

Radiological Differences between HIV-Positive and HIV-Negative Children with Cholesteatoma

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List of Abbreviations/Acronyms

CT	Computed tomography
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
MAC	Mastoid air cell
MACS	Mastoid air cell system

Abstract

Introduction

HIV-positive children are possibly more prone to developing cholesteatoma. Chronic inflammation of the middle ear cleft may be more common in patients with HIV and this may predispose HIV-positive children to developing cholesteatoma. There are no studies that describe the radiological morphology of the middle ear cleft in HIV-positive compared to HIV-negative children with cholesteatoma.

Aim

Compare the radiological differences of the middle ear cleft in HIV-positive and HIV-negative children with cholesteatoma.

Method

A retrospective, cross-sectional, observational analytical review of patients with cholesteatoma at Red Cross War Memorial Children's Hospital over a 6 year period.

Results

Forty patients were included in the study, 11 of whom had bilateral cholesteatoma and therefore 51 ears were eligible for our evaluation. HIV-positive patients had smaller ($p=0.02$) mastoid air cell systems (MACS). Forty percent of HIV-positive patients had sclerotic mastoids, whereas the rate was 3% in HIV-negative ears ($p<0.02$). Eighty-two percent of the HIV-positive patients had bilateral cholesteatoma compared to 7% of the control group ($p<0.02$). There was no difference between the 2 groups with regards to aeration of the middle ear cleft, bony erosion of middle ear structures, Eustachian tube obstruction or soft tissue occlusion of the post-nasal space.

Conclusion

HIV-positive paediatric patients with cholesteatoma are more likely to have smaller, sclerotic mastoids compared to HIV-negative patients. They are significantly more likely to have bilateral cholesteatoma. This may have implications in terms of surveillance of HIV-positive children, as well as, an approach to management, recurrence and follow-up. HIV infection should be flagged as a risk factor for developing cholesteatoma.

PART A: RESEARCH PROTOCOL

Radiological Differences between HIV-Positive and HIV-Negative Children with Cholesteatoma

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Introduction

Development of the mastoid air cell system (MACS)

At 7 months' gestation the bony spaces in the middle ear cleft are filled with mesenchyme. Regression and resorption of the mesenchyme and pneumatisation of the MACS proceed only if middle ear pressure is normal. Regression by apoptosis seems to be a pre-programmed event, occurring simultaneously with osteoclastic and osteoblastic bony remodelling to pneumatise the mastoid.¹

The mastoid antrum is the first recognisable mastoid air cell (MAC) and is completely formed at 34 weeks' gestation. At birth there are usually no other air cells and the mastoid air cell system develops in three distinguishable phases from birth until puberty, when the MACS reaches its adult capacity (12cm² by planimetric evaluation). The first phase occurs within the first year of life. There is rapid pneumatisation and the air cells increase the volume of the antrum by 1.5-2.5cm² so that the total volume is 3.5-4cm². The second phase occurs from 1 to 6 years of age and is characterised by a linear increase in aeration of 1-1.2cm² per year. The final, slower phase continues into the third decade;^{2,3} thereafter it decreases substantially.³ This growth pattern seems to be mirrored in different ethnic populations .²

The association between arrested pneumatisation and a small mastoid with middle ear pathology has long been accepted by otologists. Controversy exists whether mastoid aeration is genetically predetermined or whether environmental factors influence its formation. Proponents for genetic determination argue that in a large cohort of unselected mastoids, the aeration followed a normal bell shaped distribution.⁴ They also point out that measurements of MACS prior to and after the introduction of antibiotics did not demonstrate a difference.² This is further supported by a study of children with secretory otitis media. All the children in the study had otitis media with effusion. Secretory otitis media may be preceded by acute otitis media and this study was designed to determine whether acute otitis media affects MAC pneumatisation. It involved two arms: in one arm the children had a history of preceding acute otitis media and the other arm they did not. The children with a history of acute otitis media showed normal development of their MACS; however the other group had smaller

MACS and a greater tendency to develop middle ear atelectasis.⁵ This study suggests that children who develop secretory otitis media without a preceding acute otitis media may be predisposed to negative middle ear pressure and hypoventilation due to their genetically programmed smaller MACS.⁵

The counter argument of environmental factors influencing mastoid pneumatisation is endorsed by the finding that in children born through meconium stained amniotic fluid, there is a foreign body giant cell reaction in the middle ear mucosa. This results in granulation tissue formation, hyperplastic mucosa and secretions in the middle ear. The aeration and drainage pathways become blocked and may induce retraction pockets and predispose patients to recurring otitis media.⁶ This is further supported by a study of children with recurrent acute otitis media or otitis media with effusion in whom tympanostomy tubes were placed. They found that a small baseline MACS was associated with a young age at the time of first diagnosis of otitis media.⁷ Slow MACS growth was seen in children requiring multiple sets of ventilation tubes and a small final MACS size was a risk factor for chronic and recurrent infections.⁷ Animal studies also support the fact that chronic inflammation of the middle ear cleft causes hypocellularity of the MACS and increased thickness of the cortical bone.⁸ It is clear that chronic inflammation of the middle ear mucosa affects mastoid pneumatisation^{7,9,10}. However it is still unclear whether Eustachian tube dysfunction plays a role.¹⁰

The most likely explanation for varying MACS sizes seems to be that although genetically predetermined with a normal distribution of sizes in a population, pneumatisation of the mastoid is strongly influenced by environmental factors.^{1,11}

The middle ear and MACS may be considered a homogenous unit as diffusion of gases between the two areas is so rapid that significant differences between different areas of the middle ear cleft do not exist.¹² The middle ear exchanges gases with two compartments: the nasopharynx via the Eustachian tube and blood through the middle ear mucosa.¹³ It seems these two systems work in a complementary fashion, where the mucosa is responsible for regulating small pressure changes and this is augmented periodically by the Eustachian tube to equalise higher pressure differences.¹⁴ It is

possible that a larger MACS requires less frequent openings of the Eustachian tube to maintain ambient pressure.¹⁵

A mathematical model has been presented to establish the effect the MACS volume has on buffering middle ear pressure changes¹⁶ and clinically, this model showed that the MACS may serve as gas reservoir and regulate pressure changes in the middle ear.¹⁵

Small MACS are associated with chronic ear disease but controversy exists as to whether it is the cause or the result of chronic inflammation. A radiological study of patients with unilateral cholesteatoma and a control group of normal patients revealed that in the patients with cholesteatoma; the MACS in both the affected and unaffected ears were smaller than the control group.¹⁷ A subsequent study echoed these sentiments and reported that patients with unilateral cholesteatoma have decreased pneumatisation and reduced patency of the Eustachian tube in the unaffected ear. The contralateral ear also had an increased risk of middle ear disease.¹⁸ This suggests that a small middle ear cleft makes one prone to cholesteatoma formation.

Theories pertaining to the aetiopathogenesis of cholesteatoma formation

1. The Invagination Theory

This was originally proposed by Wittmaack and is the most widely accepted theory.¹⁹ The theory describes pars flaccida retraction pockets forming secondary to negative middle ear pressure and Eustachian tube dysfunction, repeated inflammation, small mastoid air cell volume or habitual sniffing. Retraction pocket advancement with accumulation of desquamated keratin results in cholesteatoma formation. Tos described cholesteatomas based on this theory as: (1) attic cholesteatoma arising from the pars flaccida; (2) tensa retraction cholesteatoma involving the entire pars tensa; and (3) sinus cholesteatoma, a localised postero-superior retraction pocket extending into the sinus tympani.²⁰

2. The Immigration Theory

Immigration theory describes cholesteatoma formation secondary to tympanic membrane epithelial migration or invasion through a defect in the tympanic membrane. It has been corroborated by animal studies.²¹

3. The Squamous Metaplasia Theory

Chronic irritation and inflammation may cause pluripotent epithelial cells in the middle ear to become keratinising.²²

4. The Papillary Ingrowth Theory

Keratin-filled microcysts or buds form in the basal layer of epithelium and invaginate into the middle ear. Retraction pockets and/or perforations are not a pre-requisite. This has been substantiated by clinical, experimental and animal studies.²²

Other factors that may contribute to cholesteatoma formation

Age

Cholesteatoma in children is considered to be more aggressive than in adults as it tends to be more extensive, causes greater ossicular chain pathology, has higher recurrence rates and is more difficult to eradicate.²³⁻²⁵ The causative factors in children are considered to be unstable and immature Eustachian tube function, higher rates of otitis media, and a temporal bone that is still developing.²⁵ The epidemiology of acquired cholesteatoma is unclear; however, reduced incidence is associated with better access to primary care and improved social circumstances.^{23,24}

Socioeconomic

In developing countries like South Africa, patients present with advanced disease and poor preoperative hearing thresholds. In a recent audit at our institution 8% of children presented with subperiosteal abscesses and a further 4% had intracranial sepsis.²⁶ Early diagnosis and surgical intervention could prevent these complications from occurring. Furthermore, hearing rehabilitation in resource limited settings can prove challenging as there is limited access to hearing aids, especially bone conduction hearing aids, making this a major public health concern.

HIV

Otological manifestations are commonly associated with Human Immunodeficiency Virus (HIV) infection. In a study of almost 1000 HIV-positive patients in India,

otological manifestations were present in 20% and chronic suppurative otitis media was present in 13%.²⁷ Another study of children presenting to an otology clinic with chronic otorrhoea noted that cholesteatoma was present in 20% of children and over 50% of the children were HIV-positive.²⁸

A recent audit at our institution showed 15% of children between the ages of 2-14 years old with cholesteatoma were HIV-positive.²⁶ The Western Cape Provincial prevalence rate of HIV is 1.1%.²⁹ Although our sample size was small it raises an interesting question about whether HIV plays a role in the aetiology of cholesteatoma. Chronic inflammation of the middle ear cleft may be more common in patients with HIV and this may affect mastoid air cell pneumatization and may predispose the children to developing cholesteatoma. To our knowledge there is very little literature available on cholesteatoma in HIV-positive patients.

