clinical practice, they provide a good research tool, as well as a clinical screening tool for early detection of sensorineural hearing loss which correlate with cochlear mechanical loss (Martin *et al*, 1990).

6.2. CLASSES OF OTOACOUSTIC EMISSIONS

There are two main groups of otoacoustic emissions:

1.Spontaneous otoacoustic emissions (SOAEs) occur in the absence of acoustic stimulation. They are pure tones of about 20 dB SPL found in the quiet ear canal (Kemp *et al*, 1990) and seem to result from feedback oscillation between overactive sensory elements and the middle ear. They can be modulated by pressure on the tympanum (Kemp, 1981). SOAEs have limited clinical implications as they are found in only 40 to 60% of healthy ears (Kemp *et al*, 1990). They may be the most sensitive indicators of sensorineural hearing loss only when they are present (Moulin *et al*, 1991).

2. Evoked otoacoustic emissions (EOAEs) are elicited by low-to-moderate-level test sounds delivered through a probe that is sealed securely to the ear canal. EOAEs proved to be extremely useful from a theoretical point of view and have been used extensively to elucidate some of the basic problems in cochlear physiology, leading to the concept of “active process” within the

cochlea (Davis, 1983). They are present in virtually all normal ears and can be further separated into three subclasses according to the type of stimulus used to elicit them:

a.Transient evoked otoacoustic emissions(TEOAE's) are generated in response of a stimulus of short duration and are present in most normal ears.

b.Stimulus frequency otoacoustic emissions (SFOAEs) are elicited by low-level, long-duration tones and occur at the frequency of the stimulus.

c.Distortion product otoacoustic emissions(DPOAE's) occur when simultaneous signals of different frequencies (F1 and F2) are presented to the ear. F1 and F2 are separated by an optimum ratio of 1.22 (Harris *et al*, 1989) and the power in the spectrum in the ear canal is sampled at the difference 2F1-F2. The levels of the sounds presented are at the intensity of 70 dB SPL (Sun *et al*, 1995). The ear creates a 3rd signal in the form of an otoacoustic emission generally at the frequency of 2F1-F2 (Kemp and Brown, 1984). The application of DPOAEs to the prediction of hearing status was investigated (Allen and Levitt, 1992; Avan and Bonfils, 1993; Gaskill and Brown, 1993; Gorga *et al*, 1993; Harris and Glattke, 1988; Kimberley and Nelson, 1989). Because of their frequency specificity, DPOAEs were regarded as very attractive and were thought to be able to predict hearing thresholds for narrow frequency range.

Some reports (Lonsbury-Martin and Martin, 1990; Lonsbury-Martin *et al*, 1993) found a moderately good correlation between DPOAEs amplitude and pure tone thresholds. The primary advantage of DPOAE analysis is that they can be recorded in the presence of more severe sensorineural hearing loss than TEOAE's (Norton, 1992; Probst *et al*, 1991; Whitehead *et al*, 1992).

6.3 . CLINICAL APPLICATIONS OF OTOACOUSTIC EMISSIONS

Only the applications relevant to the present study will be discussed in detail

6.3.1. Presbycusis:

The influence of age on the incidence and the threshold of EOAEs was demonstrated by Bonfils, Bertrand & Uziel (1988). EOAEs were present in 100% of tested ears (N=151 subjects) until the age of 60. The incidence of EOAEs fell to 35% in subjects older than 60 years-old, whilst a threshold elevation is noticed above 60 years of age, and this in a manner that is independent of pure tone thresholds. This finding was later confirmed by Kimberley *et al* (1994) and Stover & Norton (1994).

The decrease in EOAEs incidence and the increase in EOAEs threshold with increasing age seems to be directly related to the decrease in hearing sensitivity due to presbycusis. These results are in accordance with a previous report (Bonfils *et al*, 1987) showing that EOAEs were not observed in patients with sensorineural hearing loss when subjective click threshold shifts

more than 30 dB HL have occurred. EOAEs could be recorded in old subjects (>60 years of age) with relative preservation of hearing.

6.3.2. Noise-induced hearing loss:

Noise exposure has been shown to reduce otoacoustic emissions (Balatsouras, 2004; Zhang *et al*, 2004).

Attias (1998) recorded DPOAEs from 76 military personnel (137 ears) aged between 17 and 41 years. Ears with normal audiograms, but with a history of military noise exposure, had DPOAEs that were significantly decreased in amplitude as compared to the ears of normal hearing non-exposed to noise subjects. These ears also had an increased absence of DPOAEs as compared with the ears of the normal hearing non-exposed to noise subjects. Although, in general, the DPOAE amplitudes and spectral frequency ranges reflected the audiometric NIHL configurations, in a number of cases DPOAEs were present for hearing losses up to 75 dB HL. This is evidence that DPOAEs are being generated by intact outer hair cells and that the damage is being caused at the level of the inner hair cells onwards (Sone,1998).

6.3.3. Ototoxicity

(I) Aminoglycosides

The effect of AGs on OAEs has been well investigated by a number of workers:

Mulheran & Degg (1997) measured PTA thresholds over 0.25 and 12 kHz as well as DPOAEs growth function in 15 young patients suffering from cystic fibrosis, receiving cumulative doses of gentamicin. The results were compared with those obtained from 36 control volunteers of similar age. Fourteen of the CF patients had normal hearing, but there was a significant elevation of the stimulus levels required to generate a 2f1-f2 DPOAE = - 10 dB SPL at 4 kHz. It was believed that this elevation may represent one of the earliest changes in outer hair cell performance caused by gentamicin, although it may also be due to the CF condition itself.

Stavroulaki *et al* (2002) compared PTA and OEAs results in detecting ototoxicity in children with cystic fibrosis who had gentamicin and concluded that PTA findings were normal for all groups at baseline and remained normal in the CF-gentamicin group after treatment. The CF-gentamicin group had significantly lower OAES.

Stavroulaki *et al* (1999) also found in children receiving AG that TOAEs were reduced while PTA and ABRs remained the same.

Finally, Sone *et al* (1998) in a histological study in CF patients found on examination of the cochlea that OHCs in some subjects appeared intact while the spiral ganglia in some patients appeared to have been damaged. This is evidence that AGs could also be acting at the level of the spiral ganglia/afferent synapse.

(ii) Cisplatin

Stavroulaki *et al* (2001) conducted a study on children receiving cisplatin to try to detect whether the first cisplatin infusion is ototoxic. They used OAEs (TOAEs and DPOAEs) as well as PTA to detect early signs of ototoxicity. Their results show that a significant high-frequency hearing loss is identified in children even after one low-dose cisplatin-infusion session.

Hatzopoulos *et al* (2001) conducted a study to evaluate the use of DPOAEs responses in the detection of cisplatin-induced ototoxicity in an animal model. The post-treatment DP-gram data showed significant reduction of the signal to noise ratios in the majority of the frequencies tested, across all tested protocols. Morphological analyses indicated that the inner hair cells remained intact, while several types of alterations were observed in the arrangement of the stereocilia in the outer hair cells.

6.4. RESULTS

The use of DPOAEs in determining the early sites and mechanisms of aminoglycoside ototoxicity in the CF patient group reviewed above, formed part of the original rationale for their use in this study. However, review of all the DPOAE data in conjunction with the PTA and HFPTA data after completion of this study, showed the data to be very heterogeneous. For the relatively small groups of patients receiving no AGs (n=17) and those receiving AGs (n=33), the effect of heterogeneity was further compounded by the reduction in the numbers of patients going forward to have their DPOAEs measured. In the case of those receiving no AGs, this was reduced to n=12

and for those receiving AGs down to n=27. This heterogeneity was evident in terms of:

- the age range of patients
- the wide variation in aminoglycoside (and other drugs) received
- the incidence and degree of prior exposure to noise
- the degree of likely PTA threshold elevation due to presbyacusis
- the degree of likely PTA threshold elevation due to noise exposure

Therefore, robust correlation with treatment factors was going to be difficult to justify given these other cofactors and reduced patient numbers. Despite these limitations, the potential use of DPOAEs in categorical diagnosis of possible retrocochlear lesions in the current patient group was seen as a valuable analysis exercise.

As the concensus appeared to be that for PTA thresholds over -10 to 30 dB HL there would be a negative correlation between DPOAE amplitude and PTA threshold so that at 30 dB HL little or no emission would be expected to be seen if the OHCs were the primary lesion site (Harris and probst, 1997; Kimberley, 1997; Robinette and Durrant, 1997; Gaskill and Brown, 1993). Over 40-60 dB HL no DPOAE would expect to be generated especially with F1 and F2 stimuli levels below 60-70 dB SPL.

Consequently based on these criteria in this results section, consideration of the 2F1-F2 DPOAE and its near equivalent PTA frequencies are used as likely evidence of auditory lesions occurring at the IHC onwards. This is

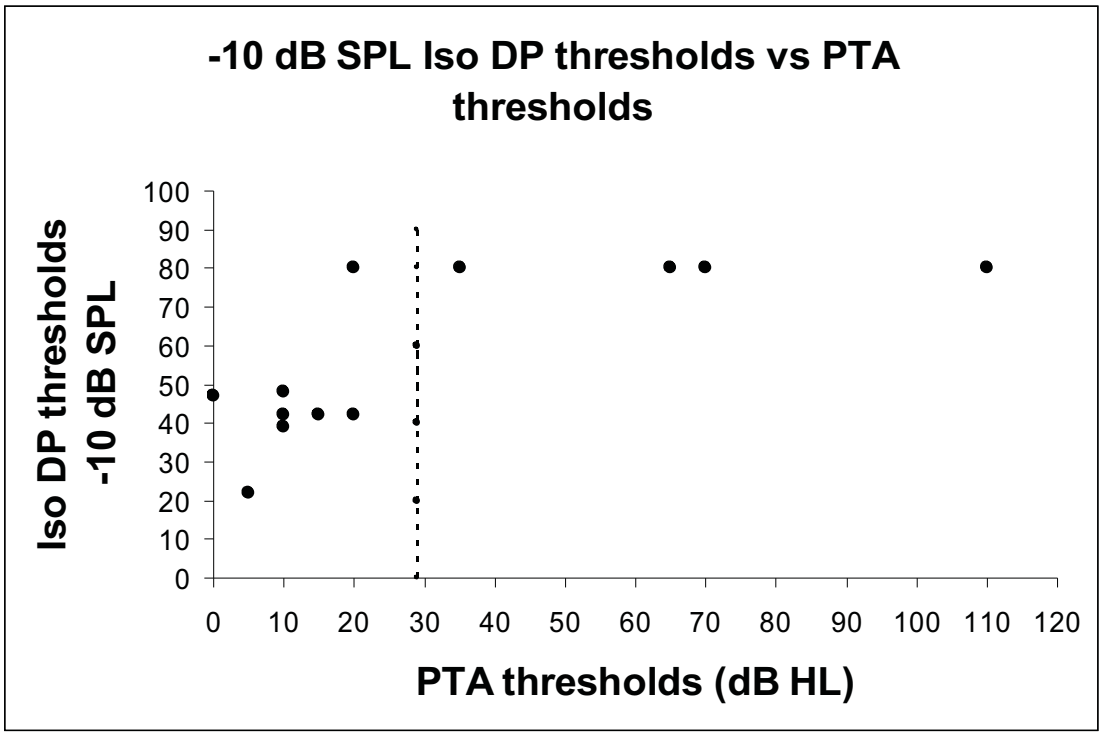
particularly true if the iso DP of –10 dB SPL is generated by stimulus below 60 dB SPL.

6.4.1. The non Gentamicin treated group

The DPOAEs are plotted against the PTA thresholds for the frequencies of 2, 4 and 6 kHz (figures 6.1, 6.2 and 6.3 respectively) for the right and left ears. The maximum stimulus was 70 dB SPL and when there was no emission the value was set to 80 dB SPL. The dotted line indicates the 30 dB HL criteria.

Figure 6.1 **2 kHz**

Right ear



Left ear

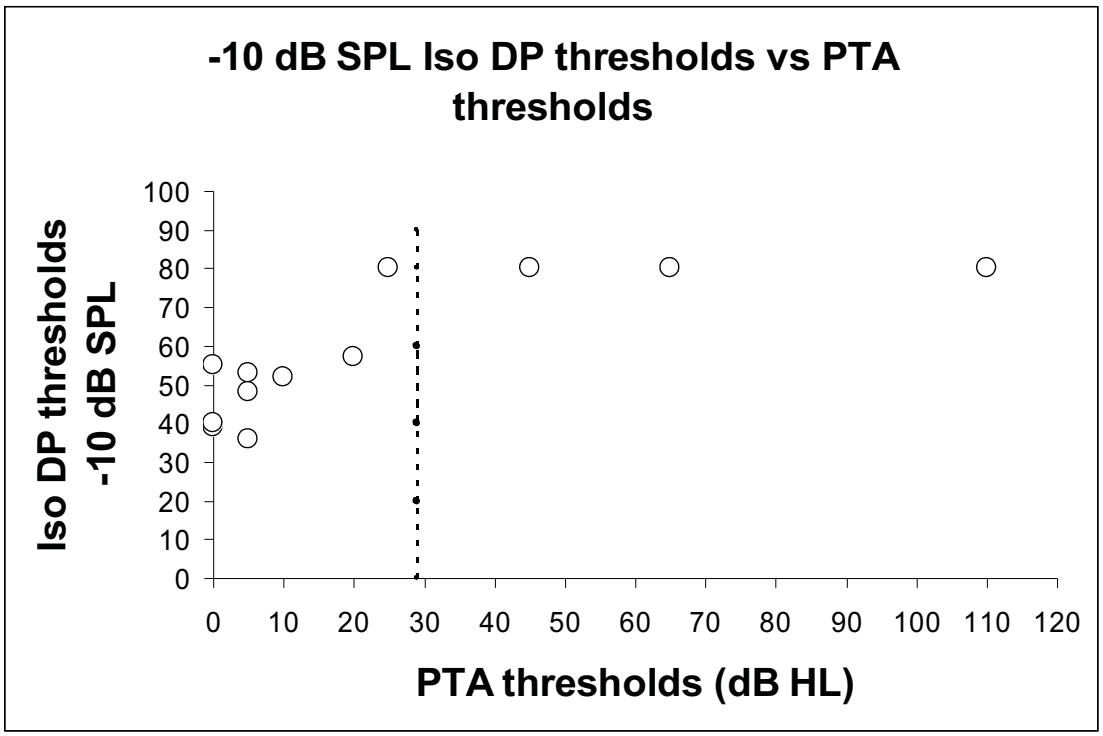
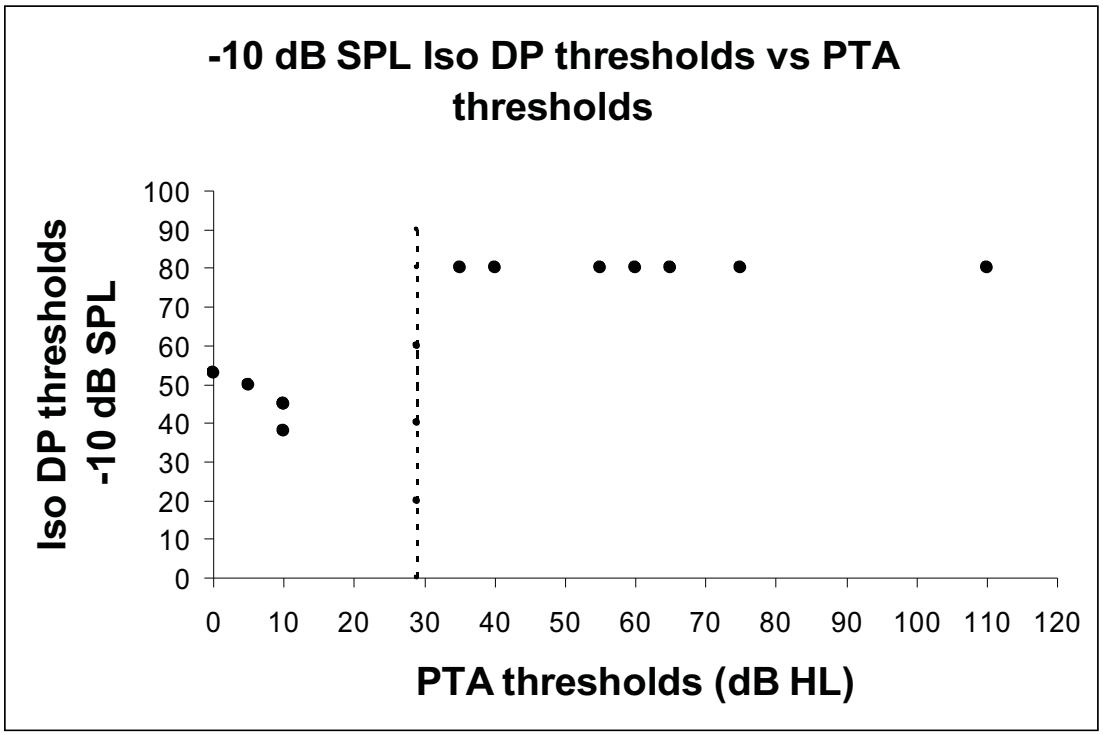


Figure 6.1. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 2 kHz. The dotted line represents the nominal line of 30 dB HL.