With this paper we hope to better describe and define the radiological manifestations of cholesteatoma in HIV-positive versus HIV-negative children.

Hypothesis

The null hypothesis is that there are no radiological differences in pneumatization of the MACS between HIV-positive and negative children with cholesteatoma.

Aims and objectives

The primary outcome is to determine whether there is a difference in mastoid pneumatization in HIV-positive compared to HIV-negative children with cholesteatoma.

Secondary outcomes are: establishing whether children with HIV develop cholesteatoma at a younger age, have more aggressive disease by radiological imaging, whether bilateral disease is more common and if Eustachian tube obstruction is more prevalent.

Research Design: Material and methods

The study consists of a retrospective, cross-sectional, observational analytical review of patients with cholesteatoma at Red Cross War Memorial Children's Hospital from 2008-2015, and compares the radiological findings of children with HIV infection to those without HIV infection.

A folder review will be done to obtain the following information: age at diagnosis, gender, HIV status and whether the child had unilateral or bilateral middle ear disease (including chronic mucosal disease) or cholesteatoma. We will examine their radiological images using the hospital's digital imaging system.

Inclusion criteria

Patients with cholesteatoma who had had a computed tomography (CT) scan prior to their first surgical procedure will be included in the study.

Exclusion criteria

Patients in whom the HIV status is unknown. Patients who have craniofacial malformations, including cleft palate and Down's syndrome will also be excluded.

The following data were collected

- Age at time of CT scan
- Sex
- Unilateral or bilateral disease (in which case, each ear was reviewed separately):
- Patients who have chronic mucosal otitis media of the contralateral ear will also be documented, although the radiological changes of that ear will not be evaluated in the descriptive analysis
- HIV status
- Radiological features:
 - a. Pneumatisation of the mastoid bone (descriptive and quantitative)
 - This will be documented as well, moderately, poorly pneumatised or sclerotic.

- It will be done independently by two investigators and the investigators will be blinded to the patients' HIV status. The investigators will standardise their results.
 - One investigator will be an experienced otologist and one, a third year resident.
 - The mastoid air cell volumes of both the patients' ears will be determined using Philips IntelliSpace Portal 7.0®, a computerised software programme utilised by the radiology department. The volume of the mastoid air cell spaces will be calculated by mapping the MACS, excluding the middle ear volume, in cubic millimetres (mm³).
 - For the purposes of accurate volume measurement, only CT temporal bone scans with 1mm cuts will be used.
 - The volumes will be calculated by 2 investigators, blinded to the HIV status of the patients.
 - To assess inter-rater reliability, each investigator will independently assess 10% of the other investigator's sample.
 - A third investigator (also blinded to patient HIV status) will independently evaluate 10% of the entire sample.
 - One investigator will be a radiologist and one will be third year resident, an experienced otologist will be the third investigator.
 - The MAC volumes of all the ears will be calculated.
- b. Ventilation of the middle ear cleft
 - c. Status of the ossicular chain
 - d. Fallopian canal dehiscence
 - e. Semicircular canal dehiscence
 - f. Dehiscence of the tegmen
 - g. Dehiscence of the posterior fossa
 - h. Patency of the Eustachian tube
 - i. Postnasal space patency at the level of the choana (coronal image)
 - Noted as soft tissue occlusion of the postnasal space: <50%, 51-75%, 76-90%, 91-100%

Statistical analysis

All statistical analyses will be performed using Stata version 12 (StataCorp, USA). Unpaired t-test or Wilcoxin rank sum tests (depending on the distribution) will be used to test for differences in continuous variables between HIV-positive and HIV-negative patients. Fisher's exact will test to determine whether there was an association between two categorical variables. $P < 0.05$ will be taken as the level for significance.

To assess the relationship between size of MACS and HIV status we will perform simple linear regression and adjust for age using multivariable linear regression. The inter-rater agreement between rater's measurements of MACS will be assessed using concordance correlation coefficient. 95% confidence intervals were reported with point estimates where relevant.

Outcomes

The findings of this paper will be the first paper in the literature on cholesteatoma to describe the radiological features of mastoid pneumatisation and bony erosion from cholesteatoma in children with HIV infection. It is an important paper because cholesteatoma may behave differently in HIV-positive children.

Implications

The findings from this paper will elucidate whether otological surveillance of children with HIV infection should include a heightened awareness for the possibility of cholesteatoma formation and its complications.

Data safety and monitoring and privacy and confidentiality

Only the principal investigator and supervisor will be able to collect data from the medical records. Radiological evaluation will be performed by the principle investigator, a radiologist and the supervisor. Information collected will be recorded on an electronic data sheet. The computer used for this purpose is password protected. Confidentiality will be maintained.

Risks and Benefits

There is no direct benefit for the patients, however knowledge gained from this research may benefit others in the future. There is no risk incurred by the patients.

Ethics

Ethics approval will be obtained from the Human Research Ethics Committee at the University of Cape Town.

Informed Consent Process

This is not necessary due to the retrospective nature of the study.

Timeline

The expected time to collect the data for the study is 3 months.

Dissemination of research

This paper will be submitted to an Otolaryngology journal for publication.

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PART B: LITERATURE REVIEW

Introduction

Cholesteatoma continues to be a major public health concern, especially in developing countries.^{1,2} The favoured model for its aetiopathology is the retraction pocket theory described by Tos.³ He surmised that chronic middle ear hypoventilation, with or without Eustachian tube dysfunction, may lead to pars flaccida retraction.³ As the retraction pocket advances, the neck becomes blocked by shed epithelial cells that are unable to migrate laterally and a cholesteatoma sac forms. Growth of the sac proceeds into the meso- and epitympanum and into the mastoid air cells (MAC).⁴

The mastoid air cell system (MACS) serves as a gas reserve and regulates middle ear pressure changes.⁵ Consequently, patients with small MACS are predisposed to otitis media with effusion, not preceded or incited by an acute inflammatory process and have a greater tendency to develop negative middle ear pressure and atelectasis.⁶

There is a definite association between hypocellularity of the MACS and cholesteatoma formation.^{7,8} A radiological study of patients with unilateral cholesteatoma and a control group of normal patients revealed that in the patients with cholesteatoma, the MACS in both the affected and unaffected ears were smaller than the control group.⁸⁻¹⁰ The side with the cholesteatoma had significantly decreased pneumatisation^{7,9-11} and reduced patency of the Eustachian tube compared to the unaffected ear.⁷ The contralateral ear also had an increased risk of middle ear disease.^{7,9} This suggests that a small middle ear cleft makes one prone to cholesteatoma formation.

Although it is controversial whether MACS development is genetically predetermined, or whether environmental factors play a role, increasingly more studies suggest a genetic predisposition strongly influenced by environmental factors as evidenced by the above findings.⁷⁻¹⁰ Likewise, the epidemiology of acquired cholesteatoma is unclear but reduced incidence is associated with better access to primary care and improved social circumstances.^{4,12}

Objectives

The aims of this review are to evaluate the literature on MACS pneumatisation to assess what factors are involved in its final determination.

Literature Search Strategy

PubMed was searched using the following search terms:

- Mastoid pneumatisation (pneumatization) AND genetics
- Mastoid pneumatisation (pneumatization) AND hereditary factors
- Mastoid pneumatisation (pneumatization) AND acquired factors
- Poor mastoid pneumatisation (pneumatization) AND acquired factors
- Mastoid pneumatisation (pneumatization) AND inflammation
- Mastoid pneumatisation (pneumatization) AND infection
- Mastoid pneumatisation (pneumatization) AND Human Immune Virus
- Mastoid pneumatisation (pneumatization) AND acquired factors
- Mastoid pneumatisation (pneumatization) AND size
- Mastoid pneumatisation (pneumatization) AND cholesteatoma
- Mastoid pneumatisation (pneumatization) AND chronic otitis media
- Mastoid pneumatisation (pneumatization) AND Eustachian tube dysfunction
- Chronic otitis media AND hypocellular mastoid
- Mastoid development AND pneumatisation (pneumatisation)
- Middle ear AND gas exchange
- HIV AND atopy
- HIV AND adenoidal hypertrophy
- HIV AND Eustachian tube dysfunction
- HIV AND otitis media with effusion

Inclusion Criteria

Papers were included that described the process of mastoid pneumatisation or the factors affecting mastoid pneumatisation. It included animal studies and laboratory temporal bone studies. Studies on middle ear gas exchange, its importance and the consequence of its pathology were also included.

Exclusion Criteria

Papers that were not in English were excluded, as well as papers exploring pneumatisation and tympanoplasty surgery.

Interpretation of Literature

Genetically pre-programmed pneumatisation of the MACS induces regression and resorption of the mesenchyme that initially fills the middle ear cleft, by apoptosis. This proceeds simultaneously with osteoclastic and osteoblastic bony remodelling.¹³ There is evidence to show that apoptosis of osteoblasts is significantly suppressed in the face of chronic inflammation of the middle ear cleft. Osteoblast apoptosis may be an essential step for bone resorption in MAC pneumatisation.¹⁴

Not only may the MACS vary within a population;¹⁵ it may even differ between the 2 mastoid processes of a single individual.¹⁶ The air cell system may encompass the whole mastoid process as well as other parts of the temporal bone, or it may be acellular or hypocellular.¹⁶

Morphology of Mastoid Hypocellularity

The different types of mastoid hypocellularity may be distinguished using a combination of macroscopic, radiological and histomorphological investigations, allowing for a detailed analysis of the architecture and relationship of the pneumatised and non-pneumatised portions of the mastoid.¹⁶ Types 1 and 2 hypocellularity represent a primary hypocellular condition with incomplete pneumatisation during development augmented by environmental factors, whereas Type 3 hypocellularity represents a secondary hypocellular condition as a result of bony obliteration from chronic infection (namely mastoiditis) post MAC development.¹⁶

Type 1 hypocellularity is characterised by 2 main features. Firstly: there is no clear boundary between the pneumatised and non-pneumatised portions of the bone, and the two parts are connected by openings of different sizes and it is not always possible to distinguish air cells from marrow spaces. Secondly: the non-pneumatised bone is substantially different from normal spongy bone and the thickened trabecular bone decreases the size of the marrow spaces.¹⁶

Type 2 hypocellularity portrays a well-defined boundary between the pneumatised and non-pneumatised mastoid portions. The boundary may be slightly sclerotic but the architecture of the non-pneumatised spongy bone is not altered.¹⁶

Type 3 hypocellularity consists of normal pneumatisation of large parts of the temporal bone, with a hypocellular mastoid process. The air cells in the mastoid are confined to its upper portion. There is a clear distinction between the pneumatised and diploic (apical) parts, which are separated by dense bone. Bone architecture in the spongy apical bone shows almost normal architecture.¹⁶ The dense bone is not composed of compact, sclerotic bone; but fine, densely packed trabeculae filling the former air cells; probably caused by bony remodelling and proliferation.¹⁶

It may be difficult to distinguish between the types of hypocellularity radiologically. Type 3 hypocellularity represents an end-stage form of chronic mastoiditis by bony remodelling. A single mastoid may portray a combination of either Type 1 or 2 with Type 3 hypocellularity.¹⁶

Various theories dominate the literature regarding the aetiopathophysiology of the hypocellular mastoid process.