Figure 6.2 **4 kHz**

Right ear



Left ear

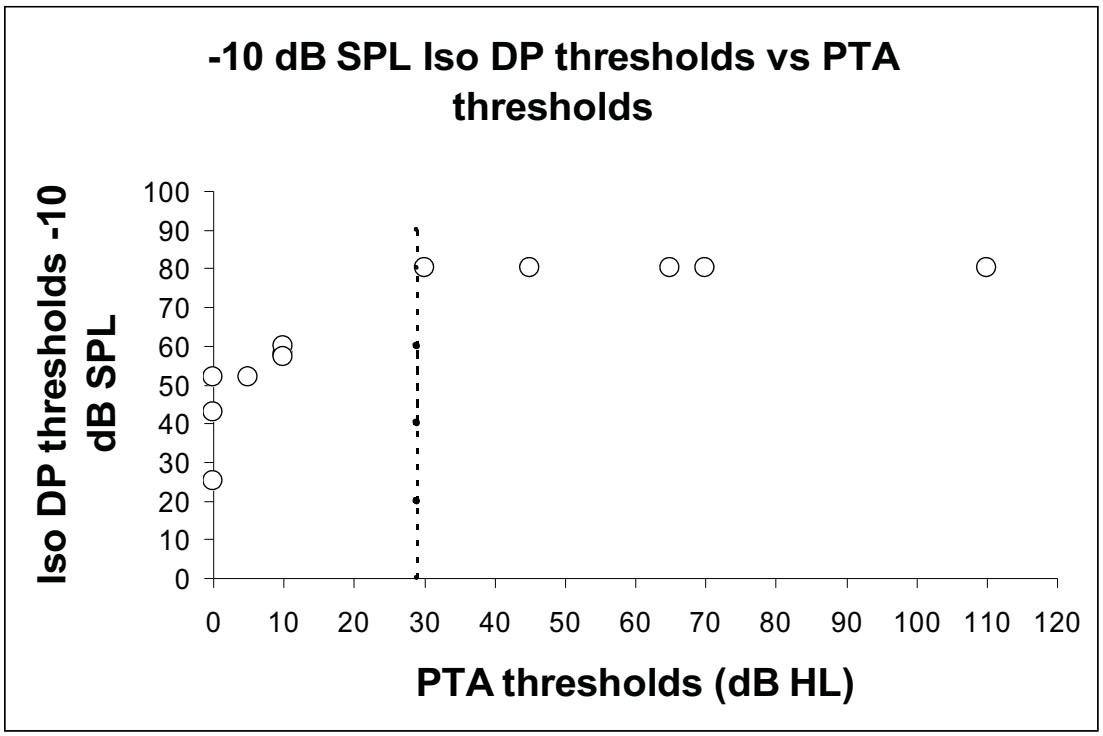
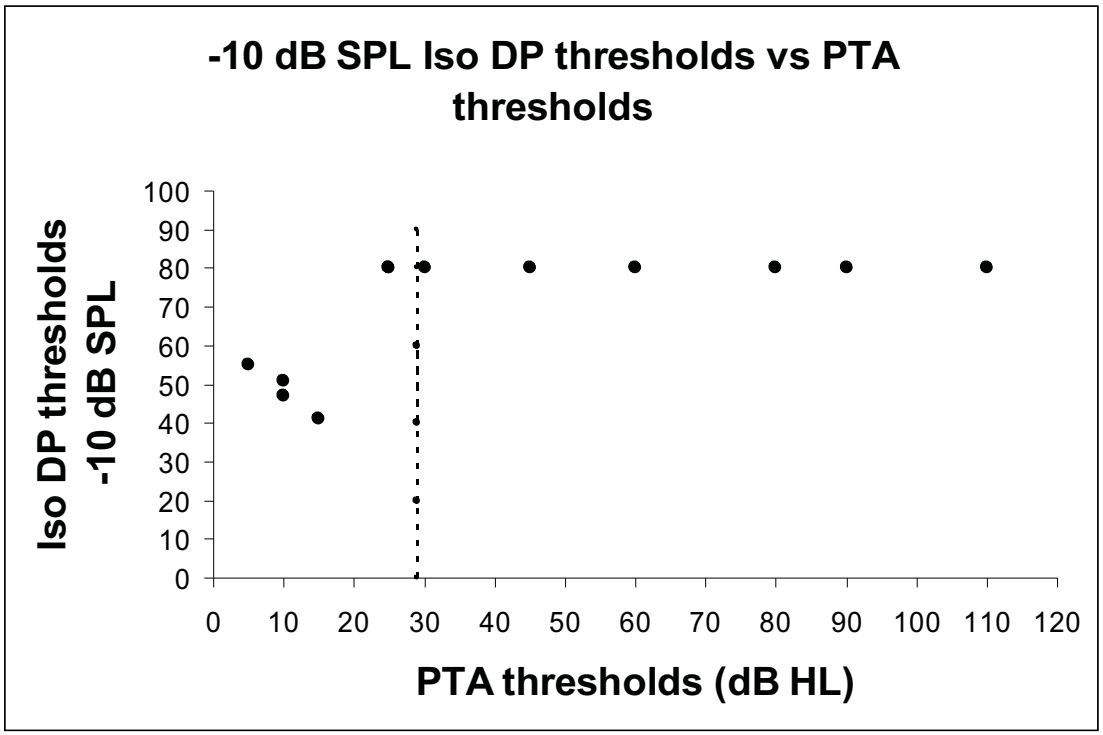


Figure 6.2. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 4 kHz. The dotted line represents the nominal line of 30 dB HL.

Figure 6.3 **6 kHz**

Right ear



Left ear

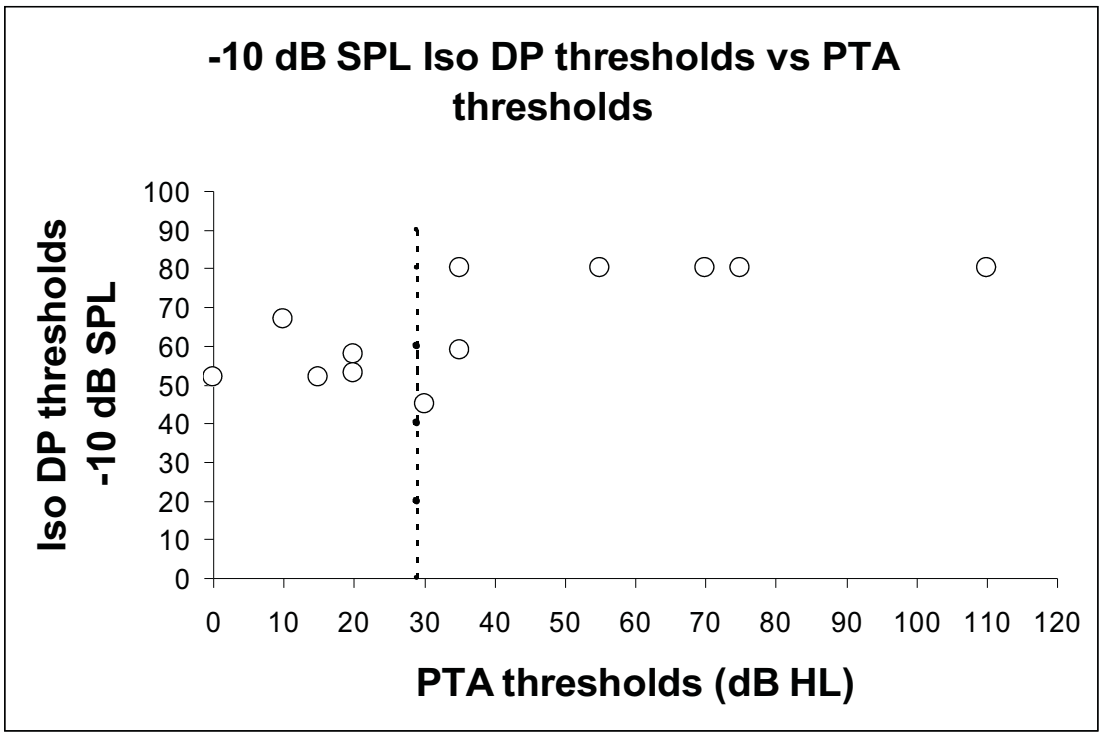


Figure 6.3. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 6 kHz. The dotted line represents the nominal line of 30 dB HL.

In Figures 6.1 – 6.3, varying proportions of present/absent DPOAEs are seen. For most figures it is evident that above 30 dB HL no emissions could be recorded as reported by earlier workers. Table 6.1 shows the ratio of DPOAEs present vs the total number of patients. Of most interest are the two points in the Left ear Figure 6.4 and summarised in Table 6.2 which occur at PTA thresholds = 30-35 dB HL. These are taken as evidence of IHC/nerve damage due to their association with PTA thresholds above 30 dB HL. They are shown in table 6.2.

Table 6.1

	Right ear	Left ear
2 kHz	7/12	8/12
4 kHz	4/12	7/12
6 kHz	4/12	7/12

Table 6.1 shows the ratio of the number of emissions present vs the number of patients tested in the non gentamicin treated group.

Table 6.2

	Right ear	Left ear
2 kHz	0	0
4 kHz	0	0
6 kHz	0	2

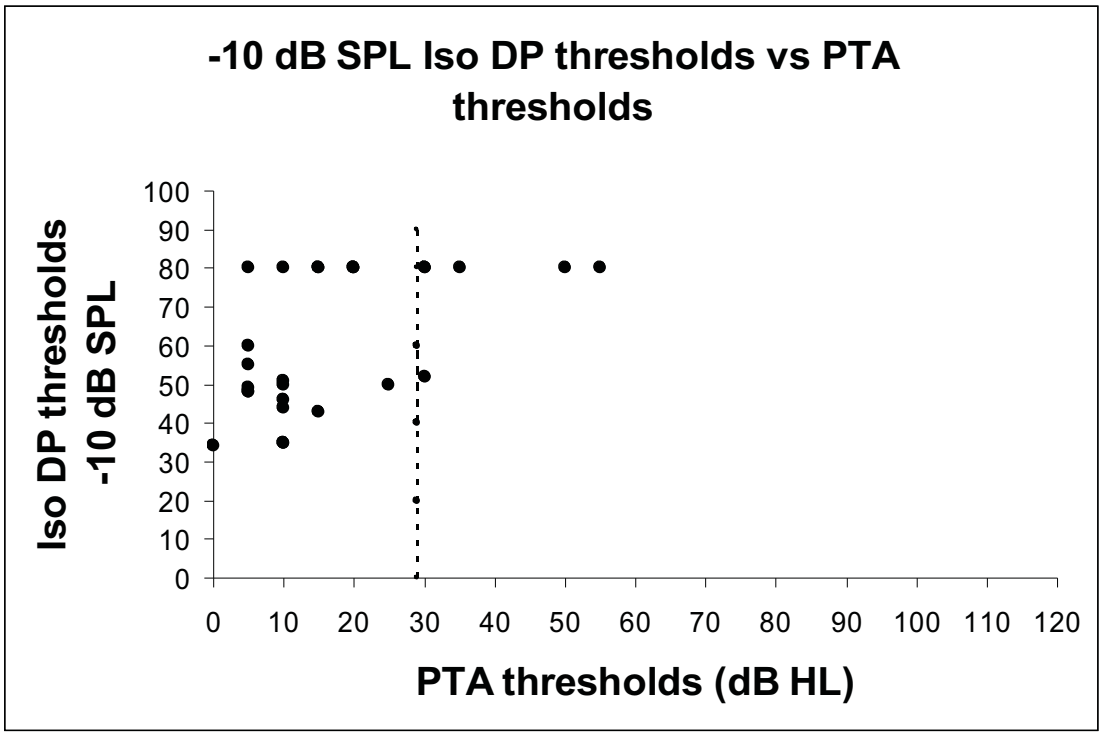
Table 6.2 indicates that in the non gentamicin treated group, only 2 patients with a HL>30 dB HL had evidence of emissions in the left ear at 6 kHz. These two patients were aged 48 and 68 years old whose DPOAEs did not reflect passive movement of the basilar membrane (Nelson and Kimberley, 1992). The 48 years-old female had NHL and had a total categorical risk of 6. The 68 years-old female had MM and had a total categorical risk of 4.

6.4.2. The Gentamicin treated group

The -10 dB SPL iso DP F2 values were plotted against the PTA thresholds for the frequencies of 2, 4 and 6 kHz in figures 6.4 - 6.6 for the right and left ears. The maximum stimulus was 70 dB SPL and when there was no emission the value was set to 80 dB SPL. The dotted line indicates the 30 dB HL criteria.

Figure 6.4 **2 kHz**

Right ear



Left ear

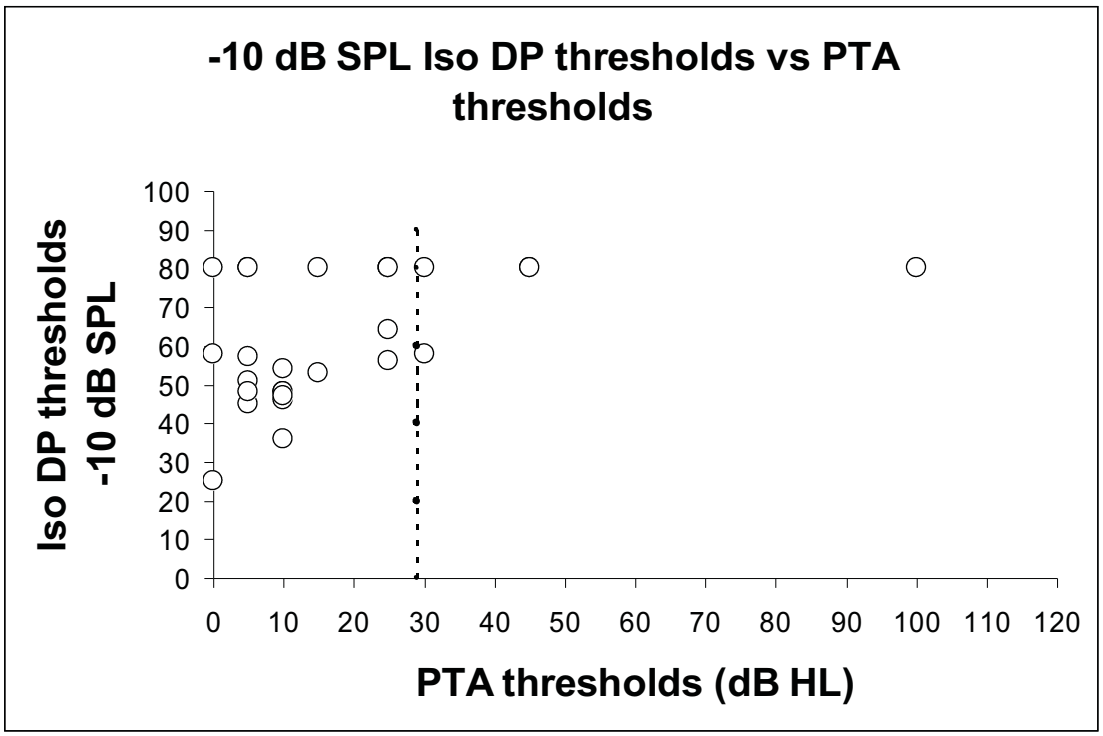
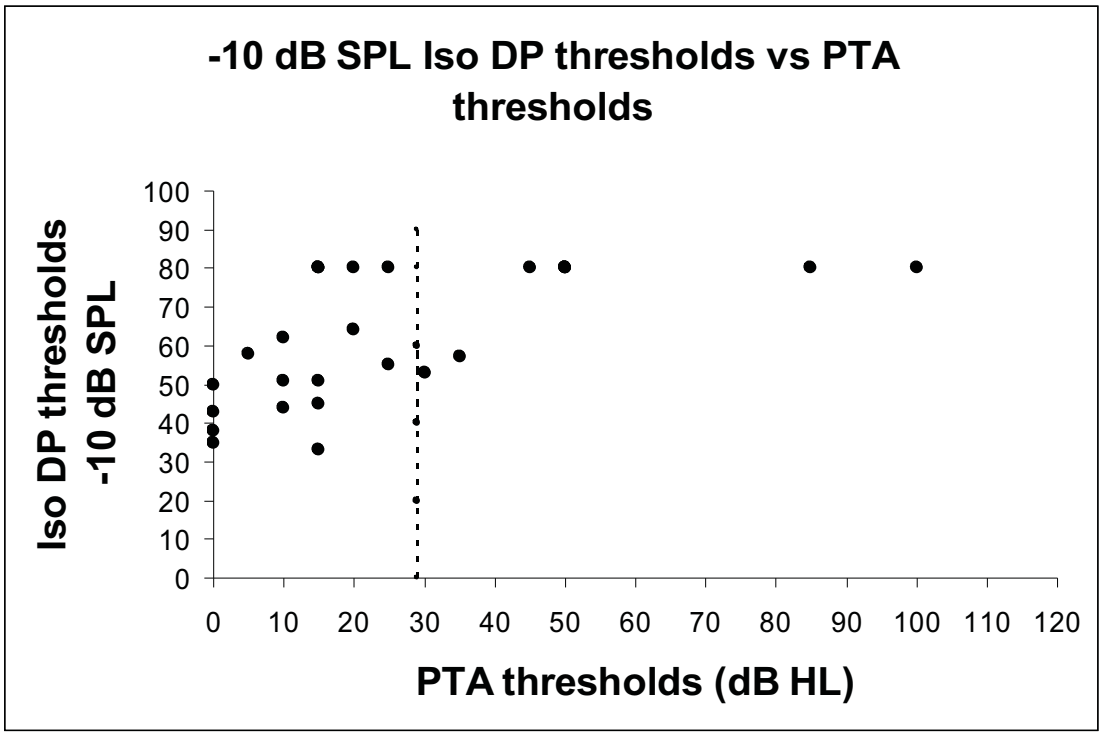


Figure 6.4. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 2 kHz. The dotted line represents the nominal line of 30 dB HL.

Figure 6.5 **4 kHz**

Right ear



Left ear

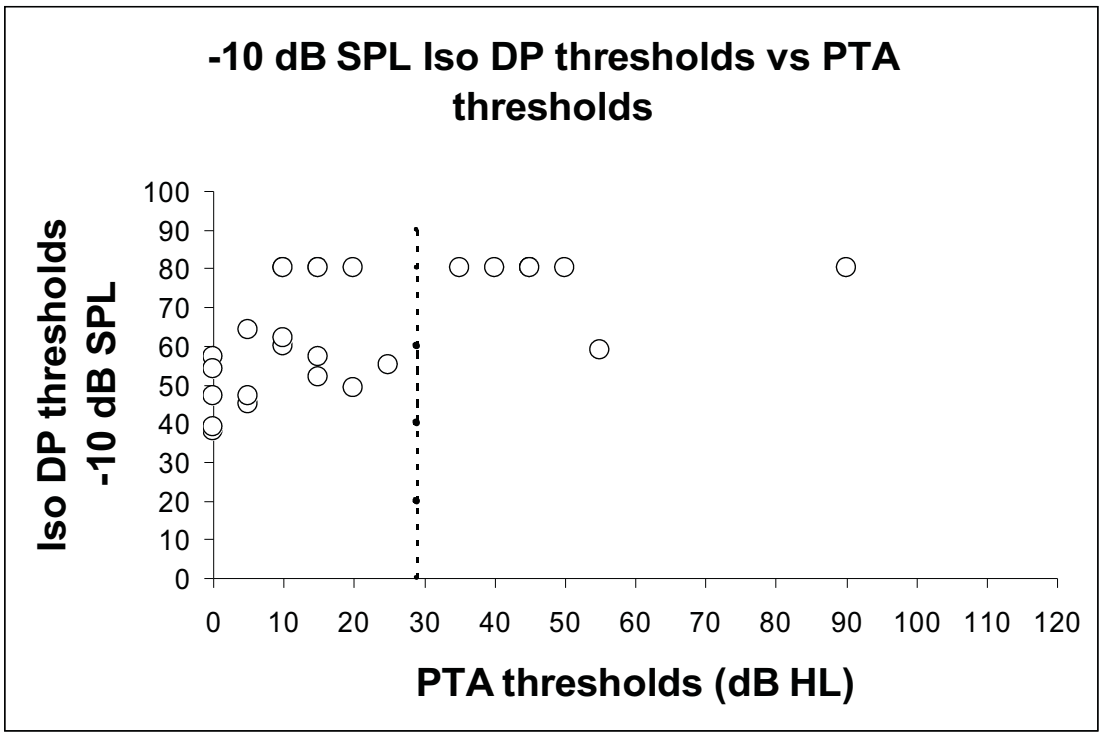
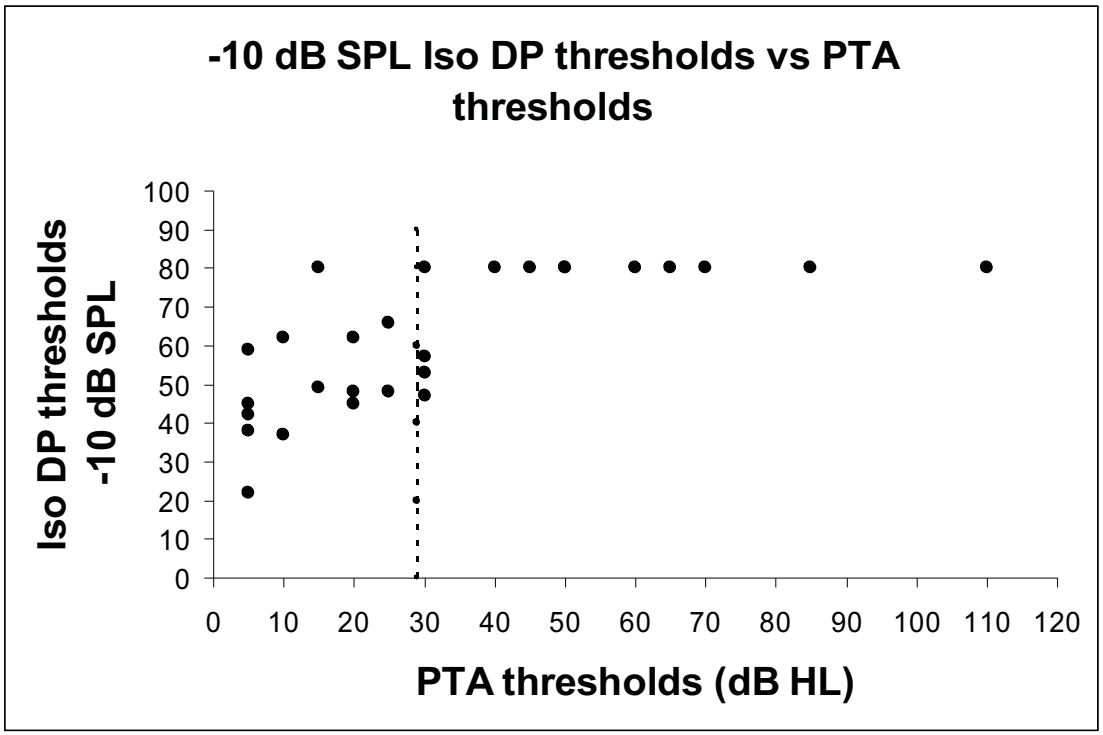


Figure 6.5. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 4 kHz. The dotted line represents the nominal line of 30 dB HL.

Figure 6.6 **6 kHz**

Right ear



Left ear

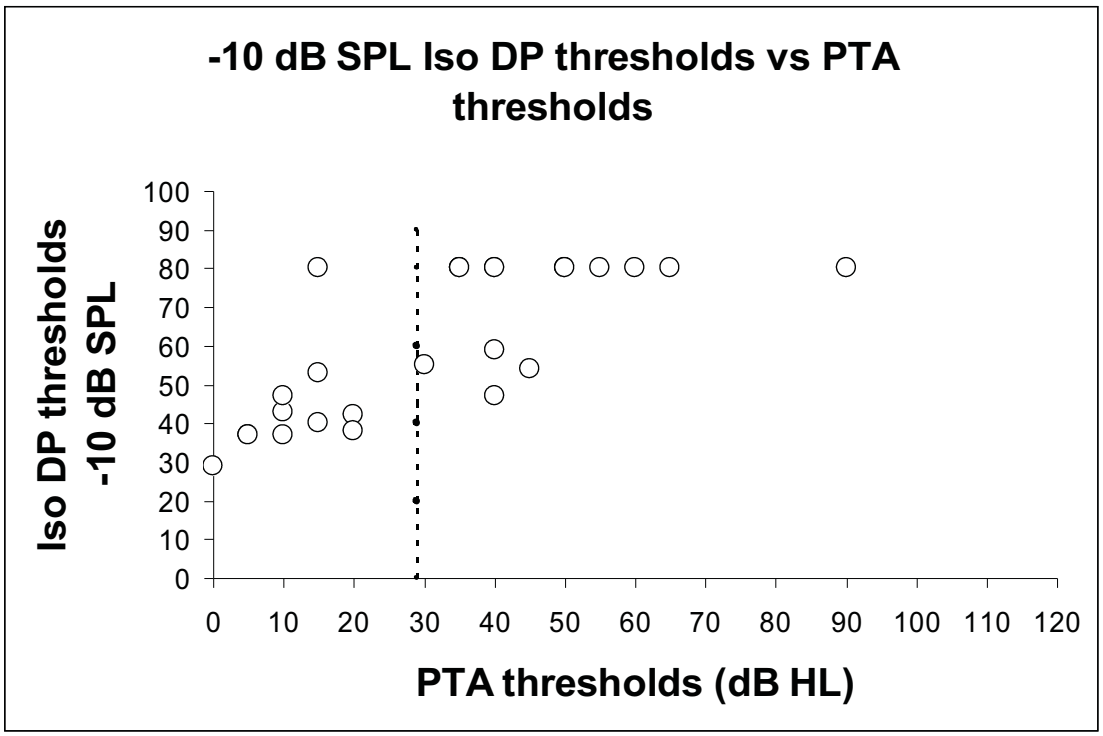


Figure 6.6. The threshold intensity necessary to generate a DPOAE at -10 dB SPL is plotted against the PTA thresholds for the frequency of 6 kHz. The dotted line represents the nominal line of 30 dB HL.

Overall, the varying proportions of present/absent DPOAEs were higher than for the non-AG treated group as shown in Table 6.3. Again it was evident above PTA thresholds of 30 dB HL many -10dB SPL iso DP values could not be recorded.

What was further apparent in the AG treated group was the presence of individuals returning -10 dB iso DP values at all frequencies. These are taken as further evidence of IHC/nerve damage possibly due to AG therapy. The proportions of these patients were shown in Table 6.4.

Table 6.3

	Right ear	Left ear
2 kHz	13/27	15/27
4 kHz	15/27	15/27
6 kHz	16/27	13/27

Table 6.3 shows the number of emissions present vs the total numbr of patients tested in the AG treated group.

Table 6.4

	Right ear	Left ear
2 kHz	1	1
4 kHz	2	1
6 kHz	3	4

Table 6.4 shows the numbers of emissions present for each frequency when PTA>30 dB HL.

Table 6.5: Summary of patients cofactor data for iso DP with elevated PTA thresholds.

Patient	Age	Diagnosis	AG dose (g)	CR	Iso DP Right	Iso DP left	PTA right/left
1	25	ALL	1,08	5	50	55	25/30
2	40	APML	5,84	3	45	45	30/40
3	61	CML	7,84	3	45	55	30/45
4	67	CML	5,82	3	55	60	30/40

In total out of 27 patients from the AG treated group tested, 4 patients had emissions with PTA>30 dB HL. The age range of the patients with evidence of IHC/nerve damage was 25-67 years old. The gentamicin dose the patients with evidence of IHC/nerve damage received was compared with the gentamicin dose of the other 23 patients using the Mann-Whitney test. The p value was 0.3, non significant.

The categorical risk of the patients with evidence of IHC nerve damage were 5, 3, 3 and 3.

6.5. DISCUSSION

Overall, these results were difficult to interpret because of the low number of patients (12 for the non AG treated group and 27 for the AG treated group). In addition, 4-8/12 in the non-AG group and 11-14/27 had no emissions in the AG treated group. This absence of emissions was as expected in the literature and indicates their lack of correlation with PTA thresholds above 30 dB HL. In fact the plots above showed that for some frequencies between 0 – 25 dB DPOAEs could not be generated with F1 and F2 stimuli up to 70 dB SPL. This again shows that DPOAEs alone cannot be relied on as an

objective measure of cochlear sensitivity. This absence of DPOAEs could reflect the passive biomechanical factors cited by Kemp (1997) especially when PTA thresholds fell between 0-10 dB HL.

The categorical use here of individual iso-DP data to indicate post OHC deficit is of interest from both a mechanistic and clinical point of view. In the case of the two patients in the non AG treated group, the threshold elevations of 30-35 dB HL with -10 dB SPL iso DP values below 60 dB HL are indicative of the hearing deficit being due to factors at sites down from the OHCs (Kemp, 1997; Harris and Probst, 1997; Kimberley *et al.,*1997; Robinette and Durrant, 1997).

This does not rule out some deficit occurring at the level of the OHCs, but it importantly points to the IHC/afferent synapse and possibly more central loss. This is supported by the previous reports following noise exposure by Attias (1998). The mechanism here maybe involve chronic low level excitotoxicity at the nerve due to presbycusis, that spared the OHCs (Henderson and Hamernick, 1995; Prasher and Canlon, 1999).

6.5.1. Non AG treated group: involvement of malignancy ?

Only two subjects had DPOAEs at frequencies were the PTA had thresholds above 30 dB HL. By the criteria of Nelson and Kimberley (1992) these emissions with -10 dB SPL iso DPs produced with F2 below 60 dB SPL are taken as evidence of active as opposed to passive OHC activity. In this group, the two patients suffered from multiple myeloma and Non Hodgkin's

lymphoma. The patient with NHL, aged 48 years-old had normal PTA thresholds, but elevated thresholds on HFPTA. The patient with multiple myeloma, aged 68 years-old had normal PTA and HFPTA thresholds. It is not considered likely that these malignancies contributed to the evidence of post OHC deficit. In these cases AGs can be discounted as contributing to deficit.

6.5.2. The AG treated group

In this group, the further evidence of deficit post OHC was apparent in four patients whose age related hearing loss could be taken to account for the PTA thresholds between 30-55 dB HL where DPOAEs could be clearly elicited with stimuli between 40-60 dB SPL. This again is taken as indicative of active generation by the OHCs rather than a predominantly passive process by Basilar Membrane movement inducing a distortion product (Nelson and Kimberley, 1992; Kemp, 1997).

The categorical risk for patients with emissions and those without is not significantly different (p value=0.2, Mann-Whitney test). Explicit interpretation of effects of categorical risk is also made more difficult by the reduction of data.

In this group, AGs are a possible factor in increased PTA thresholds whilst leaving OHCs relatively unaffected, although we cannot fully rule out other factors (eg. other drugs given in this study). From Table 6.5 the range of AGs these four patients received was from 1.1 to 7.8 g and was not significantly

different (p=0.3, Mann-Whitney test) from the other patients and the categorical risk was also comparable with the other patients.

This result is important as the vast majority of the literature supports a primary effect of AGs at the OHCs from high dose animal models (Forge and Schacht, 2000). This then suggests that if AGs were acting in these patients to affect auditory sensitivity, then they were acting down from the OHCs, possibly at the IHC afferent synapse at the NMDA glutamate receptor (Basile *et al.,* 1996). Indirect supporting evidence for the IHC/afferent synapse as a primary or parallels site along with OHCs for ototrauma is again provided by Attias (1998). The finding of Sone (1998) in cochleae obtained from CF cadavers also points to the post OHC involvement in some patients.

It is possible that the AGs here did not play a major role in the differential deficit between PTA thresholds and the thresholds for DPOAE generation but they are a major recognised risk factor with the dosages given here falling within the range associated with ototoxicity in the literature cited previously (Cone (1982); Kahlmeter and Dahlager (1984)).

6.5.3 Other Risk Factors.

In the gentamicin treated group, the patients with evidence of nerve/IHC damage suffer from ALL, APML, and two patients have CML. Of interest, the patients with CML have no evidence of hearing loss in the speech frequency range or in the high frequency range (10-16 kHz). The patient with APML has evidence of loss both in the speech frequency range and in the high

frequency range. The patient suffering from ALL had bilateral perforated tympanic membranes. She had mild losses on standard PTA and loss on HFPTA.

The drugs received by patients with haematological malignancy with the best documented evidence of neurotoxicity are the vinca-alkaloids.

In the gentamicin treated group, only the patient suffering from ALL with an IHC/nerve damage had 6 mg of vincristine. There is no common drug between these patients with evidence of IHC or nerve damage. Except for one patient, the CR for those patients with evidence of nerve damage was not different from the median CR of 3.5 for the gentamicin treated group (N=27).

In addition, the patients here may well have suffered from other risk factors as covered in Kennet *et al*, 1983; Moore & Smith, 1984; Moffat, 1987; Vernon & Brummet, 1977; Darrouzet & De Lima Sobrinho, 1962; Hawkins *et al*, 1975.

6.6 Summary

In summary this part of the study investigating DPOAE generation could not provide much resolution of effect of therapy on OHCs due to the reduced number of patients exhibiting measurable emissions. It did however provide novel data that provided evidence that OHCs in humans may not be the first site of action in all cases of ototrauma, including noise and aminoglyocides. In particular, the presence of quite strong emissions at 35 -55 dB HL are considered as compelling evidence of this and fits the criteria cited by other

workers (Harris and Probst, 1997; Robinette and Durrant, 1997). This may have important implications for the future development of other therapies in targeting the cochlea with putative protectants against a range of ototraumas in humans (Prasher and Canlon, 1999).

CHAPTER 7: INCIDENCE AND RISK OF VESTIBULOTOXICITY IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES DETERMINED BY COMPUTERISED DYNAMIC POSTUROGRAPHY.

7.1. INTRODUCTION

7.1.1. Aims of the study

In this chapter, the results are presented from the part of the study that aimed to determine the incidence of balance deficit in patients with haematological malignancy. Specifically this was with the expectation that AG therapy would lead to an increased incidence of vestibulototoxicity in those patients receiving it. In contrast to most previous studies of the effects of aminoglycosides on balance (Kahlmeter and Dahlager, 1984; El-Bakry, 1998). Computerised Dynamic Posturography (CDP) was employed. Importantly this enabled not just the contribution of the vestibular apparatus to postural control but also visual and somatosensory contributions also (Black and pesznecker, 1993; Mishra *et al*, 2009)The use of CDP has considerable advantage in that changes are usually detected early due to change in peripheral vestibular input to the brain. This then alters the adaptive use of vision and somatosensory inputs before hair cell damage. This is detected by use of the differential sensory input test battery offered by CDP (Black and Pesznecker, 1993).

In a prospective study using CDP by Black & Pesznecker (1993) to investigate AG vestibulotoxicity in a generalised hospital study it appeared that balance problems following AG exposure would reach up to 50%.