Genetic theory of mastoid hypocellularity

The argument for genetic determination of the variations of MACS pneumatisation in the population was introduced by a large cohort of unselected mastoids, where the aeration followed a normal bell shaped distribution.¹⁵ Additionally, it has been noted

that measurements of MACS prior to and after introduction of antibiotics did not demonstrate a difference.¹⁷

This argument is further supported by a study of children with secretory otitis media. All the children in the study had otitis media with effusion. Secretory otitis media may be preceded by acute otitis media and this study was designed to determine whether acute otitis media affects MAC pneumatization. It involved two arms: in one arm the children had a history of preceding acute otitis media and the other arm did not. The children with a history of acute otitis media showed normal development of their MACS; however the other group had smaller MACS and a greater tendency to develop middle ear atelectasis.⁶ This study suggests that children who develop secretory otitis media without a preceding acute otitis media may be predisposed to negative middle ear pressure and hypoventilation due to their genetically programmed smaller MACS.⁶

Another study compared the sizes of MACS in children and their parents; as well as the MACS sizes in dizygotic and monozygotic twins. They found there to be a significant correlation between the sizes of the children's MACS and that of their parents, if their parents had similarly sized MACS. This result lends further support to the argument of genetic predetermination of MACS size.¹⁸ However, if the parents had dissimilarly sized MACS, there was no correlation between the parents' MACS size and that of their offspring.¹⁸ In those cases, the children's MAC volume agreed with one or other parent or had a value somewhere in-between that of their parents¹⁸, suggesting perhaps that MACS size is a complex trait and that genetic susceptibility may well be mediated by a host of environmental factors. Not mentioned in the paper, is that the children and parents obviously shared the same environmental space. Consequently factors like overcrowding, malnutrition, poor access to health care services and sanitation would apply equally to each individual family and therefore environmental factors may well affect families concordantly, influencing the similar size of their MACS. Alternatively, there may have been some social movement amongst the parents prior to them having their children and this may account for the discordance in their MACS size.

An early review of the inheritance of the pneumatization of bone examined the literature on MACS aeration in monozygotic and dizygotic twins and found there to

be greater concordance in monozygotic compared to dizygotic twins. However, these studies did not use an objective or calculable method to assess the MACS and this is a great limitation to their findings.¹⁸ Dahlberg and Diamant explored the similarities of MACS sizes between monozygotic and dizygotic twins, using planimetric X-ray films to measure the pneumatisation more objectively and again found greater concordance among monozygotic twins.^{15,18} The MACS sizes in dizygotic twins varied in much the same way as the general population.¹⁸ Considering that dizygotic twins are raised in the same milieu, the difference in MACS size may be accounted for by genetic inheritance. However, the variation between the parents and their offspring was less than the variation between dizygotic twins, suggesting that foetal and perinatal factors may play a significant role.¹⁸ Using their results from the twin studies, they suggested that inheritance is 1.56 times more influential than environmental factors when determining MACS size.¹⁸

Development of the mastoid air cell system

Development of the MACS is well described: at 7 months gestation the bony spaces in the middle ear cleft are filled with mesenchyme. Regression and resorption of the mesenchyme and pneumatisation of the MACS proceed only if middle ear pressure is normal.¹³ Development of the MACS occurs in three distinguishable phases from birth until puberty when the MACS reaches its adult capacity (12cm² by planimetric evaluation). The first phase occurs within the first year of life. There is rapid pneumatisation and the air cells increase the volume of the antrum by 1.5 - 2.5cm² so that the total volume is 3.5 - 4cm². The second phase occurs from 1 to 6 years old and is characterised by a linear increase in aeration of 1 - 1.2cm² per year. The final, slower phase continues into the third decade,^{17,19} thereafter it decreases substantially.¹⁹ This growth pattern seems to be mirrored in different ethnic populations.¹⁷

It seems reasonable to deduce that any pathophysiological insult occurring within the phases of development may affect the final outcome of MACS size.

Mucosal inflammation and the environmental theory of mastoid hypocellularity

Children born through meconium stained amniotic fluid may have perinatally induced toxic contamination of the middle ear cleft with amniotic fluid cellular content. This causes a foreign body reaction and initiates a giant cell reaction. Granulation tissue and pseudocysts block the ventilatory routes of the middle ear. The trapped secretions may inhibit resistance to infection and an ongoing cycle of inflammation, granulation tissue and obstruction ensues.²⁰

The effect of middle ear mucosal inflammation inhibiting mastoid pneumatisation was illustrated by an animal study where paraffin liquid was instilled into the left tympanic cavity a few days after birth. The experimental side was markedly hypocellular and sclerotic compared to the control side in all cases.²¹ Similarly, glycerine was instilled unilaterally in 4 groups of piglets at different time intervals after birth. Pneumatisation was inhibited in all the experimental ears and the later the initiation of the inflammatory process, the less the degree of pneumatisation suppression. They concluded that chronic inflammation inhibits pneumatisation. The study also illustrates that the point in time during development of the mastoid at which the inflammation begins plays an important role in the final determination of mastoid size.²²

A radiological study of patients between the ages of 1 and 18 years old with otitis media (with and without effusion) found that patients without an effusion had greater age-matched MACS. However the size of the MACS in this group of patients did show great variability and this suggests a genetic cause. On the other hand, a large percentage of the group of patients with an effusion had a MACS volume <5 millilitres (cm³) and the conclusion was made that not only the timing of the disease, but also the duration may impact on final MACS.²³

These discoveries were explored clinically, and researchers corroborated their findings that a greater degree of mucosal inflammation was associated with lower middle ear total pressure and a smaller area of mastoid air cells.²⁴ The corollary for this finding is that an intervention performed during mastoid development for patients with mucosal inflammation may halt or reverse pneumatisation suppression.²⁵ They tested this clinically by inserting ventilation tubes into the tympanic membrane, collecting a mucosal specimen at the time of the procedure, and monitoring pneumatic space

volume. Their findings echoed their previous results. However, mucosal oedema resolved within 2 months of treatment and the pneumatisation process was re-initiated in patients with severe inflammation after 1.5 - 2 years.²⁵ This is a strong argument for the environmental theory of mastoid pneumatisation and more particularly highlights the findings by Wittmaack in the early twentieth century.²⁶

A more recent study of the benefit of ventilation tubes for otitis media with effusion (OME) after a 5 year follow-up period showed that a smaller MACS size was associated with multiple episodes of OME and multiple ventilation tube placements.²⁷ Obviously children requiring multiple sets of ventilation tubes have persistent OME and ongoing inflammation.²⁷ It does raise the question as to the natural history and treatment of OME in preventing suppression of mastoid growth. Is it a self-limiting condition, or does ventilation tube placement stimulate the recovery process? It appears that OME following an episode of acute otitis media (AOM) and AOM in themselves do not predispose to small MACS.⁶

Atopic rhinitis represents a chronic inflammatory disorder affecting the respiratory epithelium of the nasal cavity and paranasal sinuses. It seems appropriate to assume the same disorder may affect the respiratory epithelium of the middle ear cleft, with allergens gaining access to the middle ear via the Eustachian tube. Recently it was shown that atopic individuals had smaller MACS volumes compared to control patients. They were also 4 to 5 times more likely to have an abnormal tympanogram (type B or type C).²⁸ It is a subject that needs to be explored further; but the concept of atopy induced otitis media is a perfect example of a genetic predisposition initiated by environmental triggers affecting final MACS size.

Human immunodeficiency virus

There is some controversy as to whether patients infected with HIV have a higher prevalence of allergic disease.^{29,30} There may be an association between a higher CD8-positive cell count and the prevalence of symptomatic allergic disease.²⁹ The mechanism behind this is that CD8- positive cells stimulate Immunoglobulin-E (Ig-E) synthesis,²⁹ which may predispose these patients to an atopic phenotype.

However, it is unclear whether the raised IgE levels are caused by HIV infection or whether they are the result of atopy,³¹ and considering that atopy is a common condition, the probability of dual pathology is high.³⁰ A recent prospective study of HIV-positive and negative children in South Africa showed no significant difference in skin prick test positivity between the 2 groups.³⁰ However, there was a higher prevalence of chronic rhinitis in the HIV positive patients, possibly caused by immunological dysfunction.³⁰⁻³²

Chronic inflammation of the middle ear cleft may be more common in patients infected with HIV and this may affect mastoid air cell pneumatisation and may predispose the children to developing cholesteatoma. In a study of almost 1000 HIV-positive patients in India, otological manifestations were present in 20% and chronic suppurative otitis media was present in 13%.³³ Another study of children presenting to an otology clinic with chronic otorrhoea noted that cholesteatoma was present in 20% of children and over 50% of the children were HIV-positive.¹ However, there are no studies available that describe mastoid development in children born with HIV.