Vestibtulotoxicity occurred precipitously and without warning. Importantly they found evidence that both vestibulo-ocular (VOR) and vestibulospinal (VS) systems were affected.

For differential diagnosis of the vestibular, visual and somatosensory systems, CDP employs a test battery. A brief outline of the test battery and what each component measures is given below. The physiology and anatomy of the postural control has already been covered in Chapter 1 section 1.5.

7.1.2. Posturography

(i). Postural control is determined by the integration of three main inputs: vestibular, visual and somatosensory (Arenberg, 1993). The brain integrates the information received from these three main afferent inputs and transmits control signals via the motor efferents to the muscle groups of the whole body to maintain balance and postural control. If one or more of the three inputs is missing or giving misleading information, the postural control will be affected.

(ii). The Equitest CDP equipment uses a dynamic controlled platform coupled to accelerometers in its base. The controlled platform movements (posterior-anterior-plane only) are designed to isolate the contribution from each sensory input (Nashner, 1993a, 1993b, 1993c). At rest, everyone presents a “rest sway” and at rest without the platform being driven the CDP system measures the self referenced internal “whole body system sway noise” at rest. The “rest sway” is specific to each person, but normally remains within a cone of 12.5^0. Outside this the subject will fall.

(iii). Sway is normal at rest and is due to the small and asynchronous variations in muscle group activity. Cerebellar activity generates corrective signals to these muscle groups but in turn induces sway that has to be corrected. This constant activity when upright is necessary to keep the body around a centre of gravity (COG). The sway angle and the limits of stability lie within 12.5^0 of the vertical plane (Shepard *et al*., 1993).

(iv). A percentile scoring in the Sensory Organisation Testing (SOT) tests is used where '100%' is equivalent to completely stable with no detectable sway and '0%' is equivalent to a fall being registered.

(v). Sensory Organisation Testing (SOT) battery

The six SOT conditions present increasing levels of challenge to the processing capability of the whole postural control system. The SOT test uses the 'noise signal' at rest within each system as the reference point for each subject. It completely removes visual system input by the subject closing their eyes, or attempts to cancel out visual and proprioreceptive components of the input signals by sway referencing their input. This can be done with eyes open and sway referencing the visual surround to ankle movement or by sway referencing the ankle proprioceptive input by attempting to keep ankle joint movement at 90^O with closed eyes. The former condition has the perceptual effect of telling the eyes that there is no apparent movement.

Sway referenced movement of the balance plate uses the subjects sway signal to move the platform in synchrony with the ankles to give the perception via the proprioreceptive pathway that the ground is stationary (Shepard *et al*., 1993).

The six SOT conditions are:

Condition 1: Eyes open, horizon stable, platform stable: measures the normal sway, the “internal system noise”

Condition 2: Eyes closed, horizon stable, platform stable: measures the normal sway when the visual inputs are removed. The subject has to rely on his somatosensory and vestibular inputs only.

Condition 3: Eyes open, horizon swayed, platform stable: the visual informations are altered by the horizon sway and the subject has to rely on his somatosensory and vestibular inputs.

Condition 4: Eyes open, horizon stable, platform swayed: the somatosensory informations are altered by the platform sway and the subject has to rely on his vestibular and visual inputs.

Condition 5: Eyes closed, horizon stable, platform swayed: the visual informations are absent and the somatosensory informations are altered by the platform sway. The subject only has his vestibular inputs to rely on.

Condition 6: Eyes open, horizon swayed, platform swayed: the visual and proprioceptive informations are altered and the subject has to rely on his vestibular inputs.

The SOT results show the patients ability to use sensory inputs appropriately. It gives an indication of the patient's ability to maintain stable as the sensory inputs available for use (vision and somatosensory/proprioception informations) are manipulated. The SOT does not, however, test the peripheral senses performance. Therefore results suggesting a vestibular dysfunction pattern cannot differentiate between peripheral and central dysfunction. Also the test does not readily detect a patient who has a well-compensated unilateral vestibular lesion (Shepard *et al*, 1993). It is also important to look at the raw data, particularly the waveforms of the patients who fall to see if this shows a free fall every time or if they make corrections before falling. If corrections are present, the patient must have had some vestibular feedback before the eventual fall. If the time before falling increases for successive runs, this suggests a learning process (Shepard *et al.*, 1993).

In summary SOT conditions 1-2 establish the level of "system noise" with the system at rest. Conditions 1-3 are primarily concerned with the assessment of the visual system. In Conditions 4-6, by removing or cancelling the visual and proprioceptive components, the contribution made by the vestibular system can be better inferred (Shepard and Telian, 1996). Table 7.1 summarises the 6 SOT conditions and their significance.

Table 7.1. Summary of the six SOT conditions and their significance.

Condition	***Visual***	***Proprio***	***Vestib***	***System tests***
1	VIS ✓	PROP ✓	VESTIB ✓	Normal at rest input
2	VIS □	PROP ✓	VESTIB ✓	Visual absent others normal
3	VIS ≈	PROP ✓	VESTIB ✓	Visual misled by negative feedback to horizon. Others normal at rest
4	VIS △	PROP ≈ Ankles remain at 90°	VESTIB △	Visual and proprioreceptive senses stimulated by using own sway as driving stimulus. Proprioception misled by platform following sway movement and nullifying it by providing negative feedback.
5	VIS □	PROP ≈ Ankles remain at 90°	VESTIB △	Visual input absent. Proprioreception misled by platform and nullified by platform feedback. Vestibular system stimulated.
6	VIS ≈	PROP ≈ Ankles remain at 90°	VESTIB △	Sway noise provides signal Mislead visual and proprio by sway reffing horizon and platform. Vestibular stimulated.

SYMBOL KEY: ✓ = Normal baseline; □= Absent; △ = Sensory Stimulation; ≈ Sensory stimulus nullified using sway referenced movement of visual surround or ankle proprioreception nullified by synchronous driving of the sway platform.

7.1.3. SOT Normative values

The SOT scores of patients with haematological malignancy in this study were compared with the means and 95th percentiles of an American control group provided by the manufacturer. These SOT values are reported to be normally distributed and over age 20-59 yrs do not change. Some changes in SOT score are seen over 60-80 and the system takes this into account when making pass/fail decisions or low/normal ratios (Jacobson *et al*, 1993). These are shown in Table 7.2.

The results from the SOT test battery are then used to infer disturbance within the system and in the present study it was assumed the primary damage site was the vestibular system.

Table 7.2. Means + (95th percentiles) SOT scores (taken from Handbook of Balance Function Testing, 1993)

SOT CONDITION	20-59 (n=112)	60-69 (n=54)	70-79 (n=29)
1	94 (90)	94 (90)	89 (70)
2	92 (85)	92 (85)	86 (63)
3	91 (86)	89 (80)	88 (82)
4	82 (70)	82 (77)	78 (69)
5	69 (52)	69 (51)	62 (45)
6	67 (48)	67 (49)	53 (27)

7.1.4. SOT sensory ratios

These ratios give information about the relative performance of each sensory component in determining postural control.

The somatosensory ratio

The somatosensory ratio is derived from Condition 2/Condition 1 and normally returns a value greater to or about 1. A low ratio means relative sway increases with the eyes closed and indicates that the subject makes poor use of the remaining somatosensory references.

The visual ratio

This derives from the ratio of Condition 4/Condition 1 and is based on assessing whether sway increases when somatosensory cues are inaccurate. A low ratio would mean relative sway increases further when the somatosensory cues are nullified and this low score is taken as indicating that the subject is making poor use of visual references.

The vestibular ratio

This derives from the ratio of Condition 5/Condition 1 and measures whether sway increases when somatosensory cues are inaccurate. A low ratio would mean relative sway increases further again when visual cues are removed and somatosensory cues are nullified. A low score is taken as indicating that the subject makes poor use of vestibular cues, or that vestibular cues are unavailable. The significance of these tests along with the determinant means and 95th percentiles are given in Tables 7.3 and 7.4 respectively.

Table 7.3: Summary of SOT ratios and their significance

Sensory analysis and Ratio name	***Ratio pair***	***Significance***
Somatosensory	Condition2/condition1	Does sway increase when visual cues are removed?
Visual	Condition4/condition1	Does sway increase when somatosensory cues are inaccurate?
Vestibular	Condition5/condition1	Does sway increase when visual cues are removed and somatosensory cues are inaccurate?
Visual Preference	Condition3+6/condition2 +5	Do inaccurate visual cues result in increased sway compared to no visual cues?

Table 7.4. Means + (95^{th} percentiles) SOT ratios derived from Handbook of Balance Function Testing

SOT RATIO	***20-59 yrs (n=112)***	***60-69 yrs (n=54)***	***70-79 yrs (n=29)***
COND2/ COND 1 = SOM	0.98 (0.95)	0.98 (0.95)	0.96 (0.9)
COND4/ COND 1 = VIS	0.87 (0.77)	0.87 (0.77)	0.87 (0.77)
COND5/ COND 1 = VEST	0.73 (0.57)	0.73 (0.57)	0.73 (0.57)

7.1.5. Clinical manifestations of vestibulotoxicity

A significant number of patients have to receive aminoglycoside antibiotics because of life–threatening infections, during which long periods are often spent hospitalised. It is possible that when the patient becomes well enough to be mobile that any symptoms of postural dysfunction are first noted and are often incorrectly attributed to the patient's general debility. An accurate diagnosis of vestibulototoxicity is in such cases often delayed or overlooked. If the patient is ambulatory, dysequilibrium is the usual manifestation of vestibulotoxicity. Vestibulotoxic subjects may show an ataxic gait, stumble easily or loose their balance when turning quickly and may complain of transient positional vertigo. They rarely have prolonged episodes of rotatory vertigo unless the ototoxicity is asymmetrical or they had preexisting vestibular asymmetry.

Patients may also complain of oscillopsia, the sensation that objects jump around with head movement (Carey and Wichter, 1977; Crawford, 1952; Dayal *et al*., 1979; Dayal *et al*., 1974). This symptom is caused by loss of vestibulo-ocular reflex (VOR) function and the inability of patients to successfully stabilise objects on the retina. As VOR loss continues, patients must rely more on accurate proprioceptive input for postural control. They eventually experience balance problems when they try to walk in darkness or on uneven surfaces.

7.1.6. Haematological malignancy and vestibular dysfunction

There are very few references in the clinical and experimental literature relating a link between blood malignancies and vestibular dysfunction. The literature for this is summarised here.

Smith *et al*., (1991) reported a patient with atypical chronic myeloid leukaemia presenting with the sudden onset of profound deafness. The patient only survived 8 months. Detailed histological examination of the temporal bone performed at necropsy showed loss of ganglion cells and afferent nerve fibres in the cochlea and vestibule associated with extensive fibrosis and new bone formation in the labyrinthine spaces. Hustert *et al*., (1997) reported on a 60 year old patient who presented with unilateral deafness, high pitch tinnitus, facial paralysis and rotatory vertigo. The diagnosis of primary Non-Hodgkin lymphoma of the internal auditory canal was made by histology after removal of the tumor (translabyrinthine route). El Bakry (1998) suggested abnormal response to caloric testing in one patient with blood malignancy with no explicit information of the findings.

In an experimental study by Kano (1998), an animal model was used to establish tumor infiltration into the temporal bone. Rat thymic lymphoma cells were inoculated into the cisterna magna of rats. The rats were decapitated and after fixation, decalcification and staining, sections were histologically examined by light microscopy. Two major routes of tumor cell infiltration into the inner ear were found: the cochlear aqueduct and the internal auditory canal. Tumor cells infiltrated Rosenthal's canal via the tractus spiralis

foraminosus and passed through Rosenthal's canal and the osseous spiral lamina into the scala tympani.

However, tumor cells did not infiltrate the organ of Corti through the habenula perforata. Tractus spiralis foraminosus and habenula perforata functioned as a barrier against tumor infiltration. In a few cases, tumor cells infiltrated over the macula cribrosa into the subepithelial space of the utricule and saccule. The macula cribrosa functioned as a barrier.

7.1.7. Vestibulotoxicity determined by subjective symptoms and signs

Gailiunas *et al*., (1978) performed a study on patients undergoing chronic haemodialysis and receiving gentamicin at a dose of 1 to 1.5 mg/kg/day. Thirty per cent of patients developed clinically detectable vestibular toxicity. The criteriae for vestibular toxicity were made from subjective symptoms of vertigo as well as "objective" findings of unsteadiness and inability to walk unassisted in the acute stage.

7.1.8. Vestibular toxicity determined by caloric testing

Most studies of human vestibulotoxicity have been evaluations of vestibular function using low-frequency vestibular stimuli, usually low-frequency, calorically induced nystagmus. Although the caloric stimulus tests predominantly low-frequency VOR responses, the portion of the VOR system more severely affected by acute and chronic ototoxicity, the sensitivity and specificity of the caloric test for symmetrical losses of vestibular function are poor (Furman *et al*., 1988). Normal intrasubject caloric nystagmus test-retest

variability responses are high, requiring a 50% reduction in nystagmus slow-phase velocity from baseline in order to reliably detect transitory or progressive bilateral changes in vestibular function (Reder *et al.,* 1977). In a study of streptomycin vestibulototoxicity, caloric and sinusoidal rotation tests were equally sensitive for monitoring the degree of nystagmic depression (Reder *et al*., 1977; Wilmot, 1973). The problem seen with caloric testing are reduced with rotational testing with several studies supporting the use of more controlled rotational stimuli produce lower intersubject and intrasubject variability than caloric testing (Black & Pesznecker, 1993; Jongkees and Hulk, 1950).

7.1.9. Vestibulotoxicity determined by Computerised Dynamic Posturography

The incidence of vestibulototoxicity using CDP has rarely been established. Black and Pesznecker (1993) did a prospective study on hospitalised patients receiving gentamicin, comparing them with a control group suffering from the same condition who did not receive gentamicin. Unfortunately they did not specify the dose and the amount of courses of AGs these patients had. To try and establish whether these patients had signs of vestibulotoxicity, they performed the vestibulo-ocular reflex testing and the sensory conditions testing of the CDP. They found that 12.5% of patients tested had abnormal VOR responses and that 50% of patients had abnormal responses on CDP SOT conditions. Additionally, in one subject, an abnormality in SOT condition 6 appeared two weeks before VOR changes appeared.

Given the improved early resolution and sensitivity of CDP in assessing vestibular performance, it is surprising that very few studies using CDP have been carried out in aminoglycoside exposed patients. The Balance Centre at Leicester Royal Infirmary provided an opportunity to partly address this. This study enabled complementary investigation of incidence and risk of gentamicin vestibulotoxicity alongside its cochleotoxicity covered in the previous chapters.

7.2. RESULTS

Figures 7.1 to 7.6 illustrate the results obtained for both non AG and AG groups. For SOT Conditions 1-6, all scores are expressed as a percentage of sway within the cone of stability referred to earlier, where 100% is completely stable and 0 % represents a fall due to the subjects COG falling outside the cone of stability. In these plots all points are plotted as SOT score vs age along with the age related 95th percentile. These were derived from the results cited by Jacobson *et al* (1993) given in Table 7.2

7.2.1. SOT Conditions 1-3: Assessment of contribution of the Visual System

Figures 7.1-7.3 illustrate the SOT scores used in assessing the role of the visual system in postural control. Figure 7.1 illustrates the distribution of SOT Condition 1 which represents the amount of 'at rest' sway with no modification of sensory input. All parameters were normal when compared against the normative results cited in the Handbook of Balance Function Testing. This overall postural 'system noise' was used as reference for subsequent conditions.

Figure 7.1

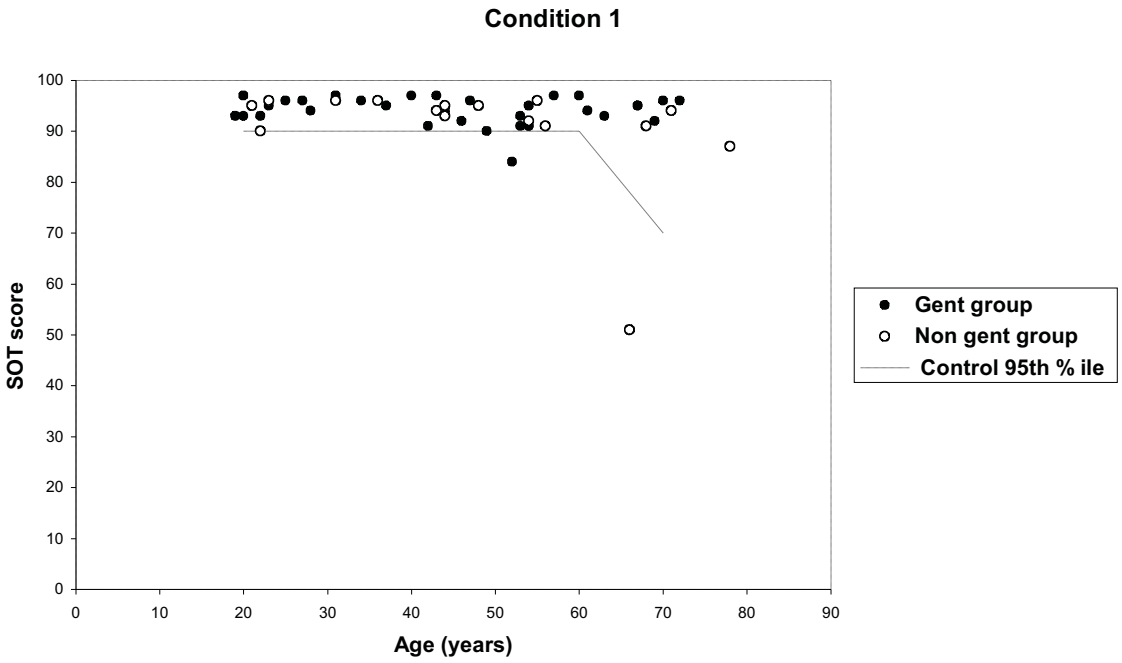


Figure 7.1. The distribution of SOT Condition 1 values is comparable to controls. The mean SOTs for both non AG and AG patients = 93, non AG =91and AG with a standard error (SE) of 2.65, AG treated =94, SE=0.48. The proportion of subjects in both groups falling below the 95th percentile was not significant (see Table 7.5). The overall distribution of those above and below the 95th percentile by χ^2 test (=0 and p=1) was not significantly different from the US controls (Jacobson *et al*, 1993).