Role of the Eustachian tube

The physiological function of the MACS is that of middle ear pressure regulation. It seems as though normal pressure is maintained in the middle ear by the complimentary actions of the MACS and the Eustachian tube. The MACS is responsible for maintaining homeostasis through constant regulation of small pressure changes and the Eustachian tube augments this with intermittent regulation of higher pressure changes.^{34,35} In periods of transient or persistent Eustachian tube dysfunction and negative middle ear pressure; a larger MACS delays the advent OME secondary to middle ear mucosal haemorrhage.^{35,36}

Animal models show that in ears with normal functioning mucosa, occlusion of the Eustachian tube does not impair MAC pneumatisation. Presumably this is because normal middle ear mucosa allows gas exchange to proceed independently of the Eustachian tube and maintain a normal pressure environment, such as is required for pneumatisation to occur.^{37,38}

Eustachian tube dysfunction in the presence of mucosal inflammation

Problems arise in the presence of mucosal inflammation. Whether or not Eustachian tube dysfunction contributes to poor MAC pneumatisation in this setting is controversial. There is evidence to show that both Eustachian tube occlusion³⁹ and a patulous Eustachian tube⁴⁰ are associated with smaller MAC volumes. However, there is also evidence to show no correlation between auditory tube occlusion and poor mastoid pneumatisation.⁴¹ Unfortunately the technique used to assess auditory tube patency in this study was not able to determine whether the Eustachian tubes were patulous. It is possible that both an occluded and a patulous Eustachian tube are the result, rather than the cause of general inflammation of the respiratory mucosa in the upper respiratory tract. However, this is difficult to measure clinically and perhaps laboratory based studies may shed further light on this theory.

Eustachian tube dysfunction, adenoidal hypertrophy and HIV-infection

HIV is active in the lymphoid tissue throughout the period of clinical latency.⁴² Adenoidal size in HIV-positive children with good immunological function has been shown to be greater than that of a cohort of HIV-negative control patients.⁴² However, HIV- positive children with poor immunological status have significantly smaller adenoidal tissue compared to the control group.⁴² The differences in size correlated with the clinical progression of the disease and may be used as a prognostic marker.⁴²

Identification of Needs for Further Research

Further histologic and longitudinal clinical studies are needed to clarify the outcome after contamination of the middle ear space by amniotic fluid cells in terms of susceptibility to chronic inflammation and/or infection of the middle ear and final MACS.

More research regarding HIV and mastoid pneumatisation is required. Considering that otological conditions manifest commonly in these patients, pneumatisation may be arrested. There is scant research on HIV and cholesteatoma. HIV is prevalent in Sub-Saharan Africa and studies elucidating whether there is an association between

HIV infection and cholesteatoma formation may alter the necessity of otological surveillance in this population.

The area of atopy and allergy induced inflammation of the middle ear cleft also requires further evaluation, especially considering that there is an increasing prevalence of atopy in developed countries⁴³ which may impact on otological health. The rise of atopy has been attributed to altered antimicrobial exposure and an early antibiotic debut (<24 months), particularly if exposed to cephalosporins or erythromycin.⁴³ A proposed mechanism for antibiotic induced susceptibility to atopy is that they alter the normal microflora of the gut, which are essential in normal immune programming.⁴⁴

There may be a benefit in sampling middle ear mucosa in atopic children to see whether it shows eosinophilic inflammation. Future efforts aiming to address whether MAC development may benefit from regular anti-allergy therapy in the pre-pubertal period might constitute a new guideline for the treatment of otitis media in atopic individuals.

There is no literature available on whether radiotherapy of the head and neck region as a child affects mastoid pneumatisation. There is also no literature on whether chronic inflammatory diseases, like cystic fibrosis affect mastoid pneumatisation. It is well documented that children with cystic fibrosis have poor development of their paranasal sinuses but they seemingly do not have an increased incidence of chronic ear disease. Since pneumatisation is arrested by inflammation of the mucosa, one would expect both radiotherapy and chronic inflammatory conditions of the upper aerodigestive tract to affect MACS.

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PART C: ARTICLE MANUSCRIPT

Radiological Differences between HIV-Positive and HIV-Negative Children with Cholesteatoma

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Abstract

Introduction

HIV-positive children are possibly more prone to developing cholesteatoma. Chronic inflammation of the middle ear cleft may be more common in patients with HIV and this may predispose HIV-positive children to developing cholesteatoma. There are no studies that describe the radiological morphology of the middle ear cleft in HIV-positive compared to HIV-negative children with cholesteatoma.

Aim

Compare the radiological differences of the middle ear cleft in HIV-positive and HIV-negative children with cholesteatoma.

Method

A retrospective, cross-sectional, observational analytical review of patients with cholesteatoma at Red Cross War Memorial Children's Hospital over a 6 year period.

Results

Forty patients were included in the study, 11 of whom had bilateral cholesteatoma and therefore 51 ears were eligible for our evaluation. HIV-positive patients had smaller ($p=0.02$) mastoid air cell systems (MACS). Forty percent of HIV-positive patients had sclerotic mastoids, whereas the rate was 3% in HIV-negative ears ($p<0.02$). Eighty-two percent of the HIV-positive patients had bilateral cholesteatoma compared to 7% of the control group ($p<0.02$). There was no difference between the 2 groups with regards to aeration of the middle ear cleft, bony erosion of middle ear structures, Eustachian tube obstruction or soft tissue occlusion of the post-nasal space.

Conclusion

HIV-positive paediatric patients with cholesteatoma are more likely to have smaller, sclerotic mastoids compared to HIV-negative patients. They are significantly more likely to have bilateral cholesteatoma. This may have implications in terms of surveillance of HIV-positive children, as well as, an approach to management, recurrence and follow-up. HIV infection should be flagged as a risk factor for developing cholesteatoma.

Introduction

HIV-positive children are possibly more prone to developing cholesteatoma.¹ Cholesteatoma continues to be a major public health concern, especially in developing countries.^{1,2} The favoured model for its aetiopathology is the retraction pocket theory describe by Tos.³ He surmised that chronic middle ear hypoventilation, with or without Eustachian tube dysfunction, may lead to pars flaccida retraction. As the retraction pocket advances, the neck becomes blocked by shed epithelial cells that are unable to migrate laterally and a cholesteatoma sac forms. Growth of the sac proceeds into the meso- and epitympanum and into the mastoid air cells (MAC).⁴

The mastoid air cell system (MACS) serves as a gas reservoir and regulates middle ear pressure changes.⁵ Consequently, patients with small MACS are predisposed to otitis media with effusion, not preceded or incited by an acute inflammatory process, and have a greater tendency to develop negative middle ear pressure and atelectasis.⁶ There is a definite association between hypocellularity of the MACS and cholesteatoma formation.^{7,8}

Radiological evaluation of patients with unilateral cholesteatoma compared to a control group of normal patients revealed that in patients with cholesteatoma, the MACS in both the affected and unaffected ears were smaller than the control group.⁸⁻¹⁰ The side with the cholesteatoma had significantly decreased pneumatisation^{7,9-11} and reduced patency of the Eustachian tube compared to the unaffected ear.⁷ The contralateral ear also had an increased risk of middle ear disease.^{7,9} This suggests that a small middle ear cleft makes one prone to cholesteatoma formation.

Although it is controversial whether MACS development is genetically predetermined, or whether environmental factors play a role, increasingly more studies suggest a genetic predisposition strongly influenced by environmental factors as evidenced by the above findings.⁷⁻¹⁰

Otological manifestations are commonly associated with Human Immunodeficiency Virus (HIV) infection. In a study of almost 1000 HIV-positive patients in India, otological manifestations were present in 20% and chronic suppurative otitis media

was present in 13%.¹² Another study of children presenting to an otology clinic with chronic otorrhoea noted that cholesteatoma was present in 20% of children and over 50% of the children were HIV-positive.²

Chronic inflammation of the middle ear cleft may be more common in patients with HIV infection and this may affect mastoid air cell pneumatisation and may predispose the children to developing cholesteatoma. However, there are no studies available that describe mastoid development or radiological features in children born with HIV infection.

This study aims to compare the radiological differences of the middle ear cleft in HIV-positive and HIV-negative children with cholesteatoma. The primary outcome is to determine whether there is a difference in mastoid pneumatisation. Secondary outcomes are: establishing whether children with HIV infection develop cholesteatoma at a younger age, have more aggressive disease by radiological imaging, whether bilateral disease is more common and if Eustachian tube obstruction is more prevalent.

Material and methods

The study is a retrospective, cross-sectional, observational analytical review of patients with cholesteatoma at Red Cross War Memorial Children's Hospital from 2008-2015, comparing the radiological findings of children with HIV to those without HIV.

Patients with cholesteatoma who had a high resolution computed tomography (CT) scan prior to their first surgical procedure were included in the study. There was a change in policy between 2008 and 2015, requiring patients to have radiological imaging prior to surgery and consequently, not all patients had radiological imaging prior to surgery. Patients in whom the HIV status was unknown, patients who had craniofacial malformations, including cleft palate and Down's syndrome were excluded.

Both folder review and analysis of the electronic radiological images were necessary to obtain the information: age at diagnosis, sex of the patient, HIV status and whether the child has unilateral or bilateral middle ear disease (chronic otitis media, either mucosal disease or cholesteatoma), unilateral or bilateral cholesteatoma. The radiological features noted were:

- Size of the MACS (mm³)
- Pneumatisation of the mastoid bone (descriptive)
 - Good
 - Moderate
 - Poor
 - Sclerotic
 - Cannot comment
- Ventilation of the middle ear cleft
 - Aerated
 - Opacification of middle ear and mastoid
 - Opacification of the middle ear
 - Opacification of mastoid air cells
 - Cannot comment
- Status of the ossicular chain
 - Present
 - Malleus and incus present
 - Malleus present
 - Nil
- For each of the structures below we commented on whether the bony structure was intact, dehiscent or could not comment:
 - Fallopian canal dehiscence
 - Semicircular canal dehiscence
 - Dehiscence of the tegmen tympani
 - Dehiscence of the posterior fossa
- Patency of the Eustachian tube
- Postnasal space patency at the level of the choanae

Volumetric analysis

Only CT temporal bone scans with cuts not more than 1 millimeter (mm) thick were used for accuracy purposes. This excluded 6 patients in whom the cuts were too large. Of the 34 patients whose MAC volumes were calculated, the ear of 1 patient was not included because it had been previously operated on. In each patient the volumes of both ears were calculated.