Figure 7.2 for SOT Condition 2 (eyes closed balance platform stable) shows an expected slight decrease in the SOT for both non AG and AG groups down to about 90, representing an expected increase in sway. Both parametric and non parametric measures of this distribution as summarised in Table 7.5 and

were borderline significant when compared to the control results cited by Jacobson *et al* (1993). Similarly, the overall distribution of those above and below the 95th percentile by χ^2 test (=2.06 and p=0.15) was not significantly different from the US controls (Jacobson *et al*, 1993).

Figure 7.2.

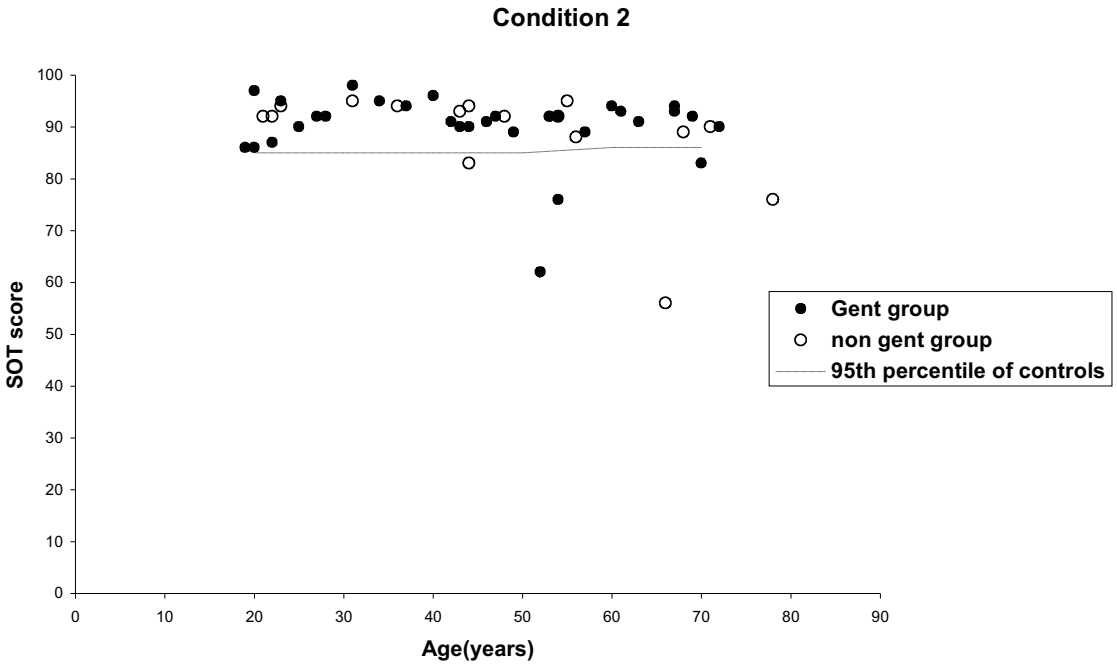


Figure 7.2. The distribution of SOT Condition 2 values is comparable to controls. The mean SOTs for all patients = 90, non AG =88 (SE=2.42) and AG treated =91 (SE=1.14). The proportion of patients with haematological malignancy overall and in both groups falling below the 95th percentile were not significant (see Table 7.5).

Figure 7.3 for SOT Condition 3 (eyes open moving visual surround, balance platform stable) shows an overall decrease in SOT score, the five subjects

outside the 95 % limit are clearly separate from the main cluster of SOT scores. When these five outliers (three non AG treated two AG treated) are removed there was no significant difference between the two treatment groups and the SOT control mean of 91. Similarly, the overall distribution of those above and below the 95th percentile by χ^2 test (=0.91 and p=0.34) was not significantly different from the US controls (Jacobson *et al*, 1993).

Figure 7.3

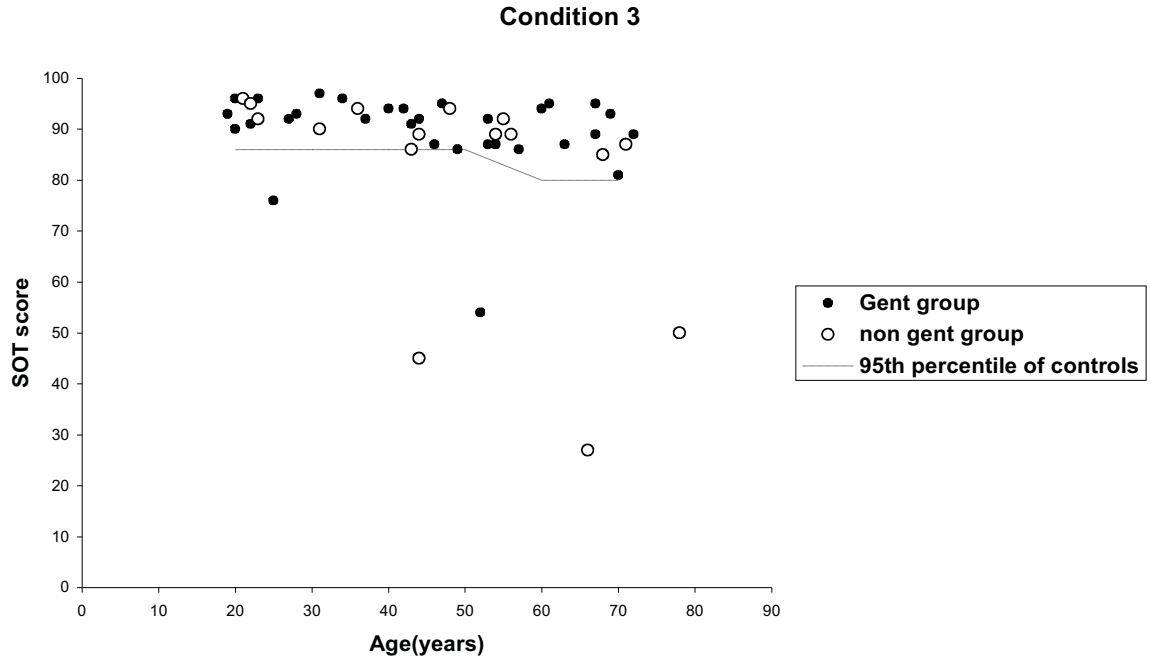


Figure 7.3. The distribution of SOT Condition 3 values is comparable to controls. The mean SOTs for all patients = 87, non AG = 81 (SE=5) and AG treated = 90 (SE=1.36). The proportion of patients with haematological malignancy overall and in both groups falling below the 95th percentile was not significant (see Table 7.5).

7.2.2. Further statistical analysis of Non AG and AG treated groups means above and below 95th percentile for SOT Conditons 1-3.

7.2.2.1. Comparison of above and below 95th percentile means

As indicated above in this analysis those SOT values falling above and below the 95th percentile were considered separately and then analysed to see if there were any differences in means between the two treatments. Over Conditions 1 to 3, the means of all SOT values *above* the 95th percentile for both treatment groups, were not significantly different from each other. These were directly comparable with the overall mean values obtained in the original US controls study (Jacobson *et al*, 1993) given in Table 7.4.

Despite the small numbers of those who fell below the 95th percentile in Conditions 2 and 3, the means SOTs of the Non Gent and Gent treated groups were also comparable suggesting no differential effects of gentamicin treatment. These are summarised in Table 7.5.

7.2.2.2. Correlation of gentamicin exposure with SOT score

For the gentamicin treated group alone there was no significant correlation when total gentamicin exposure vs SOT value was analysed for the complete data set over SOT conditions 1- 3. The hypothesis was that with increasing AG exposure, there would be evidence of a decrease in SOT score. There was no evidence of this. As with the audiometric findings, this suggests that there is no linear relationship between exposure and effect on vestibular performance. These values are summarised in Table 7.5.

7.2.2.3. Distribution of values above and below 95th percentile for SOT Conditions 1-3 in Non AG and AG treated groups .

As indicated above, the overall numbers falling below the 95th percentile, were not significantly different from the values expected from the US controls sample distribution (Jacobson *et al*, 1993). The numbers between the Non AG and AG above and below the 95th percentile were also tested and as shown in Table 7.5 were not significantly different. This is also illustrated in Figure 7.7 which follows consideration of SOT Conditions 4-6.

Table 7.5. Summary of statistical test results for SOT Conditions 1-3 Means + (95th percentiles) SOT ratios derived from Handbook of Balance Function Testing (1993).

SOT Condition	***Above 95th ile mean vs Controls values (p-value)***		***Comparison of Non AG vs AG treated means above/below 95th % ile***	
	Non AG treated	AG treated	Above 95th %ile	Below 95%ile
1	93 vs 94 (ns)	94 vs 94 (ns)	93 vs 94 (ns)	N/A
2	92 vs 92 (ns)	92 vs 92 (ns)	92 vs 92 ns)	72 vs 74 (ns)
3	91 vs 91 (ns)	91 vs 91 (ns)	91vs 91 (ns)	41 vs 65 (ns)
SOT Condition	Chi squared value above/below 95th percentile (p-value)		Correlation coefficients for AG group Total AG vs SOT score	
1	X^2 =0 , p = 1		-0.253 (ns)	
2	X^2 =2.06, p=0.15		-0.177 (ns)	
3	X^2 =0.91,p= 0.34		0.037 (ns)	

Table 7.5 summarises the evidence to show that over SOT conditions 1-3, there was no effect of haematological malignancy or its treatment on postural performance and that this extended to both treatment groups. There was some evidence of spread of SOT values of those in both non AG and AG treated groups below the 95th percentile. However, there was no clear difference between the means of these groups when compared by individual t-test. The correlation tests also showed there to be no evidence between of more subtle effects of AG exposure on the SOT score.

7.2.3. SOT Conditions 4 –6

The SOT Conditions 4–6 provide greater challenge to overall postural control and this was reflected in the marked increase in the number of patients who fell below the 95th percentile with these test conditions. This is evident from Figures 7.4-7.6

Figure 7.4 shows the effect of SOT Condition 4 when the proprioceptive input form the ankles is partly nullified by the sway platform being driven in such a way as to keep the ankles at 90^{o} to the horizontal. The most noticeable effect of this more demanding condition results in a larger failure rate than a nominal 5%. In the non AG group 5 out of 16 and 15 out of 33 in the AG treated group fell below the 95^{th} percentile. By X^2 test (=38 and $p<0.0001$) this difference in proportion is well above what would be expected by normal distribution alone. Comparison of the overall patient with haematological malignancy group ratio above and below the 95^{th} percentile with the US control Study (Jacobson *et al*, 1993) was found to be highly significant at $p<0.0001$. This is clear evidence that patients with blood malignancies regardless of whether they have received AG therapy displayed significant deficit in postural control.

Figure 7.4

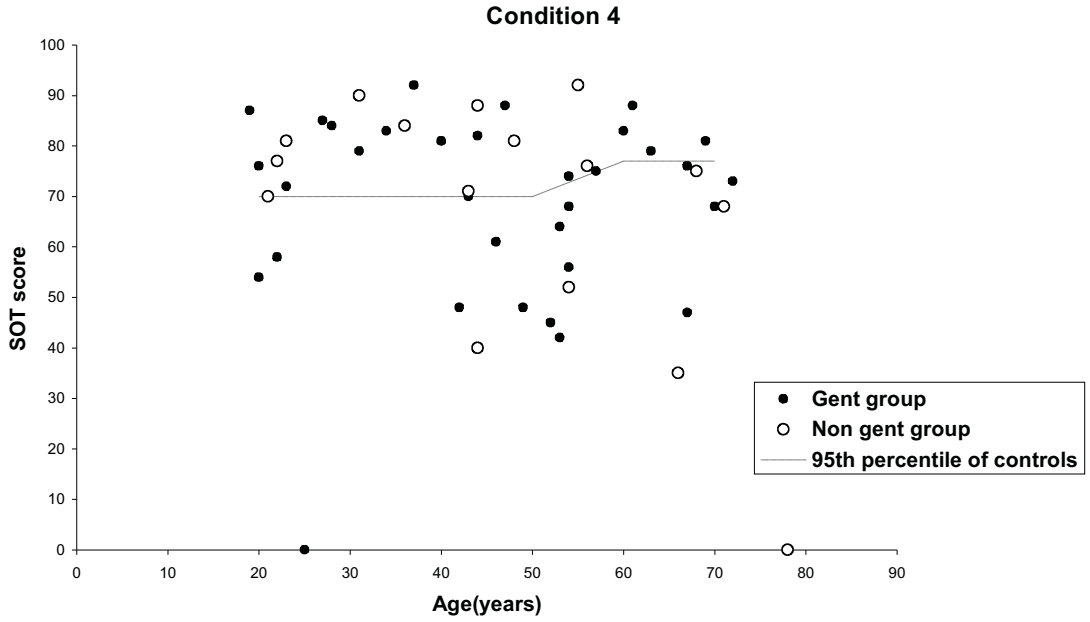


Figure 7.4. SOT Condition 4 (nullifying ankle proprioception) scores show the overall ratio of patients with haematological malignancy falling below the 95th percentile was much greater. X^2 test = 38, $p<0.0001$. Two patients completely failed the test.

This effect is even more marked in Figure 7.5, in which the effect of the proprioceptive input being partly nullified by the sway platform is further augmented by the patient closing their eyes thus removing any visual cues for controlling balance. This further demanding condition results in an increased number falling below the 95th percentile value than the expected nominal 5%. In the non Gent group 8/ 16 and 15 /33 in the Gent treated group fell below the 95th percentile. Taken together as a proportion of the whole group of patients with haematological malignancies this is well above what would be expected by normal distribution alone and this proportion of 23/49 below the

95th percentile was also highly significant (P<0.0001) by chi-squared when compared with the US controls (Jacobson *et al,* 1993).

Figure 7.5

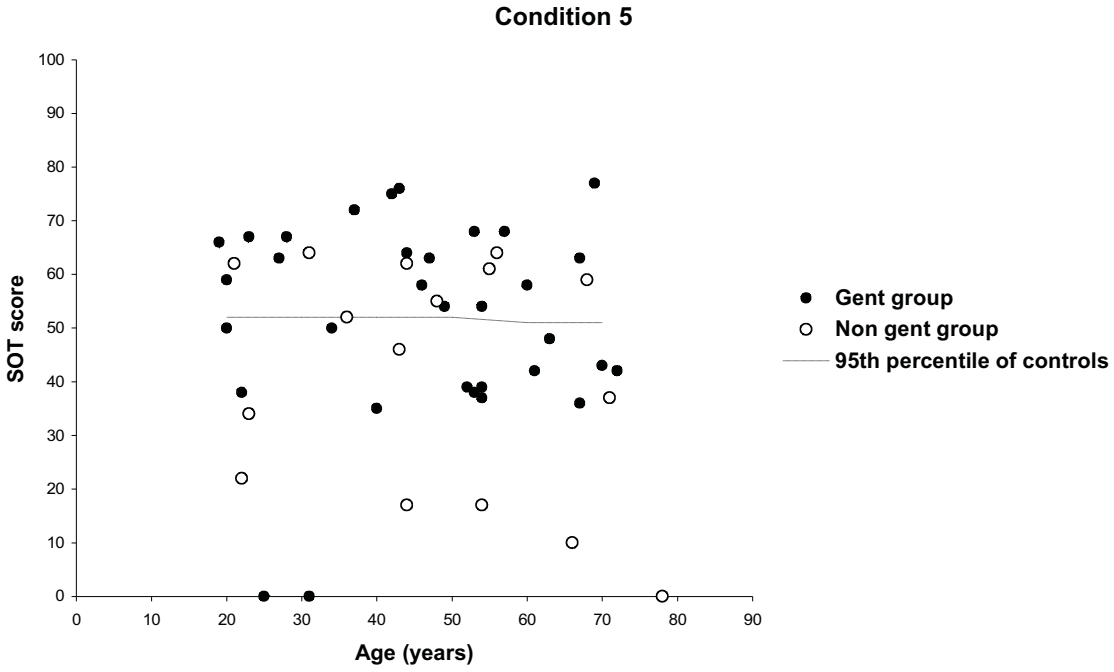


Figure 7.5. SOT Condition 5 scores again shows the overall ratio of patients with haematological malignancy falling below the 95th percentile was even greater. The X^2 test = 49.59, p<0.0001. Three patients failed the test. Interestingly, when compared with SOT Condition 5, the proportion below the 95th percentile drops in Condition 6. In Condition 6, both the visual surround and balance platform were sway referenced. The proportion below the 95th percentile for all patients with blood malignancies was 5/16 in the Non Gent group and 8/33 in the Gent treated group. Against the controls (Jacobson *et al*, 1993), the proportion of 13/49 below the 95th percentile is highly significant at p<0.0001.

Figure 7.6

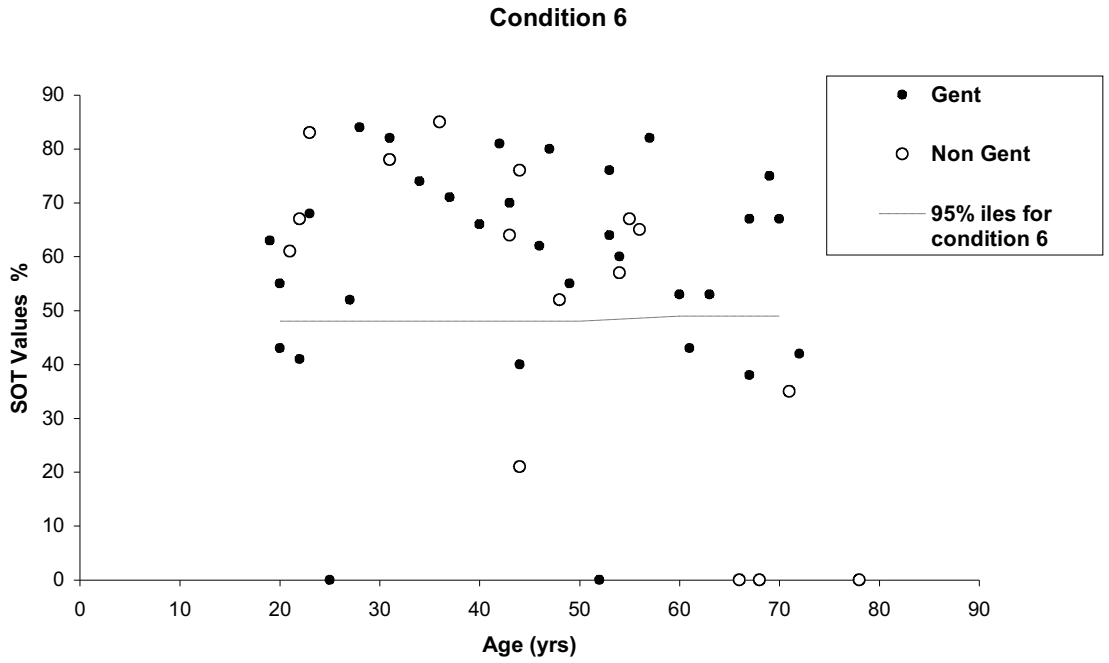


Figure 7.6. SOT Condition 6 scores again shows the overall ratio of patients with haematological malignancy falling below the 95th percentile was even greater. The X^2 test returned a value = 18, $p<0.0001$. Five patients completely failed the test.

7.2.4. Further statistical analysis of Non AG and AG treated groups means above and below 95th percentile for SOT Conditons 4-6

As with SOT Conditions 1-3 further analysis was carried out particularly to see if any effects between treatment groups were evident. This mix of parametric and non parametric tests are summarised in Table 7.6 below.

7.2.4.1. Comparison of above and below 95th percentile means

For both non AG and AG treated groups better than the 95th percentile, the means were comparable with the US data set (Jacobson *et al*, 1993). Between non AG and AG treated groups shown on the right hand side of Table 7.6 no difference was evident in above and below 95th percentile means. Similarly the means for the below the 95th percentile value were not found to be significantly different. Consequently, this result is not considered as a stronger evidence of a difference in treatment effect on SOT scores over Conditions 4-6 between the two groups.

7.2.4.2. Correlation of gentamicin exposure with SOT score

As with SOT Conditions 1-3, no significant correlation in the AG treated group alone for Total gentamicin exposure vs SOT value was found when over the more demanding SOT conditions 4-6. Again, the hypothesis that with increasing AG exposure, there would be evidence of a decrease in SOT score was not supported here. This further supports the findings over the rest of this thesis of there being no linear relationship between AG exposure and effect on vestibular performance. These values are summarised in the bottom right section of Table 7.6.

7.2.4.3. Distribution of values above and below 95th percentile for SOT Conditions 4-6 in Non AG and AG treated Groups .

Whilst there were highly significant numbers overall of patients with haematological malignancy falling below the 95th percentile over SOT Conditions 4-6, the proportions of non AG and AG treated patients below this

95th percentile were not significantly different. The Chi-squared p values for this are in the bottom left of Table 7.6. This is also illustrated in Figure 7.8 which includes consideration of the proportions above and below the 95th percentile for all SOT Conditions 1-6.

7.2.5. Correlation of categorical risk with SOT score

To establish whether there was a correlation between the categorical risk and SOT scores, a Spearman's rank correlation coefficient was used. For conditions 1-6, this was found to be non-significant both for the AG and the non AG treated groups. This is in line with what was found in the cochlea and is shown in table 7.7.

Table 7.6. Summary of statistical test results for SOT Conditions 4-6 Means + (95th percentiles) SOT ratios derived from Handbook of Balance Function Testing.

SOT Condition	***Above 95th ile mean vs Shephard values (p-value)***		***Comparison of Non AG vs AG treated means above/below 95th % ile***	
	Non AG treated	***AG treated***	***Above 95th %ile***	***Below 95%ile***
4	80 vs 82(ns)	78 vs 82 (ns)	80 vs 94 (ns)	39 vs 50 (ns)
5	60 vs 69 (ns)	64 vs 69 (ns)	60 vs 64 ns)	21 vs 37(ns)
6	69 vs 67 (ns)	67 vs 67 (ns)	69 vs 67 (ns)	11 vs 35 (ns)
SOT Condition	***Chi squared value above/below 95th percentile (p-value)***		***Correlation coefficients for AG group Total AG vs SOT score***	
4	X^2=38, p<0.0001		0.306 (ns)	
5	X^2=49.59, p<0.0001		0.250 (ns)	
6	X^2=18, p= <0.0001		0.087 (ns)	

Figure 7.7

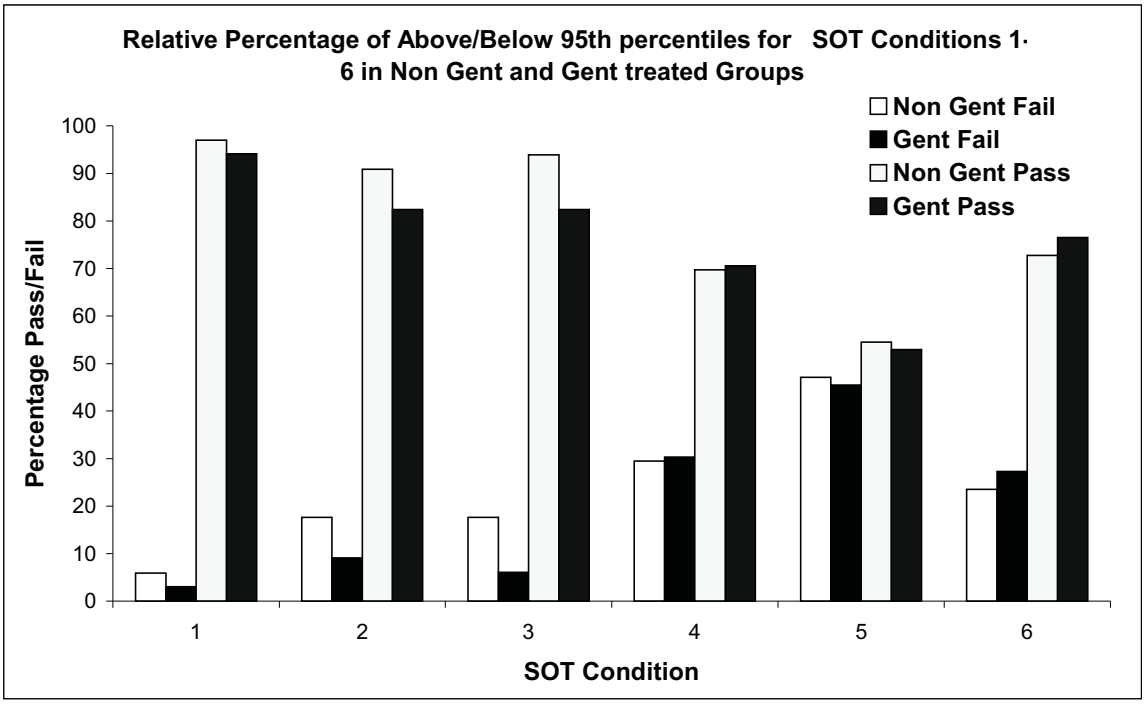


Figure 7.7 Relative proportions of patients above and below 95th percentile for both non AG and AG treated groups. This shows that the proportions of pass/fail for each treatment are comparable for both treatment groups under each SOT test condition.

Figure 7.7. shows that the relative proportions of patients above and below the 95th percentile for the SOT scores for the two treatment groups are very similar. When these ratios within each SOT test condition were analysed by chi squared test (or Fishers exact test when absolute values fell below 5) no difference was apparent in the ratios. This further supports the idea that the challenge provided by SOT Conditions to test is not related to AG therapy

alone in any direct way, but that some other aspect of the illness is leading to balance dysfunction.

Table 7.7. SOT scores vs Categorical Risk Correlation Summary

	No AG		AG	
	Correl coeff		Correl coeff	
Condition 1	0.033	NS	0.15	NS
Condition 2	0.08	NS	0.059	NS
Condition 3	0.223	NS	0.142	NS
Condition 4	0.288	NS	0.037	NS
Condition 5	0.375	NS	0.13	NS
Condition 6	0.297	NS	0.178	NS

Table 7.7. shows that there is no correlation between the categorical risk as defined in the current study and SOT score.

7.2.6. Preliminary conclusions from analysis of SOT conditions.

Taken together, the results here would seem to support the conclusion that treatment of blood malignancies or the malignancy itself, may lead to deficit in postural control. This deficit was manifest over the more demanding SOT conditions with a likely involvement of the vestibular pathway. There was no apparent difference in the incidence or degree of performance deficit between the Non-Gent treated and the Gent treated groups. Moreover, in patients receiving gentamicin therapy, there was no evidence of correlation between cumulative aminoglycoside exposure and the severity of balance dysfunction.

7.2.7. Sensory Ratio Analysis.

The data from each of the six tests were used in further analysis of the effective use of signals from the somatosensory, visual and vestibular systems. This is derived from the ratios of certain of the SOT Conditions discussed in section 7.1.4.

7.2.7.1. The SOM ratio

Figure 7.8. shows the scatter plot for the SOM ratio with 2 and 7 out of the Non Gent and Gent treated group respectively falling below the 95th percentile. The ratio 9/49 of all patients is significantly greater than above that expected from the US control data set (Jacobson *et al*, 1993) of 10/185 by chi squared($p<0.001$).

Inspection of Figure 7.8 shows that most of the SOM ratio values below the 95th percentile are only marginally so. Consideration of the means from above and below the 95th percentile for both treatment conditions reveals them to be directly comparable. Consequently, this result was not considered to be of marked clinical significance in indicating whether treatment has a profound effect on somatosensory processing. No significant correlation in the gentamicin treated group was apparent when the total gentamicin exposure vs SOT value was analysed for correlation with the SOM data.

Figure 7.8

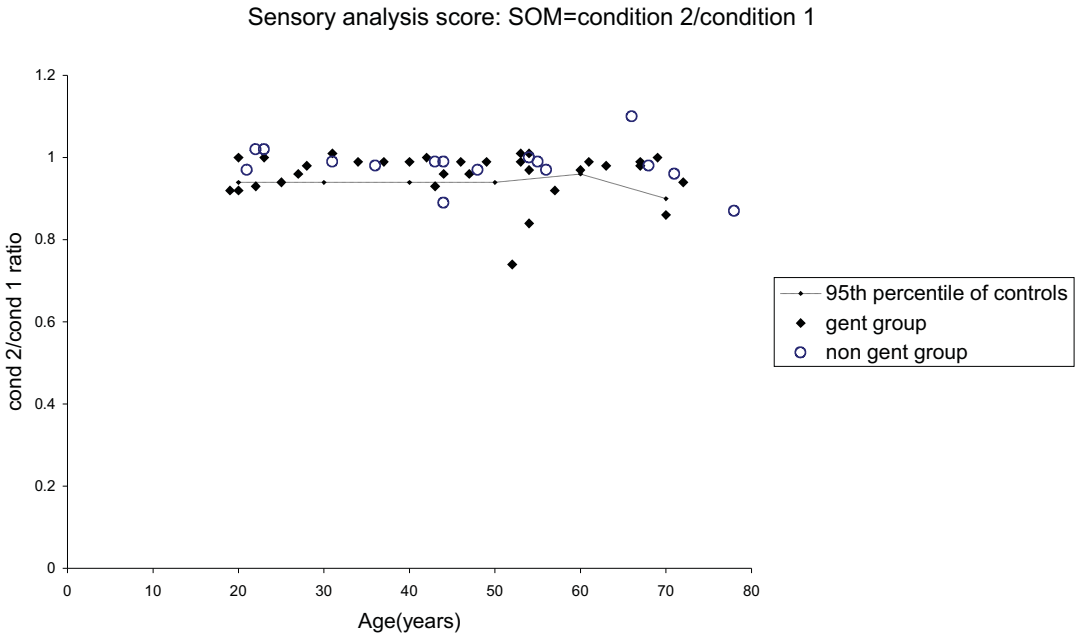


Figure 7.8. The distribution of SOM ratio from Condition 2/Condition 1 .For the above/below 95th percentile ratio the test returned a value of X^2 0.91 (p= 0.34).

7.2.7.2. The VIS ratio

Figure 7.9 shows the scatter plot for the VIS ratio with 5/16 and 16/33 of the Non Gent and Gent group falling below the 95th percentile. The overall ratio of 17/49 of all patients seen here is highly significantly above that expected from the Normative US control study (Jacobson *et al*, 1993) of 10/185 (X^2 =74.46, p<0.0001). In contrast with the results obtained for the SOM ratio above, the scatter of VIS ratios is not marginal and would clinically indicate a real visual processing deficit in all patients with blood malignancy.

Comparison of treatment group means above and below the 95th percentile did not return any significant difference. This further suggests that gentamicin does not markedly contribute to any deficit in visual processing seen in this group. No significant correlation in the gentamicin treated group was apparent when total gentamicin exposure vs SOT value was analysed for correlation with the VIS data.

Figure 7.9

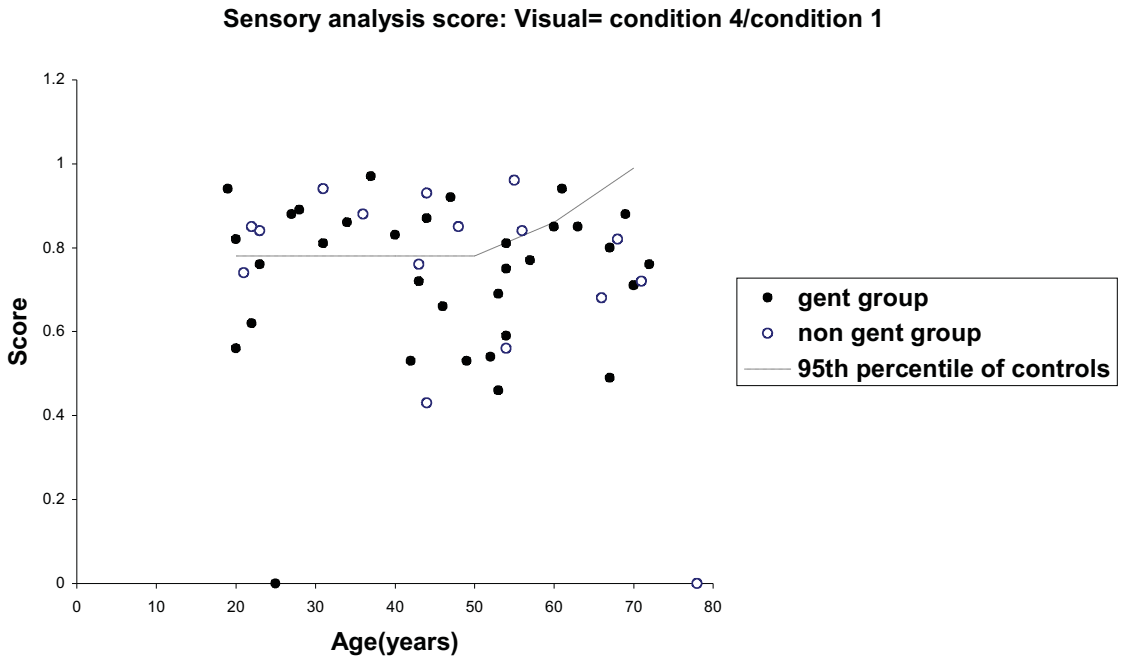


Figure 7.9. The distribution of VIS ratio from Condition 4/Condition 1. For the above/below 95th percentile ratio. $X^2 = 74.76$ ($p<0.00001$).

7.2.7.3. The VEST ratio

Figure 7.10 shows the scatter plot for the VEST ratio with 8/16 and 14/33 of the Non Gent and Gent group falling below the 95th percentile. The overall ratio here of 22/49 of all patients with blood malignancy is significantly greater than that expected from the 10/185 of the US controls (chi-squared $p<0.0001$). The scatter of VEST ratios is more marked than seen with the VIS ratio. This is taken as evidence of a clinically important vestibular processing deficit in the patients with haematological malignancy.