The MAC volumes were determined using Philips IntelliSpace Portal 7.0®, the computerised software programme utilised by our radiology department. The volume of the mastoid air cell spaces was calculated by mapping the MACS, excluding the middle ear volume, in cubic millimetres (mm³) (*Image 1*). The volumes were calculated by 2 investigators (a radiologist and a third year otolaryngology resident), blinded to the HIV status of the patients. To assess inter-rater reliability, each investigator independently assessed 10% of the other investigator's sample. A third investigator (an experienced otologist, also blinded to patient HIV status) then independently evaluated 10% of the entire sample.

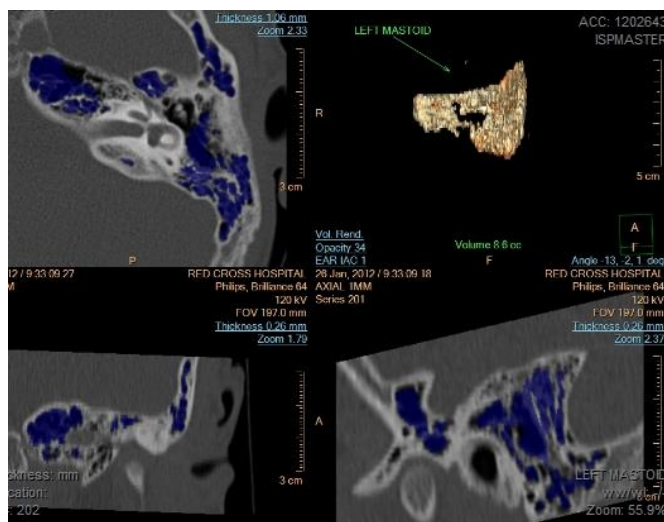


Image 1: Mapping the mastoid air cell volume using Philips IntelliSpace Portal 7.0® software

Descriptive analysis

Pneumatisation was categorised as well, moderately, poorly pneumatised or sclerotic. This was done independently by two investigators (an experienced otologist and a third year otolaryngology resident) and the investigators were blinded to the patients' HIV status. The investigators standardised their results. Aeration of the middle ear cleft was categorised into 4 categories: aerated, opacified middle ear and mastoid, opacified middle ear only and opacified mastoid only. The ossicular chain was assessed to be present, malleus and incus present, malleus present only or absent.

We were unable to obtain sagittal reconstruction images on all the patients. There is no accepted method for measuring the adenoidal pad in the postnasal space using coronal or axial CT scan images. The Eustachian tube cartilage lies in a groove between the petrous temporal bone and the greater wing of the sphenoid bone. This groove ends opposite the centre of the medial pterygoid plate at the level of the choanae. We chose to measure the patency of the postnasal space at this level using axial images. We graded the soft tissue occlusion of the post-nasal space as <50%, 51-75%, 76-90% and 91-100% occluded.

Statistical analysis

All statistical analyses were performed using Stata version 12 (StataCorp, USA). We used unpaired t-test or Wilcoxin rank sum tests (depending on the distribution) to test for differences in continuous variables between HIV-positive and HIV-negative patients. We used Fisher's exact test to determine whether there was an association between two categorical variables. $P < 0.05$ was taken as the level for significance.

To assess the relationship between size of MACS and HIV status, we performed simple linear regression and adjusted for age using multivariable linear regression. The inter-rater agreement between raters' measurements of MACS was assessed using concordance correlation coefficient. 95% confidence intervals were reported with point estimates where relevant.

Results

Fifty-nine patients and 74 ears with cholesteatoma were seen in the department from 2008 to 2015 (*Table 1*). Twenty percent of the patients were HIV-positive and the HIV status was unknown in 7% of the patients. Forty patients met both the inclusion and exclusion criteria, 11 of whom had bilateral cholesteatoma and therefore 51 ears were eligible for our evaluation. There was a male to female predominance (7:3). Only 1 HIV-positive patient was not on highly active antiretroviral therapy (HAART). The age range in the HIV-positive and negative groups were similar ($p=0.45$): 3 to 11 and 3 to 13 years old, respectively (mean of 7 and 8 years old, respectively). There was no significant difference between the HIV-positive and negative groups in terms of sex, age or side (*Table 2*).

	Total	Bilateral Cholesteatoma	Number of Ears
Number of patients	59		74
HIV-positive patients	12 (20%)	9	21
HIV-negative patients	43 (73%)	6	49
HIV-status unknown	4 (7%)		
Patients with CT Scans	40		51

Table 1: Summary of the number of patients seen in the department 2008 to 2015

	Total	HIV Negative (%)	HIV Positive (%)	P Value
Number of ears	51	31	20	
Number of patients	40	29 (72.5%)	11 (27.5%)	
Sex				0.70
Male	28 (70%)	21 (72%)	7 (64%)	
Female	12 (30%)	8 (28%)	4 (36%)	
Age				0.45
Range	3 – 13	3 – 13	3 – 11	
Median (interquartile range)	4	4	5	
Mean	7,85	8	7,1	
Side				0.39
Left	21 (41%)	11 (35%)	10 (50%)	
Right	30 (59%)	20 (65%)	10 (50%)	

Table 2: Patient demographics

Relatively more HIV-positive patients had poorly pneumatized or sclerotic mastoids: 40% of the HIV-positive ears had sclerotic MACS, whereas the rate was 3% in HIV-negative ears. This finding was significant at $p < 0.02$ (*Image 2 and 3*). Significantly more HIV-positive patients had bilateral middle ear disease ($p < 0.001$) and 82% of HIV-positive patients had bilateral cholesteatoma compared to 7% of the control group ($p < 0.05$). There was no significant difference between the 2 groups with regards to opacification of the middle ear cleft. There was also no significant difference with regard to the number of patients who had ossicular chain erosion, Fallopian canal dehiscence, semicircular canal dehiscence, tegmen dehiscence, or posterior fossa dehiscence. Patients with HIV infection did not have significantly more Eustachian tube obstruction or postnasal space occlusion (*Table 3*).

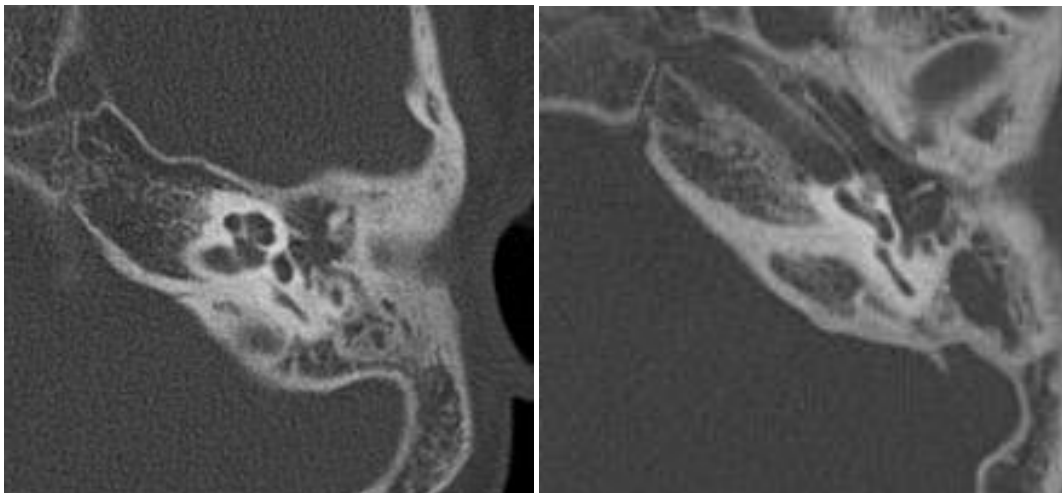


Image 2 and 3: The smaller, more sclerotic mastoid of an HIV-positive (left) compared to an HIV-negative patient (right)

	Total	HIV Negative (%)	HIV Positive (%)	P value
Pneumatisation of mastoid				<0,02
Good	3 (6%)	3 (10%)	0	
Moderate	16 (31%)	14 (45%)	2 (10%)	
Poor	14 (27%)	7 (23%)	7 (35%)	
Sclerotic	9 (17%)	1 (3%)	8 (40%)	
Cannot comment	9 (17%)	6 (20%)	3 (15%)	
Bilateral middle ear disease	21 (53.5%)	10 (34.5%)	11 (100%)	<0,001
Bilateral cholesteatoma	11 (27.5%)	2 (7%)	9 (82%)	<0,05
Opacification				0,72
Ossicular chain				0,37
Fallopian canal				1.00
SCC				1.00
Tegmen				0,63
Posterior fossa				0,15
Eustachian tube				0,08
Postnasal space soft tissue occlusion of postnasal space				0,11

Table 3: Results establishing whether there is a relationship between HIV and the outcome variable assessed

There was a significant difference between the volumes of the MACS in the two groups (*Table 4*) ($p < 0.02$). Although age was considered a strong clinical confounder for MACS, HIV status was independently associated with MACS (in cholesteatoma affected ears) with HIV-positive patients having significantly lower volumes than their HIV-negative counterparts (-386mm^3 (95% CI -59; -713), $p = 0.022$).

HIV Status	N	Mean (mm ³)	Standard Deviation (mm ³)	p50 (mm ³)	p25 (mm ³)	p75 (mm ³)	Minimum (mm ³)	Maximum (mm ³)
Negative	27	1101.3	577.8	1054.2	648.5	1260.3	327.2	2679.8
Positive	16	715.3	344.3	607.4	474.1	949.8	223.2	1438.2

Table 4: Volume differences between HIV-positive and HIV-negative MACS

Concordance correlation coefficients indicate that there was excellent agreement between the investigators (*Table 5*).

	Rho (95% CI)
Investigator 1 and 2	0.995 (0.984, 0.998)
Investigator 1 and 3	0.990 (0.951, 0.998)
Investigator 2 and 3	0.993 (0.977, 0.998)

Table 5: Inter-rater agreement for MACS measurements between the investigators

Discussion

Twenty percent of the patients seen in the department between 2008 and 2015 were HIV-positive. Although this is a small sample group, it is much higher than the Western Cape Province prevalence rate of HIV infected children in the same age group which was 1.1% in 2008.¹³ The age range in both the HIV-positive and negative groups were similar, indicating that these children present to specialist services at a similar age. However, it is unclear whether the total duration of their disease is more protracted as the finding of poorly pneumatized or sclerotic mastoids might suggest. It is recognised that otorrhoea is poorly managed and underestimated at a primary health care level and many patients spend years under their supervision prior to referral.¹

Development of the MACS

There is a significant difference in both mastoid pneumatization ($p < 0.02$) and MACS size ($p = 0.02$) between HIV-positive and negative patients, even when we adjust for age. Development of the MACS is well described: at 7 months' gestation the bony spaces in the middle ear cleft are filled with mesenchyme. Regression and resorption of the mesenchyme and pneumatization of the MACS proceed only if middle ear pressure is normal.¹⁴ Development of the MACS occurs in 3 distinguishable phases from birth until puberty when the MACS reaches its adult capacity (12cm² by planimetric evaluation).

The first phase occurs within the first year of life: there is rapid pneumatization; followed by a period of linear growth and an increase in aeration from 1 to 6 years old and a final, slower phase that continues into the third decade.^{15,16}

The process is genetically programmed but is strongly influenced by environmental factors.⁷⁻¹⁰ Any inflammatory insult occurring within the phases of development may affect the final outcome of MACS size.¹⁷ Both the timing and the duration of the inflammation impact on the final determination of mastoid size.¹⁸⁻¹⁹ It is thought the trapped secretions may inhibit resistance to infection and an ongoing cycle of inflammation, granulation tissue and obstruction ensues.²⁰

The mastoid air cell system (MACS) serves as a gas reserve and regulates middle ear pressure changes.⁵ Consequently, patients with small MACS are predisposed to otitis media with effusion, not preceded or incited by an acute inflammatory process and have a greater tendency to develop negative middle ear pressure and atelectasis.⁶ There is a definite association between hypocellularity of the MACS and cholesteatoma formation.⁷⁻⁸

Patients with HIV infection have chronic inflammatory otological disease,^{2,12} this manifests as more HIV positive patients having poorly pneumatised or sclerotic mastoids ($p < 0.02$) and may predispose them to developing cholesteatoma. The aetiology of their chronic middle ear disease is unclear but suppurative diseases are often associated with lower CD4-positive counts, while tympanic membrane retraction and otitis media with effusion are linked to higher CD4-positive counts.²¹

Aetiopathogenesis of Cholesteatoma

There are 4 theories pertaining to the aetiopathogenesis of cholesteatoma formation:²²

The Invagination Theory

This was originally proposed by Wittmaack and is the most widely accepted theory.²³ The theory describes pars flaccida retraction pockets forming secondary to negative middle ear pressure and Eustachian tube dysfunction, repeated inflammation, small mastoid air cell volume or habitual sniffing. Retraction pocket advancement with accumulation of desquamated keratin results in cholesteatoma formation. Tos described cholesteatomas based on this theory as: (1) attic cholesteatoma, arising from the pars flaccida; (2) tensa retraction cholesteatoma, involving the entire pars tensa;

and (3) sinus cholesteatoma, a localised postero-superior retraction pocket extending into the sinus tympani.³

This may explain why HIV-positive patients more commonly have mucosal disease of the other ear ($p<0.001$); and why they more often have bilateral cholesteatomata ($p<0.05$) compared to HIV negative children. They also have smaller ($p=0.02$) poorly pneumatised or sclerotic MAC ($p<0.02$), which may promote the formation of localised retraction pockets and encourage cholesteatoma formation. It is understandable that there was no significant difference between the two groups in terms of opacification of the middle ear cleft because all of the patients had active squamous disease.

The Immigration Theory

Immigration theory describes cholesteatoma formation secondary to tympanic membrane epithelial migration or invasion through a defect in the tympanic membrane.²² Considering that active mucosal disease manifests so commonly in HIV-positive patients,^{2,12} it seems entirely possible that cholesteatoma may occur secondary to epithelial migration into the middle ear cleft.

The Squamous Metaplasia Theory

Chronic irritation and inflammation may cause pluripotent epithelial cells in the middle ear to become keratinising.²² Patients with HIV infection have more than 1 reason to have chronic inflammation of the middle ear cleft: smaller ($p=0.02$), poorly pneumatised or sclerotic mastoids ($p<0.02$) and active mucosal disease.²

HIV infection is also associated with immune dysregulation and another possibility is chronic atopic mucosal inflammation. There is some controversy as to whether patients with HIV have a higher prevalence of allergic disease.^{24,25} There may be an association between a higher CD8-positive cell count and the prevalence of symptomatic allergic disease.²⁴ The mechanism behind this is that CD8-positive cells stimulate Immunoglobulin-E (Ig-E) synthesis,²⁴ which may predispose these patients to an atopic phenotype.

However, it is unclear whether the raised IgE levels are caused by HIV or whether they are the result of atopy,²⁶ and considering that atopy is a common condition, the probability of dual pathology is high.²⁵ A recent prospective study of HIV-positive and negative children in South Africa showed no significant difference in skin prick test positivity between the 2 groups.²⁵ However, there was a higher prevalence of chronic rhinitis in the HIV positive patients, possibly caused by immunological dysfunction.²⁵⁻²⁷ It is possible that the inflammation is not only limited to the nasal cavities but that it involves the middle ear cleft too. A limitation of our study is that we did not assess whether our patients had concurrent atopic disease.

The Papillary Ingrowth Theory

Keratin-filled microcysts or buds form in the basal layer of epithelium and invaginate into the middle ear. Retraction pockets and/or perforations are not a pre-requisite. This has been substantiated by clinical, experimental and animal studies²² but it is difficult to justify how this theory might predispose HIV-positive patients to cholesteatoma formation.

There was no significant relationship between having HIV infection and having bony erosion of important structures in the middle ear, compared to HIV-negative children. Various factors are responsible for the aggressiveness of cholesteatoma. Inflammation is crucial. Inflammatory cytokines induce fibroblasts to secrete cytokines that are essential in the differentiation, proliferation and migration of matrix keratinocytes.²² Inflammatory cytokines also promote angiogenesis, which is necessary for continued migration of keratinocytes into the middle ear.²² Recent studies show that squamous epithelium becomes destructive when subjected to a chronically infected environment.²² All these factors suggest that once the cholesteatoma is established in both HIV-positive and HIV-negative children, the inflammatory process rendered by the disease and concurrent infection predict its aggressiveness, rather than HIV infection itself influencing its course.

HIV Infection and Eustachian Tube Dysfunction

The physiological function of the MACS is that of middle ear pressure regulation. It seems as though normal pressure is maintained in the middle ear by the complimentary

actions of the MACS and the Eustachian tube. The MACS is responsible for maintaining homeostasis through constant regulation of small pressure changes and the Eustachian tube augments this with intermittent regulation of higher pressure changes.^{28,29} In periods of transient or persistent Eustachian tube dysfunction and negative middle ear pressure; a larger MACS delays the advent of otitis media with effusion secondary to middle ear mucosal haemorrhage.^{29,30}

Animal models show that in ears with normal functioning mucosa, occlusion of the Eustachian tube does not impair MAC pneumatisation. Presumably this is because normal middle ear mucosa allows gas exchange to proceed independently of the Eustachian tube and maintain a normal pressure environment, such as is required for pneumatisation to occur.^{31,32}

Problems arise in the presence of mucosal inflammation. Whether or not Eustachian tube dysfunction contributes to poor MAC pneumatisation in this setting is controversial. There is evidence to show that both Eustachian tube occlusion³³ and a patulous Eustachian tube³⁴ are associated with smaller MAC volumes. However, there is also evidence to show no correlation between auditory tube occlusion and poor mastoid pneumatisation.³⁵ Unfortunately the technique used to assess auditory tube patency in the above study was not able to determine whether the Eustachian tubes were patulous. It is possible that both an occluded and a patulous Eustachian tube are the result, rather than the cause of general inflammation of the respiratory mucosa in the upper respiratory tract. However, this is difficult to measure clinically and perhaps laboratory based studies may shed further light on this theory.

Although our results show no relationship between HIV status and Eustachian tube occlusion ($p=0.08$), there are a couple of limitations to our study. Firstly, the Eustachian tube is a dynamic tube and CT scan analysis is a static investigation so it may not accurately portray how the Eustachian tube is functioning. Secondly, patients may have a waxing and waning course of Eustachian tube obstruction and this would not be revealed in the CT scan examination. Further investigations are warranted to determine Eustachian tube function over time in patients with chronic middle ear disease, especially in HIV positive patients.

Although the majority of articles site the cartilaginous portion of the Eustachian tube as the obstruction point,³⁶ there is new evidence to show that the previously overlooked protympanic segment of the Eustachian tube may be responsible for tubal obstruction.³⁶ The Eustachian tube is cone-shaped and the nasopharyngeal portion has a larger aperture than the tympanic aperture.³⁶ The narrowest part of the tube lies at the junction of the proximal bony $\frac{1}{3}$ and the distal cartilaginous $\frac{2}{3}$ of the tube, known as the isthmus. It stands to reason that mucosal inflammation and recurrent infection of the middle ear may occlude the narrow lumen of the proximal bony portion of the Eustachian tube.³⁶ Otological infections are well recognised to be associated with HIV infection^{2,12} and this may contribute to Eustachian tube dysfunction. It is equally plausible that inflammation may ascend from the nasal cavities to involve the mucosa of the Eustachian tube.

There was no apparent connection between adenoidal size and HIV infection. A limitation to our study is that we did not include the viral load or CD4+ counts and therefore we are unable to determine whether the children were virally suppressed or whether they had treatment failure. HIV is active in the lymphoid tissue throughout the period of clinical latency.³⁷ Adenoidal size in HIV-positive children with good immunological function has been shown to be greater than that of a cohort of HIV-negative control patients.³⁷ However, HIV-positive children with poor immunological status had significantly smaller adenoidal tissue compared to the control group.³⁷ We are unable to say whether the lack of apparent correlation is linked to the children's immunological status.

Conclusion

The aetiopathogenesis of cholesteatoma is complicated. HIV-positive paediatric patients with cholesteatoma are more likely to have smaller, poorly pneumatised or sclerotic mastoids compared to HIV-negative patients. They are significantly more likely to have bilateral cholesteatoma and bilateral middle ear disease. This may have implications in terms of surveillance of HIV-positive children, as well as an approach to management, recurrence and follow-up. HIV infection should be flagged as a risk factor for developing cholesteatoma and a high index of suspicion should be maintained when examining an HIV-positive child with chronic otorrhoea. This paper

highlights deficiencies in the literature, where further research is warranted to gain a clearer understanding of why these patients have altered MAC development and why they are predisposed to having bilateral disease.

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PART D: SUPPORTING DOCUMENTATION

Departmental Research Committee approval



UNIVERSITY OF CAPE TOWN

Department of Surgery

Departmental Research Committee

Dr Timothy Pennel

D24 Office, Groote Schuur Hospital,

Observatory 7925, South Africa

Tel (021) 406 6168/6232/6227 Fax (021) 448 6461

Email: tim.pennel@uct.ac.za17th June 2016

Dr J McGuire

Department of Surgery
Groote Schuur Hospital
University of Cape Town

Dear Dr McGuire

RE: PROJECT 2016/022

**PROJECT TITLE: In Children with Cholesteatoma – Is there an Association
between Mastoid Pneumatisation and Human Immune Virus?**

The above proposal has been reviewed by the Department of Surgery Research Committee. I am pleased to inform you that the committee approved the scientific merit of the study, and endorse the protocol for submission to the relevant ethics committee.

Please use the above project number in all future correspondence.



Yours sincerely

A grey rectangular box containing the word "Signed" in a large, black, serif font.


DR TIMOTHY PENNEL

CHAIRMAN: RESEARCH COMMITTEE

Human Research Ethics Committee approval

	<p>UNIVERSITY OF CAPE TOWN Faculty of Health Sciences Human Research Ethics Committee</p>	
<p>Room E52-40 UIC Main Building Groote Schuur Hospital Observatory 7925 Telephone (021) 406 6492 Email: sumavah.aries@uct.ac.za Website: www.health.uct.ac.za/fhs/research/humanethics/forms</p>		
<p>20 July 2016</p> <p>HREC REF: 520/2016</p> <p>Dr T Harris Department of Otolaryngology H-53 OMB</p> <p>Dear Dr Harris</p> <p>PROJECT TITLE: RADIOLOGICAL DIFFERENCES BETWEEN HIV POSITIVE AND HIV NEGATIVE CHILDREN WITH CHOLESTEATOMA (MMeD-Candidate- Dr J McGuire)</p> <p>Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.</p> <p>It is a pleasure to inform you that the HREC has formally approved the above-mentioned study.</p> <p>Approval is granted for one year until the 30 July 2017.</p> <p>Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period. (Form's can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)</p> <p>Please quote the HREC REF in all your correspondence.</p> <p><i>We acknowledge that the student; Dr Jessica McGuire will also be involved in this study.</i></p> <p>Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.</p> <p>Please note that for all studies approved by the HREC, the principal investigator must obtain appropriate institutional approval before the research may occur.</p> <p>Yours sincerely</p> <div style="background-color: #cccccc; padding: 5px; display: inline-block; margin-top: 10px;">Signed</div> <p>PROFESSOR M BLOCKMAN CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE</p> <p>Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938</p> <p style="text-align: right; padding-top: 20px;">HREC 520/2016</p>		

Institutional approval

 <p>Western Cape Government Health</p>	<p>Dr AS Booysen Manager: Medical Services Email: Tony.Booyesen@Westerncape.gov.za Tel: +27 21 658 5788 Fax: +27 21 658 5166 RXH: RCC39</p>
<hr/> <p>Dr C McGuire Red Cross War Memorial Children's Hospital</p>	
<p>Dear Dr C McGuire</p>	
<p>APPROVAL OF RESEARCH</p>	
<p>PROJECT TITLE: RADIOLOGICAL DIFFERENCES OF HIV- POSITIVE AND HIV- NEGATIVE CHILDREN WITH CHOLESTEXTOMA</p>	
<p>It is a pleasure to inform you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.</p>	
<p>Yours sincerely,</p>	
<p>Signed</p>	
<p>Dr AS Booysen Manager: Medical Services Date: 15.09.16</p>	

Data Capture

Number	Age	Sex	HIV status	HAART	Side	Unilateral/ bilateral
		Female 0	Negative 0	No 0	right 1	Unilateral 0
		Male 1	Positive 1	Yes 1	left 2	Bilateral 1
31914799	8	1	0	0	1	1
87805693	8	1	0	0	2	1
86055209	12	1	0	0	1	1
115665028	5	1	0	0	2	1
15376312	6	1	0	0	1	1
38036034	6	1	0	0	2	0
88034772	6	1	0	0	2	0
13297965	6	1	0	0	1	0
88615125	8	1	0	0	2	0
43790385	4	1	0	0	1	1
11479011	6	1	0	0	2	0
14673875	10	2	0	0	1	1
14673875	10	2	0	0	2	1
114285778	13	1	0	0	1	0
86759727	12	1	0	0	1	0
28718393	3	1	0	0	1	1
28718393	3	1	0	0	2	1
38769386	9	1	0	0	1	0
16248262	5	2	0	0	1	0
89150502	4	2	0	0	1	0
88726633	8	1	0	0	1	0
113377675	12	2	0	0	1	0
119057602	6	1	0	0	1	1
12813085	10	1	0	0	1	0
87650545	10	2	0	0	2	0
43754555	9	2	0	0	1	1
15890775	9	1	0	0	1	0
86587870	10	1	0	0	2	0
106743875	12	2	0	0	1	0
88908884	7	2	0	0	1	1
86587870	11	1	0	0	2	0
86376597	11	1	1	1	2	1
86376597	11	1	1	1	1	1
86419157	5	1	1	1	1	1
86419157	5	1	1	1	2	1
22901359	6	2	1	1	1	1
34771162	3	1	1	1	1	1

34771162	3	1	1	1	2	1
88512322	11	1	1	1	2	1
88512322	11	1	1	1	1	1
						cant comment previously operated
112185244	10	2	1	1	1	
112185244	10	2	1	1	2	1
88465638	9	1	1	1	2	1
88465638	9	1	1	1	1	1
24701732	8	2	1	1	2	1
24701733	9	2	1	1	1	1
116422718	6	1	1	0	1	1
116422718	6	1	1	0	2	1
22901359	6	2	1	1	2	1
15536105	4	1	1	1	2	1
15536105	4	1	1	1	1	1

Pneumatisation	Comment	Aeration	Ossicular chain	Fallopian canal
well - 1	sclerotic tip - 1	opacification - ME + mastoid - 2	present - 1	dehiscent - 1
mod - 2	marrow - 2	opacification - Middle ear - 1	MI - 2	intact - 0
poor - 3	both - 3	aerated - 0	m - 3	
extensive dx - 4	neither - 4	opacification - mastoid not ME - 3	nil - 4	
	sclerotic - 5		3	1
	3	3	0	1
	1	4	2	1
	2	4	3	2
	2	4	2	2
	3	2	1	1
	4	1	3	3
	4	1	3	4
	2	2	3	1
	4	1	1	1
	1	1	2	2
cant comment			2	1
	3	1	2	1
	3	1	3	3
	2	2	2	1
	3	1	2	1
	2	2	2	1

	2	2	2	1	1
	2	1	2	1	1
	2	1	1	4	1
	3	3	2	1	1
	2	1	3	2	1
	5			4	1
cant comment			2	1	1
	2	3	2	1	1
	2	1	3	1	0
	3	1	2	2	1
	1	1	2	1	1
	2	2	3	3	1
	2	1	2	1	1
	2	1	2	3	0
done			2	3	0
	2	2	2	4	1
	2	2	2	1	1
	5		2	1	1
	5		2	1	1
	5		2	1	1
	3	3	2	4	1
	3	3	2	1	1
	5		3	1	0
	5				
prev operated		1	2	4	1
	3	1	3	2	1
	3	2	3	2	1
	5		2	2	1
	3	1	2	2	1
	3	1	1	4	1
	5		2	3	1
	5		2	1	1
3		3	2	4	0
cant comment			3	1	0

	posterior fossa	eustachian tube	SSC fistula	postnasal space soft tissue
Tegmen				
intact 0	intact 0	occluded 0	no - 0	
dehiscence 1	dehiscence 1	patent 1	yes - 1	
	0	0	1	0
	0	0	1 0	80%
				cant comment

0	1	1	0	>60%	
0	0	1	0	moderate	
0	0	1	0	moderate	
0	0	1	0		60%
0	0	1	0		100%
0	0	0	0		90%
0	0	1	0	cant comment	
0	0	0	0	<50%	
1	0	0	0	huge - does not touch septum	
0	0	0	0	huge - does not touch septum	
0	0	0	0	mod	
				huge - touch septum	
0	0	1	0	septum	
0	0	0	0	huge	
0	0	0	0		50%
0	0	0	0		50%
0	0	0	0	75-80%	
1	0	1	0	<25%	
0	0	1	0		50%
0	0	0	0	<50%	
0	0	1	0		50%
0	0	1	0		100%
0	0	1	0	unable to assess	
0	0	0	0		50%
0	0	1	0	<50%	
0	0	1	0	huge	
0	0	0	0		50%
0	0	1	1	small	
0	0	0	0	cant comment	
0	0	0	0		80%
0	0	0	0	80% occlusion	
0	1	0	0	80% occlusion	
0	0	0	0		50%
0	0	0	0		50%
0	0	1	0		100%
0	1	0	1	moderate	
0	0	0	0	moderate	
0	0	0	0	does not go down	
1	0	1	0	does not go down	
0	0	0	0		50%
0	0	1	0		20%
0	0	1	0		20%
0	0	0	0		40%
0	0	0	0		40%

0	0	0 0	huge
0	0	0 0	huge
0	0	1	0 100%
1	0	0 0	small
0	1	0 0	small

Number	Age	Sex	HIV	Volume cubmm Right	Volume cubmm Left	Cholesteatoma
31914799	8	1	0	507,8	482,6	R
43790385	4	1	0	971,2	1131,4	R
28718393	3	1	0	522,2	1090,9	RL
119057602	6	1	0	1485,4	1241	R
86587870	11	1	0	403,8	655,1	L
86055209	12	1	0	980,6	8600	R
115665028	5	1	0	4530,2	1545,2	L
15376312	6	1	0	678,4	1278,5	R
38036034	6	1	0	1018,2	2679,8	L
88034772	6	1	0	1841,4	1099,8	L
13297965	6	1	0	1162,9	1713,7	R
88615125	8	1	0	1567,2	1111,7	L
11479011	6	1	0	2992,5	1792,4	L
14673875	10	2	0	591	1054,2	RL
114285778	13	1	0	2127,5	5300	R
86759727	12	1	0	379,1	963,2	R
38769386	9	1	0	1199,7	7300	R
16248262	5	2	0	648,5	371,8	R
89150502	4	2	0	327,2	779,9	R
88726633	8	1	0	1260,3	3342,6	R
113377675	12	2	0	1065,4	1575,6	R
12813085	10	1	0	628,5	6300	R
87650545	10	2	0	5500	895,6	L
43754555	9	2	0	2248,4	2145	R
15890775	9	1	0	1027,6	1597,6	R
86376597	11	1	1	415,6	223,2	RL
86419157	5	1	1	605,3	740	RL
22901359	6	2	1	547,6	609,4	L
34771162	3	1	1	532,5	1438,2	RL
88512322	11	1	1	1072	1128,3	RL
112185244	10	2	1	x	1232,6	RL
88465638	9	1	1	736,1	572,3	RL
24701732	8	2	1	317	413,8	RL
116422718	6	1	1	827,6	580,4	RL

STATA

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STATS TABLES

Variable	Levels	n	Means	Medians	Min	Max	P25	P75	IQR	SD
Age	Negative	31	8.0	8	3	13	6	10	4	2.9
	Positive	20	7.3	7	3	11	5	10	5	2.9
$p = 0.45$	all	51	7.7	8	3	13	6	10	4	2.8

Table 1: Age by HIV Status, p-value obtained using Kruskal-Wallis test

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Sex	Male	22	71.0	14	70.0	36	70.6
	Female	9	29.0	6	30.0	15	29.4
$p = 1.00$	all	31	100.0	20	100.0	51	100.0

Table 2: Sex, p-value generated using Fisher's exact test

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
HAART	No	31	100.0	2	10.0	33	64.7
	Yes	0	0.0	18	90.0	18	35.3
	all	31	100.0	20	100.0	51	100.0

Table 3: HAART, p-value not necessary for this variable.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Side	Right	20	64.5	10	50.0	30	58.8
	Left	11	35.5	10	50.0	21	41.2
$p = 0.39$	all	31	100.0	20	100.0	51	100.0

Table 4: Side, p-value generated using Fisher's exact test

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Unilateral/bilateral	Unilateral	18	58.1	0	0.0	18	35.0
	Bilateral	13	41.9	19	100.0	32	64.0
$p < 0.0001$	all	31	100.0	19	100.0	50	100.0

Table 5: Unilateral/bilateral, p-value generated using Fisher's exact test

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Presmatisation	Good	5	16.7	1	5.9	6	12.8
	Moderate	13	43.3	1	5.9	14	29.8
	Poor	12	40.0	15	88.2	27	57.5
$p = 0.0055$	all	30	100.0	17	100.0	47	100.0

Table 6: Pneumatization, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Aeration	aerated	1	3.3	0	0.0	1	2.0
	opacification - Middle ear	3	10.0	1	5.3	4	8.2
	opacification - MEI + mastoid	17	56.7	14	73.7	31	63.3
	opacification - mastoid not MEI	9	30.0	4	21.1	13	26.5
$p = 0.72$	all	30	100.0	19	100.0	49	100.0

Table 7: Aeration, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Ossicularchain	Present	19	61.3	8	42.1	27	54.0
	MI	5	16.1	4	21.1	9	18.0
	M	4	12.9	2	10.5	6	12.0
	Nil	3	9.7	5	26.3	8	16.0
$p = 0.27$	all	31	100.0	19	100.0	50	100.0

Table 8: Ossicularchain, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Fallopiancanal	Intact	6	19.4	4	21.1	10	20.0
	Dehiscent	25	80.7	15	79.0	40	80.0
$p = 1.00$	all	31	100.0	19	100.0	50	100.0

Table 9: Fallopiancanal, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
SSChisula	No	30	96.8	18	94.7	48	96.0
	Yes	1	3.2	1	5.3	2	4.0
$p = 1.00$	all	31	100.0	19	100.0	50	100.0

Table 10: SSChisula, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{all}	% _{all}
Tegmen	Intact	29	93.5	17	89.5	46	92.0
	Dehiscence	2	6.4	2	10.5	4	8.0
$p = 0.63$	all	31	100.0	19	100.0	50	100.0

Table 11: Tegmen, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{All}	% _{All}
posteriorfossa	Intact	30	96.8	16	84.2	46	92.0
	Dehiscence	1	3.2	3	15.8	4	8.0
p = 0.15	all	31	100.0	19	100.0	50	100.0

Table 12: posteriorfossa, p-value generated using Fisher's exact test

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{All}	% _{All}
eustachiantube	Occluded	14	45.2	14	73.7	28	56.0
	Patent	17	54.8	5	26.3	22	44.0
p = 0.08	all	31	100.0	19	100.0	50	100.0

Table 13: eustachiantube, p-value generated using Fisher's exact test.

Variable	Levels	n _{Negative}	% _{Negative}	n _{Positive}	% _{Positive}	n _{All}	% _{All}
Postnasal space soft tissue	<50	4	14.8	8	47.1	12	27.3
	50-75	12	44.4	3	17.6	15	34.1
	75-90	4	14.8	2	11.8	6	13.6
	90-100	7	25.9	4	23.5	11	25.0
p = 0.11	all	27	100.0	17	100.0	44	100.0

Table 14: Postnasal space soft tissue, p-value generated using Fisher's exact test

Concordance correlation coefficient (Rho_c): measure of precision & accuracy

```
. concord rater1 rater2
```

Concordance correlation coefficient (Lin, 1989, 2000):

rho_c	SE(rho_c)	Obs	[95% CI]	P	CI type
0.995	0.003	8	0.989 1.000	0.000	asymptotic
			0.984 0.998	0.000	z-transform

```
. concord rater2 rater3
```

Concordance correlation coefficient (Lin, 1989, 2000):

rho_c	SE(rho_c)	Obs	[95% CI]	P	CI type
0.993	0.004	8	0.985 1.002	0.000	asymptotic
			0.977 0.998	0.000	z-transform

```
. concord rater1 rater3
```

Concordance correlation coefficient (Lin, 1989, 2000):

rho_c	SE(rho_c)	Obs	[95% CI]	P	CI type
0.990	0.008	8	0.973 1.006	0.000	asymptotic
			0.951 0.998	0.000	z-transform

Unadjusted simple linear regression

```
. regress Volume_cubmm HIV_pos
```

Source	SS	df	MS	Number of obs =	43
Model	1497505.91	1	1497505.91	F(1, 41) =	5.87
Residual	10458371.1	41	255082.222	Prob > F =	0.0199
Total	11955877	42	284663.738	R-squared =	0.1253
				Adj R-squared =	0.1039
				Root MSE =	505.06

Volume_cubmm	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
HIV_pos	-386.0794	159.3428	-2.42	0.020	-707.8787 -64.28008
_cons	1101.348	97.1982	11.33	0.000	905.0524 1297.644

```
. bysort HIV_pos: tabstat Volume_cubmm, stats (n mean sd p50 p25 p75 min max)
```

```
-> HIV_pos = 0
```

variable	N	mean	sd	p50	p25	p75	min	max
Volume_cubmm	27	1101.348	577.7884	1054.2	648.5	1260.3	327.2	2679.8

```
-> HIV_pos = 1
```

variable	N	mean	sd	p50	p25	p75	min	max
Volume_cubmm	16	715.2687	344.3396	607.35	474.05	949.8	223.2	1438.2

Adjusted multiple regression: adjusted for age

```
. regress Volume_cubmm HIV_pos age
```

Source	SS	df	MS	Number of obs =	43
Model	1497641.43	2	748820.716	F(2, 40) =	2.86
Residual	10458235.6	40	261455.889	Prob > F =	0.0688
Total	11955877	42	284663.738	R-squared =	0.1253
				Adj R-squared =	0.0815
				Root MSE =	511.33

Volume_cubmm	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
HIV_pos	-386.362	161.7981	-2.39	0.022	-713.3681 -59.35589
age	-.6634805	29.1426	-0.02	0.982	-59.56288 58.23592
_cons	1106.607	251.0703	4.41	0.000	599.1749 1614.039

International Journal of Pediatric Otorhinolaryngology:

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Article structure

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

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[2] W. Strunk Jr., E.B. White, *The Elements of Style*, fourth ed., Longman, New York, 2000.

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[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), *Introduction to the Electronic Age, E-Publishing Inc.*, New York, 2009, pp. 281–304.

Reference to a website:

[4] Cancer Research UK, *Cancer statistics reports for the UK*.
<http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/>, 2003 (accessed 13.03.03).

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