With the VEST ratio, comparison of treatment group means above and below the 95th percentile did not return any significant difference. As with the VIS ratio, this further suggests that treatment with gentamicin did not markedly contribute to any deficit in vestibular processing seen in this group. No significant correlation in the gentamicin treated group was apparent when cumulative gentamicin exposure vs SOT value was analysed for correlation with the VEST data.

Figure 7.10

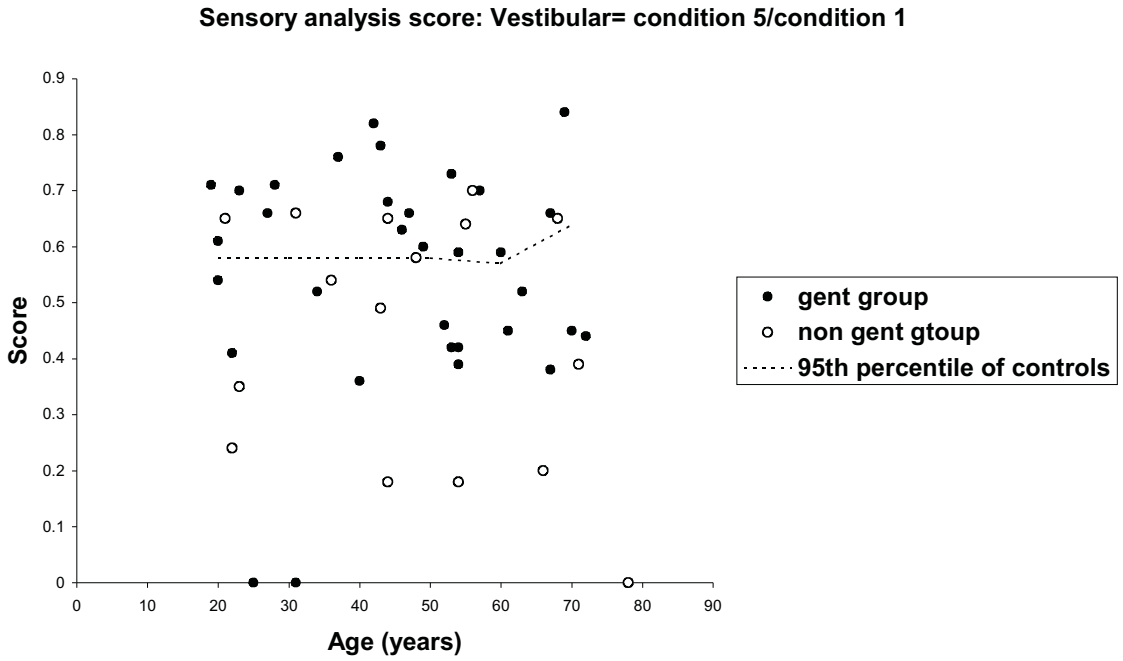


Figure 7.10. The distribution of VEST ratio from Condition 5/Condition 1. For the above/below 95th percentile ratio the X^2 test returned a value of 57.67 ($p < 0.00001$).

The proportions of Non AG gent vs AG treated subjects with SOT ratios above and below 95th percentile for SOM VIS VEST are summarised in Figure 7.11. This again shows that the relative proportions of patients above and below the 95th percentile for each of the ratios are very similar. Ratios tested by using the chi squared test or Fishers Exact test and no significant difference in any ratio was apparent.

The statistical analysis of the sensory analysis score is shown in table 7.8.

Figure 7.11

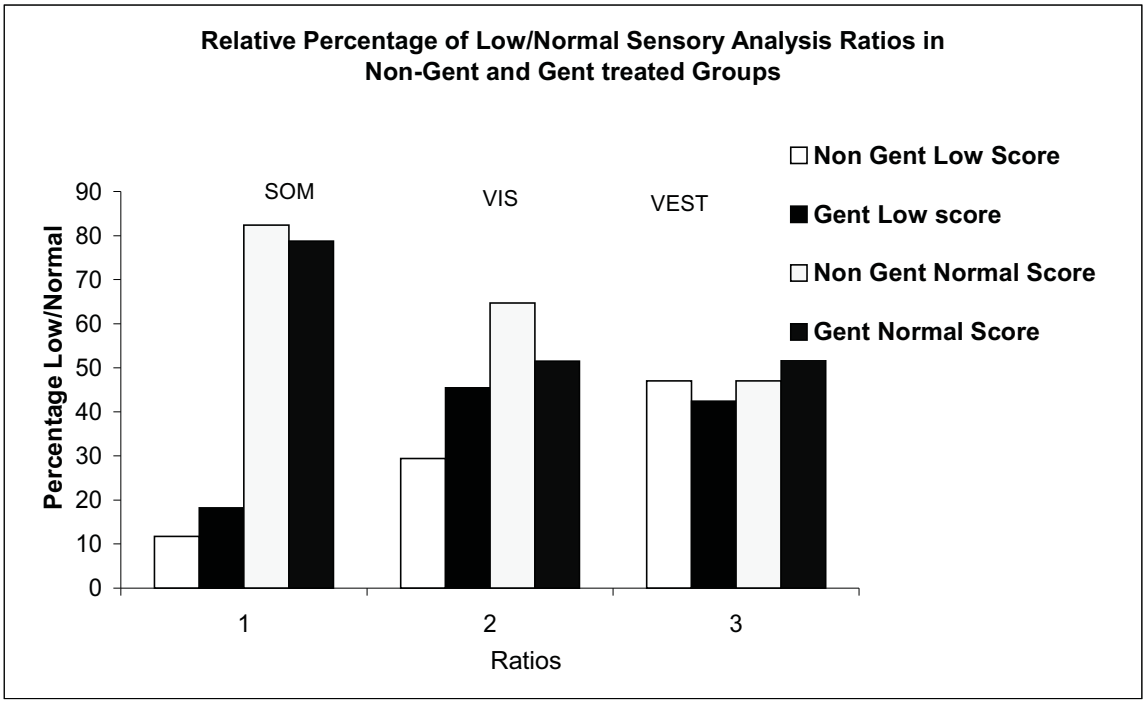


Figure 7.11. Summary of SOM VIS VEST ratio proportions for no AG and AG treated patients.

7.2.8. Preliminary conclusions from analysis of SOT ratios.

The results for the three SOT ratios SOM, VIS and VEST support the idea that treatment of haematological malignancies may further lead to deficit in postural control within both the visual and vestibular processing pathways. The reasons for this are not known and may be multifactorial (vestibulotoxicity, neurotoxicity, hypoxia, bleeding..). This apparent deficit in visual processing does not appear to have been reported before. As with the SOT condition analysis, there was no apparent difference either in the categorical incidence or the degree of performance deficit between the Non AG treated and AG treated groups. In the group receiving gentamicin therapy

there was no evidence of significant correlation between cumulative aminoglycoside exposure and the decrease in any of the above SOT ratios.

Table 7.8: Summary of statistical test results for sensory ratio analysis (SOM, VIS, VEST Means + (95^{th} percentiles) compared with controls derived from Handbook of Balance Function Testing.

Analysis	***Comparison of Non AG vs AG treated groups above/below the 95^{th} % ile***	
	Above 95^{th} % ile	***Below 95^{th} % ile***
SOM	1+/-0.01 vs 0.98+/-0	0.88+/-0.01 vs 0.88 +/-0
VIS	0.88+/-0.02 vs 0.86 +/-0	0.56+/-0.103 vs 0.59+/-0
VEST	0.63+/-0.02 vs 0.69+/- 0	0.25+/-0.05 vs 0.38+/-0
	T-test results P values for non AG vs AG: Above 95th% ile;below 95th %ile	
SOM	0.2 vs 0.92	
VIS	0.51 vs 0.8	
VEST	0.03 vs 0.08	
	T-test results P values for non AG vs non AG above vs below 95th %ile	***T-test results P values for AG vs AG above vs below 95th %ile***
SOM	0.002	0.002
VIS	0.01	0.01
VEST	0.0001	0.0001

7.2.9. Clinical management of patients symptoms

Of the 17 patients in the non AG group five successfully benefited from vestibular rehabilitation. Of the AG treated group 11 underwent successful rehabilitation for balance problems.

7.2.10. Correlation of non AG drug exposure and SOT scores

Inspection of the drugs taken by patients with balance deficit show that there was no common drug administration pattern among patients with abnormal findings on CDP for either the non AG and AG treated groups. There was no correlation between the categorical risk and SOT scores for all SOT conditions and for both the AG and non AG treated groups.

From a clinical point of view, it is important to identify which group of haematological malignancy is more at risk to develop balance dysfunction determined by CDP.

1. APML (N=3)

One patient had vestibular dysfunction evidenced by CDP. This patient also has threshold elevation on HFPTA and has normal hearing on standard PTA.

2. AML (N=11)

Five patients have balance problems. The only common medication between those 5 patients is cytarabine. Only 1 patient out of the 5 who have balance problems already had HL on HFPTA. This patient also received AG. The other 4 patients with balance deficit had normal thresholds on HFPTA. In those 5 patients with balance deficit, four received AG as part of their treatment. The balance deficit affects at least the vestibular system in all cases (2 vestibular, 2 vestibular/visual). The patient who has balance deficit as well as HL on HFPTA presents the vest/vis/somatic/pref pattern.

3. CML (N=4)

All patients with CML have normal thresholds on HFPTA. Two of them have balance dysfunction: one has pure vestibular dysfunction, the other one has vestibular/visual deficit. The only drug common to these two patients is AG.

4. ALL (N=8)

Four patients have balance dysfunction. Three of them have pure vestibular deficit (two have normal thresholds on HFPTA, one has elevated thresholds on HFPTA). One patient has vestibular/visual deficit. The drug common to those patients with balance dysfunction is methotrexate administered intrathecally.

5. NHL (N=7)

In this group, two patients have vestibular dysfunction. One patient has congenital HL and has evidence of vestibular/visual/somatic dysfunction on CDP, the other patient has normal thresholds on HFPTA and has vestibular/visual dysfunction. One could argue that the vestibular system may be affected in a patient with congenital HL. However, Tribukait *et al* (2004) stipulate that vestibular function tends to be preserved up to a point where hearing is almost absent. Hearing level correlated more closely with otolith function, especially that of the utricule, than with semicircular canal function.

6. Hodgkin's disease (N=7)

Two patients, who already had threshold elevation on HFPTA, have evidence of pure vestibular dysfunction on CDP testing. The drugs common to these two patients are: cytarabine, etoposide, melphalan, cyclophopshamide and teicoplenin.

7. Multiple myeloma (N=8)

Five patients have balance dysfunction. Two of those already showed threshold elevation on HFPTA. Two have pure vestibular dysfunction (one of them had raised HFPTA thresholds), one has vestibular/visual dysfunction, one has vestibular/visual/somatic deficit and one has visual preference. There is no common drug for those patients with balance performance deficit.

8. Aplastic anaemia (N=1)

This patient has normal values on CDP testing

9. Waldenstrom macroglobulinaemia (N=1)

This patient has vestibular/visual deficit determined by CDP.

The results of balance function for each malignancy group are summarised in table 7.9. This shows that, unlike the cochlear organ, haematological malignancy and/or its treatment affects the balance function in all subtypes of malignancy, almost in a homogeneous way. The AML, CML, ALL and MM patients have the same incidence of balance dysfunction.

Table 7.9. Summary of Malignancy types and incidence of Balance deficit

Malignancy	Balance
AML N=11	**5 (45,4%)**
AG N=8	1vest/vis-2 vest-1 ve/vis/so/pr (50%)
No AG N=3	1 vest/vis (33.3%)
APML(all AG)N=3	**1 vest (33.3%)**
CML (all AG)N=4	**1vest/vis-1 vest (WBC=153) (50%)**
ALL N=8	**4 (50%)**
AG N=6	1vest/vis-1vest (33.3%)
No AG N=2	2 vest (100%)
NHL N=7	**2 (28.5%)**
AG N=3	0
No AG N=4	1vest/vis/som-1vest/vis
HD N=7	**2 (28.5%)**
AG N=2	1 vest
No AG N=5	1 vest
MM N=8	**4 (50%)**
AG N=5	2 vest-1vest/vis
No AG N=3	1pref-1vest/vis/som
WALD N=1 (AG)	**vest/vis**
AA N=1 (AG)	0

7.3. DISCUSSION

These results revealed a number of interesting and unexpected findings in this patient group. The partly pragmatic approach to analysing the data and separating out those above the 95th percentile was used after taking statistical advice (Department of Health Sciences, University of Leicester) as details of the absolute nature of the US Equitest manufacturers control distribution were not available. From a clinical point of view, it was considered legitimate to consider those below the 95th percentile as a separate group with a likely balance disorder, as they would have been if they had come into

the balance clinic without prior knowledge of their medical history. It was clear after looking at the proportions of all patients that SOT Conditions 4-6 resulted in a greater proportion across both treatment groups showed evidence of postural performance deficit.

Given the recognised vestibulotoxicity of gentamicin, it was expected that any evidence of vestibular dysfunction would have been associated with gentamicin therapy. This was not found to be the case with the analyses done on this data. Instead it was found that both groups of non AG and AG treated patients showed similar incidence and degree of vestibular deficit. There was also very strong evidence from the SOT ratios that there may have been a visual processing deficit contributing to this.

7.3.1. SOT Conditions 1-3

SOT Conditions 1-3 did not reveal any association or signs of any postural problems. The inspection of data and consideration of proportions above and below the 95th percentile revealed no difference with the normal population. These tests partly establish a baseline for the contribution of vision to postural control and the unmasking of the ‘at rest’ contribution of the vestibular system and part of the lower limb proprioreceptive input. With visual input removed or ‘misled’ by negative feedback, there was no evidence of increased sway when the ‘at rest’ condition was maintained for the other two postural control signal components.

7.3.2. SOT Conditions 4-6

In SOT condition 4, with proprioreceptive input nullified and the vestibular and visual system driven by platform sway the incidence of those displaying a clinically remarkable increase in sway increased significantly with 33% overall performing below the SOT 4 Condition score of 70 with mean scores of 39 and 50 for the non AG and AG treated groups respectively. This was the first condition in which it was noticed that the incidence of those with measurable and defined deficit occurred equally in both treatment groups, suggesting that the gentamicin was not the single likely underlying cause. This was further supported by the fact that as shown in Table 7.6 the SOT Condition 4 means of 39 and 50 between the treatment groups were not significantly different.

The challenge provided by SOT condition 5 appeared to be the most marked in this study, with both proprioreceptive input nullified and visual system removed the vestibular system alone was then driven by platform sway. This led to an overall incidence of 47 % exhibiting a clear increase in sway with three actually falling. The mean SOT scores of the sub 95th percentile ie below 52 in the treatment group were also notably lower at 21 and 37 for non AG and AG treated groups respectively.

These results indicated that the overall deficit had a clear vestibular component but again because the proportionate incidence in both treatment groups, this could not be due to gentamicin therapy alone. As with SOT Condition 4 the absence of any correlation with gentamicin exposure in the AG treated group further supported this idea. This also meant that any effect

gentamicin was having on the vestibular system was non linear and could not be easily separated out from either the haematological malignancy and/or other therapeutic agents employed.

Interestingly in SOT Condition 6 in which both the proprioreceptive and visual system input were nullified by sway referenced movement of the platform and visual surround the incidence of poor performers decreased overall to 30%, slightly lower than Condition 4. This re-establishment of a visual input, albeit misled by sway reference seemed to result in the return of some degree of performance. This condition in the control population as shown by Table 7.2 is apparently slightly more demanding than SOT Condition 5. The equivalence of between treatment group incidence and lack of correlation with gentamicin exposure in the AG treated group alone further reinforce the conclusions about vestibulotoxicity in SOT Conditions 4 and 5.

7.3.3. The SOM VIS VEST ratios

The effective use of signals from each of these sensory contributors to postural control was established with these ratios. Whilst there was evidence of a greater overall incidence of 9/49 or 18% of patients with haematological malignancy showing poorer use of somatic signal direct inspection of the SOM ratios showed that these were not spread far from the lower percentile limit in particular contrast with the SOT conditions 4-6. For this reason this was not considered as clinically strong evidence of a direct illness and/or treatment effect on somatic processing efficiency.

This was contrasted with the unexpected and marked incidence outside the VIS ratio 95th percentile limit of 17/49 or 35 % also showing a wide scatter with sub 95th percentile means ratios of both groups about 0.56 to 0.59. This was taken as evidence that would support a clinical interpretation of deficit in visual processing.

The VEST ratio showed that overall 45 % of patients with haematological malignancy had a vestibular signal processing deficit and a broad spread of sub 95th percentile values. But again the unexpected lack of correlation with gentamicin exposure in the AG treated group ruled out a single explanation of this deficit.

Furthermore, the lack of correlation with generalised neurotoxic categorical risk as defined in this study suggested the absence of a non linear relationship of drug involvement in inducing these deficits.

7.3.4. Comparison with previous reports using CDP to detect aminoglycoside vestibulotoxicity.

The literature of aminoglycoside vestibulotoxicity measured by other techniques has been reviewed previously in Chapter 1. The only other report found in the literature review in which CDP was used to measure aminoglycoside vestibulotoxicity was by Black and Pesznecker (1993). In this study, the time course of vestibulo-spinal (VS) changes in 36 patients receiving aminoglycosides was compared with 53 hospitalised controls

suffering from the same or similar infectious conditions, but not receiving aminoglycosides. In this study, the first abnormality occurred in SOT condition 6 which presents visual-vestibular-somatosensory conflict. Of the patients taking AGs, 18 or 50% fell in condition 6 against 20 or 38% of the hospitalised controls falling in condition 6. Whilst they did not mention whether statistical analysis was performed on this group analysis here by chi squared (18:18 vs 33:20. chi square =1.32, p=0.25), shows that these proportions numbers are not significantly different.

As SOT condition 6 was the only one affected they concluded that the first manifestation of AG ototoxicity appears to be disturbed visuo-vestibulospinal interaction. However, given that the incidence of severe dysfunction was not significantly different in either group, it would appear that the state of their illness/additional or other treatment was at the very least contributory to the severe failure rate.

This study partly agrees with what was observed here in this study in that there was evidence of mixed sensory postural signal processing deficit in both the vestibular and visual systems. There was also evidence of the cause of this being due to the disease state/ other drug therapy being equal to any aminoglycoside effects.

The contrast with this study is that the incidence of falling is greater than was seen in the study described in this thesis in which more subtle measures of postural performance deficit were applied. The deficits were also seen in

SOT conditions 4 –6 and in the lower VIS VEST ratios not further discussed by Black and Pesznecker (1993). Apart from these differences between this current study and Black and Pesznecker (1993), the further evidence of non AG induced balance deficit is both clinically and scientifically important and deserves further investigation.

7.3.5. Clinical observations

Surprisingly, some patients from both the non gentamicin and the gentamicin treated groups reported *subjective* symptoms of unsteadiness but had normal SOT results. These patients' symptoms improved dramatically after vestibular rehabilitation. In contrast, other patients had no *subjective* complaint of balance problems, but had evidence of vestibular dysfunction following CDP. Their lack of symptoms may be explainable by an efficient central nervous system compensation. The decision to refer the patient for customised vestibular rehabilitation was solely based on clinical grounds and provided a major improvement or full recovery in all cases.

7.3.6. Mechanism of damage

The biochemical mechanisms considered responsible for damage discussed previously would be able to account for any damage due to aminoglycoside therapy. However, the balance deficit seen in the non AG groups cannot be accounted for and raises a number of key questions. The loss of postural control in this group would appear to be chronic as the time since finishing therapy was considerable (more than three months). This suggests that the lesion responsible for the deficit may also be permanent.

In the broadest mechanistic sense this opens up two important possibilities. The first is that the broad drug treatments used in treating this patient group result in a cumulative non-specific vestibulotoxic effect. Supporting evidence for this comes from within this study from Chapter 5 dealing with HFPTA. Here, general combined drug use leading to generalised metabolic stress at the more vulnerable high frequencies of the cochlea was also proposed to lead to ostensible threshold elevations seen in this study.

Whether there is a differential pattern of sensitivity to vestibulotoxicity in the vestibular apparatus, akin to tonotopic vulnerability seen in the cochlea, has not been established. What also needs to be established is whether any pattern of vulnerability to toxins is modulated by metabolic demand as also seen in the cochlea (Prasher and Canlon, 1999).

The other possibility was that the haematological malignancy itself, either alone or in combination with general drug therapy potentially contributes to any visuo-vestibular lesion. The possibility that the malignancy or neutropenic status indirectly contribute to balance dysfunction by making the system more sensitive to generalised drug administration/increased metabolic stress does not find support by the literature. However, this simply is likely to reflect the lack of any consideration of this possibility in previous studies.

Haematological malignancy is mentioned in the differential diagnosis of dizziness in almost all otolaryngology textbooks, there are very few reports in

the literature mentioning a link between haematological malignancy and balance disorder. This differential diagnosis of dizziness is not accompanied by any explanation regarding the likely mechanisms involved. The literature review only presents case reports of balance deficit and this only when the tumour is localised in the cerebello-pontine angle or in the inner ear itself, which was not the case in the patient group of the present study.

What is surprising is the well documented preferential vestibulotoxicity of AGs in general (Tange, 1998; Govaerts *et al*., 1990; Cone, 1982). However, in this present study gentamicin appeared to be primarily cochleotoxic from the results presented in Chapter 4. If the findings from this study are correct then there is the possibility that at least in certain patient groups, the estimates of incidence and risk of vestibulotoxicity may be partly incorrect. It would also be the case that vestibulo and cochleotoxic effects may also have previously been missed.

What is most surprising is that, looking at the different sub-types of haematological malignancies, it appears that patients who have evidence of cochlear performance deficit usually have normal balance function and vice versa. This means that in all subtypes, the majority of patients present evidence of inner ear toxicity, affecting either the vestibule or the cochlea.

Although the patients with haematological malignancies studied in the present thesis have different subtypes of haematological malignancies, there is evidence that most of them are at risk to develop balance and/or hearing

dysfunction. Hyperviscosity has been ruled out in most cases and none of the patients had evidence of brain metastasis at the time of testing. There is no consistent drug taken by all patients who have frank cochleovestibular performance deficit. This serves to highlight the important possibility of multiple interactions between disease state and therapeutic agents in producing lesions and deserves further study in its own right as it would markedly shift the understanding of mechanisms of damage in this generally underinvestigated system.

In summary, the original hypothesis about the differential effects of AG on vestibular function appears to have been substantially incorrect. Instead these novel results support an unexpected an intriguing involvement of haematological malignancy/additional drug exposure contributing to marked deficit in postural control with vestibular and visual pathway involvement.

GENERAL DISCUSSION

The project set out with the aim of establishing the incidence and risk of gentamicin ototoxicity in patients with haematological malignancy recruited during routine clinical follow up within the Haematology Dept at Leicester Royal Infirmary. This was inspired in part by the well documented ototoxicity of gentamicin which had been previously poorly characterised in this patient group. From earlier studies carried out at Leicester Royal Infirmary and other hospitals in the East Midlands it was apparent that focused study of incidence and risk of clinical aminoglycoside ototoxicity in defined patient groups was of both scientific and clinical value (Mulheran and Degg 1997; Mulheran *et al.,* 2001; Smyth *et al.,* 2005). The scientific and clinical relevance of investigation of inner ear function in the haematological malignancy patient group appears to have been borne out in the study reported here.

Notwithstanding the relatively small number of patients in this study a number of novel and significant findings were apparent. These are summarised below.

Chapter 3 set out the general patient demographic data against malignancy subtype and included a categorical ototoxic risk assessment of the drugs used in patients. It raised a secondary but interesting finding that the proportion of Asian patients presenting with haematological malignancy was lower than expected.

Chapter 4 dealt with PTA threshold elevation as the ‘gold standard’ of assessing aminoglyocide cochleototoxic risk. Once other sources of

ototrauma had been taken into account it appeared that there was a considerable risk of clinically recognised cochleotoxicity in neutropenic patients receiving aminoglycosides of about 1 in 10. This was with a median of 4g aminoglycoside exposure given over a median of 8 days. This exposure is not severe by general clinical practice. This risk appeared to be in line with reports in the literature. This incidence may contribute to further management challenge in patients identified with any serious hearing deficit once fully recovered from therapy.

The results from Chapter 5 dealt with AG treatment effects on HFPTA thresholds. The main limitation of HFPTA testing was the low number of controls. As there are no internationally accepted standard HFPTA threshold values over the frequencies of 10 to 16 kHz, the extrapolation of controls' thresholds using the exponential fitting regime seems to be the most limiting factor of the whole thesis. The lack of large scale normative threshold values made the data more difficult to interpret.

The limited results here provided some evidence of mixed effects of disease state/broad non specific drug cochleotoxicity with raised HFPTA thresholds evident in both non AG and AG treated groups. Although the amount of patients on either side of the 50^{th} percentile is roughly equal in both the non AG and the AG treated groups, the loss appears to be more profound in the AG treated group. The strongest evidence was for AG treated patients which is in agreement with the rest of the literature. The reasons for this HFPTA loss in both the non AG and the AG treated groups are not known and are

probably multifactorial. Hyperviscosity has been ruled out for most patients. As the variety of malignancies received different chemotherapy regimens, there is no obvious drug common to those people with HFPTA thresholds elevation.

The results from Chapter 6 dealt with the effects of therapy on DPOAEs. The use of DPOAEs as clinical early warning of ototoxic damage has been proposed by many workers, but their use is confounded by many variables. The presence of DPOAEs were analysed simply as evidence of another site of damage beyond OHCs when PTA thresholds were elevated. This physiological evidence of a secondary site of ototoxic action fits with experimental reports (Basile *et al.,* 1995) and other observations in humans (Eg Attias, 1998; Sone *et al*., 1998). This evidence challenges the view that cochlear OHCs are the primary damage site for aminoglycosides. This has implications for development of therapeutics to counter this damage in humans (Prasher and Canlon, 1999). In theory, no DPOAEs are supposed to be generated if PTA thresholds are more than 30 dB HL. If DPOAEs are present with a HL on PTA above 30 dB HL, this suggests retrocochlear damage as OHCs are responsible for the generation of OAEs.

Chapter 7 dealt with results from using CDP to determine treatment effects on postural control. Data for this Chapter were most extensive and not subject to the audiometric filtering required in Chapter 4-6. Although the American guidelines recommend that ENG should be used in the detection of vestibulotoxicity, CDP was used in the current study as it was less invasive for

the patients, provided information from both the sensory and motor components of the balance system and formed a valuable basis for the customised vestibular rehabilitation. This study is probably the one giving the most unexpected results as it presented very strong evidence of a broadly equivalent incidence of vestibulotoxicity in both treatment groups. It further provided evidence of involvement of deficit in the visual pathway, which was unexpected. This lends support to the idea that non specific cumulative toxicity and/or the malignancy being responsible for this balance disturbance not previously reported in the clinical or experimental literature. Additionally this part of the study directly led to sixteen patients being referred for rehabilitation with excellent outcome.

Taken together the Chapters in this thesis provide a basis for re-evaluation of the understanding of ototoxic and neurotoxic mechanisms outlined for the aminoglycosides and other therapeutic agents investigated in this study. The idea that disease state in combination with therapy may serve to modulate primary toxic effect is not new as applied in other body systems and is well documented for the cochlea. What is perhaps more novel is the idea that disease state along with broad exposure to therapeutics with a low toxicity may produce specific organ toxicity. This could be explored without recourse to new methodology and could be carried out in other well defined patient groups, ideally with pre-therapeutic measures of inner ear function.

The occult cochleovestibular damage as shown in the present study is probably of multifactorial origin. Hypoxia probably plays a role, together with multiple drugs toxicity and disease state. The present study shows that there is an obvious lack of awareness of cochleovestibular vulnerability in this patient group. Knowing that patients with haematological malignancy are at risk of developing vestibulocochlear damage, they should be asked specifically before, during and after therapy for any specific hearing and/or balance problems. Furthermore, they should have pre treatment baseline measurements of cochlear function for both speech and high frequencies as well as evaluation of balance function. The importance of the costs these tests would generate for the NHS also needs to be taken into account. The compliance of the patients to undergo this battery of tests also needs some consideration. As far as the balance testing is concerned, if CDP cannot be performed due to the general heath status of the patients, a full bedside neurootological examination should be performed by an ENT specialist. If the CDP is not available, an ENG should be performed. As with new therapy regimens life expectancy has increased for patients with haematological malignancies, quality of life becomes an important factor.

In the longer term this would assist in improved patient management by reducing the incidence and risk of iatrogenic damage to the inner ear.

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APPENDIX

Leicester Royal Infirmary
Hospital Hearing Questionnaire

Thank you for agreeing to take part in our survey. We would like you to fill in this questionnaire so that we can interpret our results from the hearing tests more accurately. The results from both the hearing tests and this survey may be useful in helping your specialist decide which drugs to choose for your treatment.

When filling in your questionnaire go at your own pace and don't worry about filling it in all at once. We want you to answer the questions by ticking the box that best applies to you. To some of the questions you need to write just a short answer. If you are unsure about any of the questions or what they might mean don't worry, just leave them out for the time being. The staff at the hospital involved in this survey and the tests in the clinic will be happy to spend time with you going over these questions. They will also be happy to talk with you about anything you might want to ask about this survey.

Section 1. About you

This part of the questionnaire asks for general information about yourself .

1.1 Name_____ **1.2** Date of Birth ____/____/____

1.3 Male ☐ Female ☐

1.4 Address

_____

_____

_____

_____

1.5 Occupation

_____

_____

_____

_____

Section 2. General Health

This part of the questionnaire asks for information about your general health and what kind of medicine you might be taking. If you are unfamiliar with any of the words don't worry, as the staff at the hospital will help explain what they mean.

2.1 Can you remember when your cancer was first diagnosed?

Yes ☐ No ☐

If yes can you tell us when ? _____Year

2.2 Have you had any difficulty with your breathing, for example do you have asthma or any allergies that make you short of breath?

Yes ☐ No ☐

2.3 Have you any problems with your heart ?

Yes ☐ No ☐

2.4 Do you have any problems with your general circulation, for example do you often have cold hands or feet even when its not very cold ?

Yes ☐ No ☐

2.5 Have you been ill with any of the following? Please tick the appropriate box if the answer is Yes.

Measles ☐ Mumps ☐ Meningitis ☐

Chicken pox ☐ Scarlet fever ☐

2.6 Are you taking any medicines at the moment? If so write down the names of the medicine below. If you are unsure of their names don't worry, bring the medicines with you when you next come to the hospital.

_____

_____

2.7 Have you ever had to take medicine for any kidney trouble? If you answered Yes, can you remember approximately when this was? Please write the approximate date(s) in below.

_____

2.8 Have you ever been given aminoglycoside antibiotics? These often have name that end in -ycin or -icin your consultant should be able to help you name these

_____

2.9 Do you take aspirin regularly Yes ☐ No ☐

2.10 Do you know whether you had a difficult birth or whether you were born prematurely. If so could you give more details.

_____

2.11 If there is anything else about your general health you would like to tell us about please write it in below.

_____

_____

_____

Section 3 Your hearing

This section asks you for information about your hearing. We are interested in this information as it will be used to help us to understand the hearing tests you will do at the LRI.

3.1 Do you often have earache, 'blocked' up ears or get a discharge from your ears?

Yes ☐ No ☐

3.2 If you answered Yes, which ear does it occur in most?

Left ☐ Right ☐

Both as much ☐

3.3 Do your ears seem to get blocked up very easily when you get a cold or the 'flu.

Yes ☐ No ☐

3.4 Have you ever seen your doctor about your ears or had an operation on your ears?

Yes ☐ No ☐

3.5 If you answered Yes, can you remember approximately when this was? Please write the approximate date(s) in below.

_____

3.6 Have you ever had bouts of light headedness or dizziness as though you felt you were going to fall over ?

Yes ☐ No ☐

If you answered yes did the dizziness ever seem to accompany the times you were receiving any of the drug treatment for blood cancer? If so, could you give more details.

_____

_____

3.7 Many people sometimes say that they can hear noises in their head or in their ears. This sensation is often referred to as **tinnitus.** These noises can sound like a TV or a radio not tuned in right or like a whistling noise or sometimes a pulsing noise.

Have you ever had tinnitus ? Yes ☐ No ☐

3.8 If you answered Yes to the last question, how often would you say that you get tinnitus ?

Hardly ever ☐

After listening to loud sound or music, for example at a rock concert or on headphones ☐

At other times, with the tinnitus lasting for more than ten minutes or so ☐

The tinnitus seems to be there nearly all the time ☐

3.9 When you have tinnitus which ear does it seem to be coming from?

Left ☐ Right ☐ Both ☐

3.10 When you have tinnitus, how loud would you say it seemed to be?

Quiet ☐ Not very loud ☐ Quite loud ☐ Very loud ☐

If you answered yes to the above questions, did the tinnitus ever seem to accompany the times you were receiving any of the drug treatment for blood cancer? If so, could you give more details.

_____

_____

3.11 Have you ever received a very sharp knock or blow to the head that resulted in a temporary loss of consciousness (this is some times referred to as concussion) ?

Yes ☐ No ☐

3.12 Have you ever been exposed to very loud noise such as a shotgun going off?

Yes ☐ No ☐

3.12 Is there anyone in your family who was born deaf or developed hearing problems at an early age?

Yes ☐ No ☐

3.13 If you answered Yes could you write below who the member of the family is and how old they were when they went deaf.

_____

_____

_____

Section 4 Hearing and Activities

In this section we want to ask you questions about your hearing when you are taking part in social and leisure activities.

4.1 When you are talking to people or perhaps when listening to the television or radio, do you find you sometimes have difficulty being able to tell what is being said ?

Yes ☐ No ☐

4.2 If you answered Yes to the last question how often would you say this was ?

Not very often ☐ Quite often ☐

Most of the time ☐ Nearly all or all of the time ☐

4.3 If you answered Yes to question 4.1, does the problem of hearing what is being said seem worse if there is any other conversation or noise going on in the background?

Yes ☐ No ☐

4.4 Below is a list of leisure and social activities that you may get involved in. If you do, tick the box next to the activity and put in the space beside it how many hours a week or month on average you spend in these activities.

Hours a week or month (please specify which)

Going to concerts ☐ _____

Going to discos/raves ☐ _____

Spending time in a workshop or working on car/bike engines

☐ _____

Playing amplified music ☐ _____

Playing in orchestra ☐ _____

Using a Walkman or normal stereo headphones to listen to music

☐ _____

4.5 If you do use a Walkman or stereo headphones could you write in the name of the particular model you use below.

_____

When you come to the hospital could you also bring in your Walkman and show us how loud you normally have the volume on, along with one of your favourite tapes.

Section 5 Any Comments ?

If you have any comments to add now that you have finished the questionnaire please fill them in below. Thank you for taking time to fill it in.

Comments: