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Systematic Review

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The Relationship between Different Types of Streptococci and Pharyngotonsillitis: A Systematic Review

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ABSTRACT

Introduction: Streptococci were initially viewed by Louis Pasteur in 1879. Brown, in 1919, created the first systematized classification of streptococci in α, β and γ. Rebecca Lancefield contributed for knowledge of streptococcal polysaccharides discovering groups and M cell wall protein. Streptococci are gram positive, catalase and oxidase negative. *Streptococci* related to pathogenesis of acute sore throat are Streptococcus β-hemolytic of the groups A, B, C, F and G.
Objective: Our objective was to make a review of the different types of streptococcus that can cause infection in the oropharynx.
Review: *Streptococcus pyogenes* is belonging to the Lancefield grouping. Skin and mucous membranes of humans are the only known reservoir in the nature of streptococcus group A. Streptococcus of Group B (SGB) was originally isolated by Nocard in 1887. The primary habitat of these bacteria is the human colon, although it may colonize the oropharynx and especially, the vagina. For this reason, in mid-1960, the SGB has become a major cause of perinatal bacterial infection, including bacteremia and urinary tract infection in pregnant women. Group C is involved in purulent pharyngitis outbreaks. According to Fox et al, *Streptococcus anginosus* is the most common isolate β-hemolytic group C in the oropharynx. Streptococcus Group C (SGC) is reported as "pyogenes-like" because it shares important virulence factors such as hemolysins, extracellular enzymes and M proteins as well as the SGA.
Conclusion: We conclude that it's important to have knowledge about the different types of streptococci to better treat patients with sore throats and problems associated with this condition.

INTRODUCTION

Streptococci were initially viewed by Louis Pasteur in 1879. He had observed under the microscope "some chain account", which in 1884 was named Streptococcus by Rosenbach. Brown, in 1919, created the first systematized classification of streptococci in α, β and γ, from standard hemolysis checked on blood agar plates. Colonies of streptococci that produce a clear halo around due to complete lysis of erythrocytes were called β-hemolytic. Strains of streptococci that produce partial hemolysis give a greenish aspect, bright; around the colony are the α-hemolytic. Colonies that are not capable of producing hemolysis are identified as γ or non-hemolytic and rarely cause infection in man.

Streptococci are gram positive, catalase and oxidase negative. They tend to grow in pairs or chains. They are anaerobic optional, although some species grow best aerobically. The cell wall composition is similar to other Gram-positive bacteria, although richer in peptidoglycans, where are inserted various carbohydrates, lipoproteins and surface protein antigen.[1]

Rebecca Lancefield contributed for knowledge of streptococcal polysaccharides discovering groups and M cell wall protein whose antigenic molecules are considered to become

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the basis for grouping these micro-organisms.[2,3] The antigens detected in the group of Lancefield consisting of cell wall polysaccharides (groups A, B, C, F and G in humans), and cell wall lipoteichoic acids (group D enterococci). Other Streptococcus, such as viridans group, have no cell wall antigen, and cannot be included in any of these groups.[4]

Streptococci related to pathogenesis of acute sore throat are Streptococcus β-hemolytic of the groups A, B, C, F and G. In bacterial pharyngotonsillitis (PT), the main single agent is *Streptococcus pyogenes*, although in the last 50 years, an increased number of human infections caused by β-hemolytic Streptococcus in groups B, C, and G, have been observed worldwide.[5,6]

OBJECTIVE

Our goal was to make a review of the different types of streptococcus that can cause infection in the oropharynx.

REVIEW

Streptococcus pyogenes (SGA)

Streptococcus pyogenes is belonging to the Lancefield grouping. Skin and mucous membranes of humans are the only known reservoir in the nature of streptococcus group A. Its pathogenicity is associated with at least two surface molecules, conferring resistance factor: The M protein and the capsular hyaluronic acid.[1]

The M protein is a flexible and fibrillar structure situated on the surface of the bacterial cell wall, which provides resistance and anti-phagocytic polymorphonuclear leukocytes protective against. Molecularly, they are dimeric and designed in a carboxyl-terminal portion. In their amino-terminal hypervariable portion is the specific nature of their serotype. Currently, there are about 170 types of M protein, found in ´´*emm*`` patterns with more than 750 subtypes.[7-9]

The capsule is another antiphagocytic hialuronic structure, offering a protection, interfering in the setting of phagocytes. There are other so-called virulence factors such as the opacity factor, hemolysin, streptolysins, streptococcal pyrogenic exotoxin, C5a peptidase and deoxyribonuclease, all with specific activities of antigenic protection.[4,10]

Streptococcus agalactiae (SGB)

β-hemolytic streptococcus or streptococcus of group B (SGB) was originally isolated by Nocard in 1887 and described as the causative agent of bovine mastitis. They are encapsulated microorganisms, diplococcus gram-positive β-hemolytic on blood agar, with most of its strains resistant to bacitracin. Differentiation among the other Lancefield groups is given by capsular polysaccharides and specific antigenic proteins. They include serotypes Ia, Ib, Ia/c, II, III, IV, V, VI, VII and VIII. The SGB produces many virulence factors, including hemolysins, encapsulated polysaccharides, C5a peptidase (only in human pathogenic strains) and some strains produce hyaluronidase and various surface proteins which stimulates the secretion of IgA and act as adhesins.[11]

The primary habitat of this bacterium is the human colon, although it may colonize the oropharynx and especially, the vagina. For this reason, in mid-1960, the SGB has become a major cause of perinatal bacterial infection, including bacteremia, amniotites, endometritis and urinary tract infection in pregnant women, as well as focal or systemic infections in newborns. The SGB also cause infections in nonpregnant adults including meningitis, endocarditis and septic arthritis.[12,13]

The PT by SGB is reported, mostly in young adults and adults with any immunosuppressive underlying disease. There were no reports of acute SGB pharyngotonsillitis in children of school age.

Streptococcus anginosus (SGC)

Group C is involved in purulent pharyngitis outbreaks. According to Fox et al, *Streptococcus anginosus* is the most common isolate β-hemolytic group C in the oropharynx, while the *Streptococcus equisimilis* has been associated with PT. Turner et al[14] reported that *Streptococcus equisimilis* showed strong evidence of being causative agent in patients with PT as *Streptococcus anginosus* appears to be part of the microbiota of the oropharynx. Al-Charrakh et al[15] in their study of the prevalence of Streptococcus β-hemolytic groups C and F in patients with acute pharyngitis, report that these groups are involved in acute PT at 6.2% of cases. Detected yet, many of these isolates showed ability to produce more than a virulence factor. According Zaoutis et al[16], Group C Streptococcus (SGC) and Group G Streptococci (SGG) are reported as "pyogenes-like" because they share important virulence factors such as hemolysins, extracellular enzymes and M proteins as well as the SGA.

Regarding the similarity between the SGC and the SGA, Shah et al[17] reported that although the β-hemolytic group C is not a common cause of acute PT, comprises similar characteristics to Group A Streptococcus, as both cause isolated exudative, PT and the like cellulitis, making them clinically indistinguishable.

Strains of Streptococcus Group C contains fibrinolysin and streptolysin, such as streptolysin O (ASO). Given this proven by Johnson et al[18] who demonstrated in their studies that the group C Streptococcus showed a strong immune response *via* high titer antibody against ASO (ASO), and that this increase concomitantly persisted in the presence of streptococcus in the oropharynx of the patient.

Although the microbiological characteristics and similar virulence between the GSC and EMS, compared to non-suppurative complications, Killian[11] reports that the GSC may is

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Complications of acute pharyngotonsillitis	
Suppurative	**Non-suppurative**
Peritonsillar and retropharyngeal abscess	Rheumatic fever
Sinusitis	Post-streptococci glomerulonephritis
	Scarlet fever

Table 1: Some complications of acute PT.

Drug	Dosage
Amoxicillin	500 mg, 3x each day, for 10 days
Amoxicillin/Clavulanate	500+125 mg, 3x each day, for 10 days
Benzathine penicillin G	1.200.000UI, IM, one dose
Erythromycin	250 mg, 4x each day, for 10 days
Clindamycin	300 mg, 4x each day, for 10 days

Table 2: Treatment for acute pharyngotonsillitis.

The major criteria for diagnosis includes	The minor criteria includes
Arthritis in several joints (polyarthritis)	Fever
Heart inflammation (carditis)	High ESR (erythrocyte sedimentation rate, an laboratory sign of inflammation)
Nodules under the skin (subcutaneous nodules or Aschoff bodies)	Joint pain (arthralgia)
Rapid, jerky movements (Sydenham's chorea)	EKG changes (electrocardiogram)
Skin rash (erythemamarginatum)	Other laboratory findings (elevated c-reactive protein, elevated or rising streptococcal antigen test)

Table 3: The Jones criteria for diagnosis of rheumatic fever.

related to acute glomerulonephritis but not with the FR. This fact contradicted a study by Haidan et al[19], who searched PT by SGC and SGG an aboriginal population, which reported that there was a high incidence of rheumatic fever *versus* low incidence of SGA infection, leading them to investigate the correlation of a group non-A, FR, suggesting in that results *in vitro*, the SGC and SGG in those conditions have the potential for initiating an autoimmune response, triggering acute rheumatic fever.

SYSTEMIC EFFECTS

Streptococcal PT, if not adequately addressed, can lead to non-suppurative complications such as rheumatic fever and glomerulonephritis (Tables 1 and 2).[1,4]

Rheumatic Fever (RF) is a rheumatic and inflammatory disease, consequence of an untreated upper airway infection for β-hemolytic streptococcus of group A, usually with immunological basis. It is a disease with significant prevalence in the population of school age, manifested as polyarthritis, carditis, chorea, erythema marginatum and/or subcutaneous nodules and is considered the leading cause of chronic valvular heart disease in young adults in developing countries.[9] The Jones Criteria guides doctors to clinically diagnose of Rheumatic fever (Table 3). According to American Heart Association (AHA), two major criteria, or one major and two minor criteria plus a previous history of sore throat infection are necessary to diagnosis.[20]

The M protein is intrinsically linked to the development of rheumatic fever, which postulated involves molecular mimicry between proteins of the host and streptococcus, where the host antibodies against streptococcal antigens, do not recognize their own structures, converging in the humoral and cellular cross-recognition. This phenomenon occurs between the M protein and myosin in cardiac tissue, resulting in aggression and cell damage.[10]

CONCLUSION

It is important to have knowledge about the different types of streptococci to better treat patients with sore throats and problems associated with this condition.

CONFLICTS OF INTEREST: None.

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Research

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Tympanoplasty Outcomes In Dry and Wet Ears

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ABSTRACT

Introduction: Chronic suppurative otitis media is a major public health problem in children and adults. Tympanoplasty is a surgical procedure which aims to prevent recurrent otorrhea and restore hearing ability.
Objective: To compare the graft uptake and the hearing outcome of tympanoplasty in non cholesteatomatous chronic otitis media between wet and totally dry ears.
Methods: A total of 108 wet ears (with mucoid discharge) and 232 dry ears (at least 3 months before surgery) with chronic suppurativeotitis media were operated on by myringoplasty between 2000 and 2014. Postoperative graft uptake and hearing gain rates were compared between both groups.
Results: Cartilage was used in 100% of wet ears and 35% of dry ears. In the other cases, temporalis fascia was the chosen graft. Anatomical success rate was 88% for the wet ear group and 87.5% for the dry ear group. The hearing gain rate was 62%% for the wet ear group and 56% for the dry ear group. Differences were found to be statistically non significant for both graft intake (p=0.9) and hearing gain (p=0.29). In the wet ear group, only age <40 years was found to be a predictive factor of audio logical success in tympanoplasty (p=0.001).
Conclusion: The success of myringoplasty is not adversely affected by the presence of mucoid ear discharge at time of surgery, and outcomes are comparable to those of the operation done for dry ears.

KEYWORDS: Tympanoplasty: Chronic otitis media; Wet ear; Cartilage graft.

INTRODUCTION

Chronic suppurative otitis media is a major public health problem in children and adults. It is characterized by recurrent middle ear discharge through a tympanic membrane perforation.[1] Tympanoplasty is a surgical procedure which aims to prevent recurrent otorrhea and restore hearing ability.[1] The literature mentions several factors that may affect surgical results, including age, perforation size and site, the status of the opposite ear, the type of graft and condition of the operated ear (dry or wet). Controversy exists about the condition of the middle ear as a prognostic factor in myringoplasty.

OBJECTIVE

The purpose of this study was to compare the graft uptake and the hearing outcome of tympanoplasty in non cholesteatomatous chronic otitis media between wet and totally dry ears, focusing on factors that may have influenced the success of surgery in wet ears.

METHODS

We conducted a retrospective study between January 2000 and December 2014 about 306 patients (340 ears) with chronic suppurativeotitis media (CSOM). All patients had a perforation in the pars tensa and underwent a primary tympanoplasty without mastoidectomy. Those with

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attic perforation or cholesteatoma were excluded from the study.

Patients were divided into two groups:

- Group 1 (Dry perforation group): when there was no evidence of otorrhea within three months before surgery.

- Group 2 (Wet perforation group): when there was any degree of discharge before surgery, granulation tissue, inflammatory, thickened or polypoidal mucosa in the middle ear. Preoperative audiometry was systematically performed.

For each patient we analyzed: age, gender, history of adenoidectomy or tonsillectomy, condition of the affected and the contralateral ear, size, type and location of the perforation, surgical technique, graft material and its placement. Size of the perforation was considered large when it exceeded the quarter area of the tympanic membrane. It was classified anterior or posterior relative to the long process of the malleus.

All tympanoplasties were performed under general anesthesia. Regarding the bilateral cases, a six months interval was contemplated between both surgeries.

Anatomical success, evaluated by microscopy, was defined as an intact graft without lateralization, retraction, inflammation or infection at the last follow-up visit with a minimum of 6 months. Patients were observed for an average time period of 30 months after surgery varying between 6 months and 6 years.

Auditory function was analyzed by performing preoperative and postoperative tone threshold audiometry at 3 months, 6 months and one year after surgery. Functional failure was defined by an air bone gap (ABG) of 0.5, 1 and 2 kHz more than 10 dB.

Hearing restoration was considered successful if the post-operative air bone gap (ABG) was less than 10 dB, calculated on the frequencies of 500 Hz, 1000 Hz and 2000 Hz. Statistical analysis was performed using SPSS® 19. We conducted Chi-square study of qualitative variables. Statistical significance was assigned to a $p \leq 0.05$.

RESULTS

In our study of 306 patients, there were 204 females and 102 males (sex-ratio=0.3). The mean age was 34.6 years [7-74 years]. Otitis media during childhood was the most frequently seen disorder, reported in 97% of cases. The mean symptoms were as follows: otorrhea (33%), hearing loss (30%) and tinnitus (11%). On examination, the perforation was wet in 32% of cases (Group 2) and totally dry in 68% of cases (Group 1) (Figure 1).

Past medical history revealed allergic rhinitis in 13% of wet ears *versus* 2% of cases in dry ears (p=0.0001) (Table 1). Previous adenoidectomy during childhood was performed in 9% of patients from Group 1 *versus* 3% of patients from Group 2 with a statistically significant difference (p=0.05).

Tympanic membrane perforation was anterior in 30%, subtotal in 28%, posterior in 19%, inferior in 15% and central in 6% of cases. In patients with wet ears, systemic and local antibiotics were delivered before surgery. Preoperative audiometry revealeda conductive hearing loss in 62%, a mixed hearing loss in 32% and was normal in the other cases. The mean ABG was 29.9 dB in group 1 and 32 dB in group 2 with no statistically significant difference (p=0.8).

All procedures were done under general anesthesia with a post auricular approach in 97% and a transmeatal technique in 3% of cases. Incision of the external auditory canal was performed between the 6 and the 12 o'clock direction and the tympanopeatal flap was detached. The ossicular chain was discontinued in 30% of cases in group 1 and 20% of cases in group 2 (p=0.05). The uncus was the ossicle the most frequently lysed in both groups.

We performed a type I tympanoplasty in 80% and a type II tympanoplasty in 20% of all cases. Cartilage graft (conchal or tragal) was used in 100% of wet ears and 35% of dry ears (p=0.02). In the other cases, temporalis fascia was the chosen

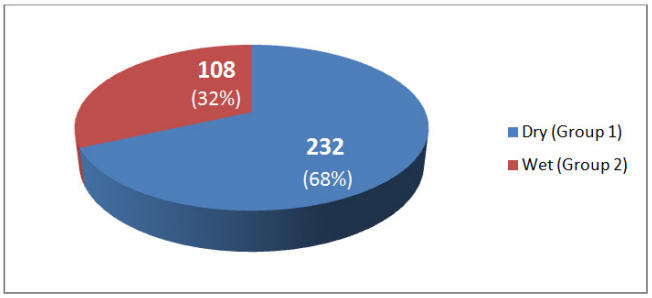

Figure 1: Patient's repartition according to the status of the middle ear.

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	Group 1 (232 ears)	Group 2 (108 ears)	Total (340 ears)	*p*-value
Allergic rhinitis	6(2%)	14(13%)	20	0.0001
Adenoidectomy	21(9%)	4(3%)	25	0.05
Otitis media in childhood	211(90%)	99(91%)	310	0.9
Perforation type: - anterior - posterior -subtotal -inferior -central	 73 42 70 39 8	 29 23 27 15 14	 102 65 97 54 22	
Tympanoplasty **-** Type I - Type II	 185(80%) 47(20%)	 87(80%) 21(20%)	 272 68	
Surgical approach - post auricular - transmeatal	 225 7	 105 3	 330 10	
Ossicular chain: **- complete** **- discontinued** * malleus * uncus * malleus+uncus * stapes	 164(70%) 68(30%) 20 32 16 -	 87(80%) 21(20%) 5 13 3 -	 251(73%) 89(27%) 25 45 19 -	0.05
Ossicular chain: - mobile - tympanoscelrosis	 142(61%) 90(39%)	 91(84%) 17(16%)	 233(68%) 107(32%)	0.003
Graft -Cartilage - Conchal - Tragal -Temporalis fascia	 82(35%) 73(90%) 9(10%) 150(65%)	 108(100%) 74(68%) 34(32%) -	 190(55%) 147(76%) 43(24%) 150(45%)	0.02
Conchal cartilage **Tragal cartilage**	165(71%) 67(29%)	73(67%) 35(33%)	238(70%) 102(30%)	

Table 1: Comparative findings between the two groups.

graft. After it was shaped, it was placed using the underlay technique, under or over the malleus handle depending on the perforation features and the anatomical conditions. Gel foam was used to support the graft and the tympanomeatal flap was reposiotioned. Meatal pack was used in all cases and removed by the third post-operative day.

On the last post-operative assessment, we observed a total graft integration without retraction, perforation or shifting in 87.5% of group 1 *versus* 88% of group 2, without a statistically significant difference (p=0.9) (Table 2). On the other hand, post-operative audiometry revealed ABG closure in 56% of group 1 *versus* 62% of group 2 (p=0.29). The mean hearing gain was 16.2 dB in dry ear group and 16.9 dB in wet ear group (p=0.9).

We also analyzed predictive factors of anatomical and functional success in wet ear group.

We concluded that age <40 years predicted better postoperative hearing gain with 74% of audiological success *versus* 39% for patients aged >40 years (p=0.001). Moreover, placing the graft under the malleus handle predicted better audiological results with 80% of success (p=0.04) (Table 3).

Other factors seemed to influence positively the outcome of tympanoplasty, without a significant correlation. These factors were: female sex, the absence of allergic rhinitis, a central perforation, a normal ossicular chain, the use of conchal cartilage and the placement under the malleus handle.

DISCUSSION

Chronic suppurative otitis media is a major public health problem in children and adults. It is characterized by recurrent middle ear discharge through a tympanic membrane perforation.[1] The main symptoms in this disease are intermittent otorrhea and hypoacusis.[2]

Tympanoplasty aims to prevent recurrent otorrhea and restore hearing ability.[1,3]

Several factors influencing surgical results in myringoplastyare mentioned in literature such including age, perforation size, perforation site, status of the contralateral ear, status of the middle ear mucosa, type of graft.[2,4]

The tympanic membrane is considered wet when one of these conditions is present:[5]

- remnant tympanic membrane is congested;

	Group 1	Group 2	*p*-value
Anatomical success - yes - no ˙retraction ˙perforation ˙shifting	 203(87.5%) 29(12%) 5 12 12	 95(88%) 13(12%) - 6 7	 0.9
Hearing improvement - yes - no	 130(56%) 102(44%)	 67(**62%**) 41(38%)	 0.29
Mean hearing gain	16.2 dB	16.9 dB	0.9
Post-operative complications: - myringitis - cholesteatoma	 9 3	 6 3	

Table 2: Anatomical & functional results of tympanoplasty in dry and wet ears.

	Anatomical success		Audiological success	
Age <40 years >40 years	 88% 86%	 *p*=0.9	 74% 39%	 *p*=0.001
Sex - male - female	 87% 95%	 *p*=0.39	 66% 65%	 *p*=0.9
Allergic rhinitis - yes - no	 82% 89%	 *p*=0.4	 52% 63%	 *p*=0.4
Perforation site: - marginal - central	 90% 100%	 *p*=0.3	 68% 70%	 *p*=0.39
Ossicular chain - normal - discontinued	 88% 85%	 *p*=0.7	 65% 47%	 *p*=0.1
Cartilage - conchal - tragal	 89% 85%	 *p*=0.6	 67% 51%	 *p*=0.1
Graft placement - under the malleus handle - over the malleus handle - sandwich	 100% 90% 87%	 *p*=0.06	 80% 51% 60%	 *p*=0.04

Table 3: Prognosis factors of tympanoplasty in wet ears.

- the middle ear mucosa is congested, polypoida or oedematous;
- perforation margins are oedematous or granular;
- discharge seen through the perforation;

For most authors, the wet or dry ear condition of the ear preoperatively was found to be of no predictive influence on anatomic or functional success.[2,6-15]

For Nagle,[15] closure rate was 88% for dry ears and 74% for wet ears without a significant difference (p=0.07). A hearing gain rate more than 10 dB was seen in 72% for dry ears *versus* 60% for wet ears (p=0.85). For Webb,[7] in a retrospective study of 205 ears, the rate of revision surgery was 26% in the wet group *versus* 40% in the dry perforation group without significant difference (Table 4).

In the study of Claes,[6] the influence of dry preoperative status of the ear was only present in patients with more than 30 dB preoperative ABG. For Shankar,[16] neither the type nor the presence of discharge in the middle ear influenced the success rate of surgery.

A meta-analysis done by Vrabec et al.[17] considering the effect of otorrhea on closure rate indicates that tympanoplasty on a discharging ear is as successful as in a dry ear.

Contrary to our results, many studies have reported that a discharging middle ear at time of surgery influences negatively the outcome of myringoplasty.[18-20] Uyar[20] found that there was a significantly higher rate of graft uptake in patients with dry ears for three months preoperatively. He thus recommends perform tympanoplasty. when the ears have been dry for at least three months. Gersdorff[21] and Pignataro[22] found a better outcome when operating on a dry ear, and both recommended medical treatment of discharging ears to control the inflammatory changes before myringoplasty. Onal et al.[23] reported that myringoplasty is more likely to be successful if the ear has been dry for at least one month.

Similarly, Pinar[18] found a significant association be-

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	Surgical success		*p-value*	Audiological success		*p-value*
	Dry ears	Wet ears		Dry ears	Wet ears	
Nagle[15]	88%	74%	0.07	72%	60%	0.85
Webb[7]	10%	7%	0.06	-	-	-
Hosni[4]	90.4%	87%	0.6	92%	91%	1
Pinar[18]	79%	64%	0.003*			
Dhar[11]	96%	84%	0.09			
De Lima[2]	94%	100%	0.3			
Claes[6]	-	-		51%	48%	0.01*
Shankar[16]	88%	80%	0.5			
Naderpour[12]	97%	94%	0.89			
Our study	87.5%	88%	0.9	56%	62%	0.29

Table 4: Graft intake and audiological success of tympanoplasty in dry and wet ears according to authors.

tween dry ear and success of tympanoplasty. Indeed, graft intake rate was 79% for dry ears *versus* 64% for wet ears (p=0.003). For this author, more than three months dry period of the ear and a middle ear risk index (MERI) less than 3 were found to be statistically significant prognostic factors that affect success rate. Takahashi[24] found that the presence of granulation tissue or oedematous mucosa in the middle earimpairs the function of transmucosal gas exchange and increases the distance between the middle ear cavity and the capillaries. This may result in surgical failure of tympanoplasty in wet ears.

On the other hand, fewstudies revealed a positive effect of ear discharge on graft uptake.

Caylan et al[25] have reported better healing of the tympanic membrane after myringoplasty in a discharging ear with 100% of success rate, while it was 75% in dry ears. They attributed such better results to the probable increase in the vascularity of the middle ear, which could have favored better healing in wet ears.

Vijeyandra[5] did a histopathological study of the remnant tympanic membrane of 20 dry and 20 wet central perforations. They concluded that in wet central perforation, all the layers of epithelium were present and there were a raised number of inflammatory cells and blood vessels. Also, the fibrous layer was present, contrarily to dry ears. According to this author, draining central perforation was not a contraindication for tympanoplasty as these anatomical conditions promote the graft intake.

Although better hearing gain and graft intake were observed for wet ears in our study, the difference was not statistically significant. We can then conclude that the wet or dry condition has no influence on anatomic or functional results.

Also, in the present study, only age <40 years and the placement of the graft under the malleus handle predicted better postoperative hearing gain with 74% and 80% of audiological success respectively. Female sex, the absence of allergic rhinitis, the normality of the ossicular chain and the use of conchal cartilage were associated with better anatomical and functional results, but the influence of the latter factors seemed to be rather weak.

Temporalis fascia and perichondrium alone often fails as graft material for tympanic membrane reconstructions because of their low stability and tendency to atrophy over the years.[26] However, cartilage graft is characterized by its resistance to resorption, retraction and negative pressure in middle ear.[26,27] Its connection to the surrounding tissue and its suitable elasticity for sound transmission make it a material of choice in tympanoplasty especially for wet ears.[29] For optimal acoustic transfer behavior, since the cartilage is formed mainly by type 2 collagen, it should be cut as thin as possible.[26]

In our study, we used cartilage (conchal or tragal) in 100% of wet ears and 35% of dry ears. In wet ear group, better anatomical and audiological outcomes were observed with conchal cartilage compared to tragal cartilage graft.

A limitation of the present study would be the fact that tympanoplasties were not performed by the same surgeon in our establishment. The type of graft as well as the graft placement technique was not the same for all operated ears. This may affect surgical results.

CONCLUSION

Based on our data, mucoid ear discharge does not interfere with the results of tympanoplasty and has no adverse effect on the surgical outcome and the postoperative hearing gain. Indeed, achieving surgical success depends more on meticulous graft placement rather than the status of the operated ear.

CONFOUNDING VARIABLES IN THE PRESENT STUDY

The results of this study may be influenced by some confounding variables:

- Type of graft: For all wet ears, we used systematically cartilage graft. This graft is well known for its stability and better resistance to retraction and re perforation. This may have

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affected the anatomical results.

- Age: For all children, tympanoplasty was performed using cartilage graft. This is due to Eustachian tube dysfunction seen in pediatric population.

- Surgeon: tympanoplasties were not performed by the same surgeon. This may have affected the surgery outcomes.

CONFLICTS OF INTEREST: None.

CONSENT STATEMENT

As our article did not publish any personal photo or information regarding any of the patient in my manuscript thus the consent is not required for the article publication.

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Review

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Defining "Best Practices" For Critical Endpoints In Preclinical Screening of New Chemical Entities For Ototoxicity Liability

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ABSTRACT

Introduction: Ototoxicity has been defined as the tendency of certain therapeutic agents and other chemical substances to cause functional impairments and cellular degeneration of the tissues of the inner ear resulting in hearing loss.
Objectives: This review is intended to provide details of a standardized preclinical assessment for ototoxicity under the current US FDA guidance documents that represents "industry best practices" for new drug application review of all new chemical entities being developed for human use.
Methods: A literature review was conducted to assimilate study strategies that represent "Industry Best Practices" for the conduct of preclinical ototoxicity evaluation for submission to regulatory drug approval agencies.
Conclusion: We have proposed a systems-approach and protocol criteria for the valid and reliable assessment of auditory function that can be easily included as a screening tool for ototoxicity within the Tiered Structure of preclinical assays required for approval of any chemical entity targeted for human use.

KEYWORDS: Ototoxicity; Auditory Brainstem Response (ABRs); Cytocochleagram; Histopathology; Otic Microscopy; Best practices.

INTRODUCTION

Defining the Problem

There are over 130 drugs or drug combinations that have a known risk liability for auditory dysfunction in humans.[1-4] In 2001, Palomar Garcia et al conducted a comprehensive literature review through 10 years of peer-reviewed publications to investigate the prevalence of drug-induced ototoxicity.[5] Cochlear and vestibular hair cell damage can be induced by numerous other factors such as acoustic trauma, aging, blast wave, irradiation, infection, heavy metals, pesticides, herbicides, organic solvents or gene mutations.[6]

There are currently no systematic or regulatory requirements for any standard preclinical studies conducted of new molecular entities (NCEs) that include analysis of all peripheral and central vestibular structures or auditory sensory organs before drug approval. Major obstacles in vestibular and hearing research include post-mortem tissue acquisition as well as neuropathological characterization prior to new drug application submission to the US Food and Drug Administration. Furthermore, inner ear structures are "safely" buried in the hardest bone of the human body and the cochlea and vestibular structures lie in different anatomical planes.[7,8] Any test article-related toxicity induced in this small compartment containing these critical end organs is rarely discovered during standard preclinical protocols

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on the journey to drug approval.

Part of the requisite risk analysis required for NDA review is to first identify the hazard. The objectives of hazard identification are to 1) produce a qualitative understanding of the potential for the system to present a risk (hearing loss, tinnitus, hyperacussis), and then 2) highlight the sources of that risk (cell damage inducing sensorineuronal deafness, or physical damage or inflammation of ossicles inducing conductive hearing loss). Once the hazard has been identified, hazard assessment is conducted. At this stage the aim is to understand and describe the plausible and critical pathways by which the substances reach the targets, the fate of the substance in the environmental media through which they are transported and the characteristics of the targets at risk (for example, apoptosis versus necrosis, secondary inflammatory responses, etc.). And the final step in the process is exposure estimation. For the small compartment of the VIIIth cranial nerve this is a challenge.

Regulatory Perspective

In July, 2001, the US FDA[7] adopted and provided approval of the ICH S7A Guidelines, titled, "Safety Pharmacology Studies for Human Pharmaceuticals" that were approved in November of 2000 by the regulatory bodies of the European Union, Japan, and USA.[9] More recently, the FDA published a notice adopting its own guidance titled, "Non clinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route: Guidance for Industry and Review Staff; Good Review Practice".[10] In it and for the first time, this guidance specifically gives details on preclinical toxicology screening for "otic" products which includes auditory brainstem response (ABR) and cytocochleogram evaluations. Interestingly, there is no legally defined or administrative policies (regulations) that clearly delineates what constitutes an acceptable ABR evaluation or sets the criteria for the processes, staining techniques, or details (full or partial cell count) of an acceptable cytocochleogram.

It's the intent of the drug regulators to allow for Industry to determine best practices. Selection of experimental variables should be based on "best judgment", experience, education, historical control data, and the reliability and validity of the resulting practices. The purpose of this review is to characterize what we believe should be the "industry best practice" with respect to both ABR evaluations, otic histopathology, and cytocochleogram parameters that will be the basis for sound and reliable drug safety determination of NCEs during the NDA process.

METHODOLOGIES AND PROCEDURES

Auditory Brainstem Response Evaluations for Regulatory Review

Regulatory agencies require an objective measure of functional changes in auditory thresholds in intact animals that can be temporally-linked to the magnitude and duration of exposure to NCEs. At least two measures of drug-induced deficits are used in the regulatory risk assessment of auditory toxicology. The degree of change in ABR data should be consistent with the post-life analysis of cellular damage defined using the cytocochleograms.

The ABR test produces a short acoustic (sound) stimulus such as a "chip", "tone", "pulse", etc., to the experimental subject and then measures *via* cutaneous electrodes the auditory evoked potentials from the inner ear, the auditory nerve and the brainstem. The test uses an automatic detection algorithm to determine if an ABR is evoked in response to the short acoustic stimulus. The data acquisition of the ABR-recordings takes place from the surface electrodes mounted at specific recording points on the animal. The analogue ABR-recordings are amplified in the external preamplifier connected to the electrodes. The amplified analogue ABR-recordings are converted into a digital signal in the Analog to Digital Converter. The digital ABR-recordings undergo data processing handled by the PC to improve the ABR-recordings. The ABR-recordings are displayed on the monitor for the operator. All ABR-recordings are then stored on the computer hard drive for later examination and diagnosis. The ABR is not a measure of perceptual deafness, only that the transduction of air wave pressure changes to electrical signals by the cochlea, and the transmission of those electrical nerve pulses are transmitted effectively to the brain, the forward progression from cochlea to brain cortex represents the process of normal information processing. The specific wave form frequencies to test are based on convention, laboratory experience, and published reports of auditory sensory ranges for the species being used.

Auditory brainstem response evaluations have been recorded in guinea pigs,[11,12] mice,[13-15] rats,[16] cats,[17-20] and non-human primates.[21] The ABR is an electrophysiological recording of transient responses following the onset of a sound stimulus presented to the ear while recording the events using standard EEG scalp electrodes. The recorded EEG voltage changes arise from within or in close proximity to various auditory brain stem nuclei and neural pathways. Psychophysical tuning curves are generated by repeated presentations of a select frequency signal (tone) at varying sound pressure levels (intensity=loudness). A minimum of 500 presentations at each step of frequency and intensity should be averaged for each subject. An evoked response requires an approximate 10 msec window of recording beginning at a sweep time of zero. This allows the AB, which spans approximately 1 to 6 msec to be visualized.

The use of animals to predict ototoxicity is intended to diminish or prevent similar cellular changes induced by drug exposures in an unsuspecting human population prescribed the drug therapeutically. Consistent and relatively more stable recordings can be achieved with the use of needle electrodes, or with chronically implanted, mechanically stabilized electrodes in animals when compared to human recording sessions. Human recordings are generally conducted with the awake patient of one

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in natural sleep. As a general rule, animal ABRs are never made with awake animals (except with the use of radio telemetry). Recording ABRs under anesthesia minimizes the undue influence of motor or movement artifacts in the EEG and under anesthesia complex and highly resolved parametric sequences of stimulation can be presented within a single session. Standard purpose-bred laboratory animals provide a smaller absolute head size and concomitant smaller brain, with proportionately thinner bone, muscle, and skin which provide a more favorable situation for recording the electrical impulses elicited by externally presented auditory stimuli. The evoked response detected on the scalp of an anesthetized laboratory animal tends to have larger absolute magnitudes as compared to humans, and there are more favorable signal-to-noise ratios in the signal detection.[22] Therefore, the overall gain required in the computer software amplifier system can be reduced and the amount of averaging of the number of stimulus-response pairings required to extract a clear and reliable response is less than that typically required from human subjects.

The brainstem evoked responses should be recorded from a minimum of three scalp electrodes by standard waveform analysis systems. These represent a series of sound volume or intensity-dependent neural potentials which originate and are time-locked or phase-locked to primary auditory pathways from transduction to central mediated information processing nuclei. The recorded and amplified scalp voltage waveforms have an identifiable series of peaks and troughs with latencies of approximately 10 msec from the stimulus onset.

Because neural synchrony is the basic requirement to produce an ABR, tone-burst sounds must be short (usually 4 or 5 cycles long) with relatively fast rise/fall times. The intervals between peaks are approximately 1 msec. For a given experimental subject, the peak latencies for any given stimulus or tone are unchanging over successive trials or recording sessions. Reliable waveforms can be recorded and reproduced over repeated presentations of the identical sound stimulus. There is general agreement that the brainstem response recordings reflect a series of neural events that when averaged and summarized provide 5 distinct peaks and troughs that represent a series of neural codes from the initial transduction of sound energy to electrical energy within the cochlea (Wave 1), the combined output of sensory cell voltage potentials to the cochlear nucleus (Wave II), and further transmission of the neural code to the superior olivary nucleus (Wave III) to areas around and within the inferior colliculus of the brainstem (Waves IV and V).

Since the critical biomarker in toxicity testing is the structural/anatomical changes that can be linked to the duration and magnitude of exposures to an NCE, the selection of Wave I, originating from within the cochlea is the major covariate in the analysis. Wave I of the ABR reflects the graded, generator potential of sensory hair cells within the cochlea. As summarized by Buchwald based on the preponderance of data detailed in published reports appearing in peer-reviewed scientific journals, Wave I of the ABR is produced by acoustic generator potentials of hair cells within the cochlea of cats,[17,18,23-26] rats,[24] mouse,[13-15] non-human primate[21] and guinea pigs.[11,12]

The middle ear is separated from the outer ear by the tympanic membrane (ear drum). The tympanic membrane is composed of three layers. From inside, the membrane is covered by a mucous membrane formed by the pavement epithelium; in the middle layer there are blood vessels which regulate the degree of the stratum's thickness. The auditory ossicles are a set of bones of tight tolerance connected together by tensor muscles. Changes to the elasticity of the tympanum, inflammation within the middle ear that compromises the muscle tension and close tolerance between the maleus, incus, and stapes that make up the ossicles may produce significant changes in auditory thresholds as measured by the ABR but do not induce any cell injury within the cochlea. Therefore, ABR changes in frequency thresholds may not be definitive evidence of direct cytotoxicity in the cochlea. To differentiate between the ABR threshold shifts due to conductive hearing losses and sensorineuronal cytotoxicity associated with drug administrations, the cytocochleogram becomes critical. Such objectively verifiable electrophysiological data can then be compared to the degree of cell injury or cell death verified post-mortem using standardized histopathology of prepared auditory tissues that are the basis of the cytocochleogram.

There are a variety of computer-based software programs that can be utilized for the collection, consolidation, and archiving of ABR data for regulatory review. In general, ABR testing of auditory function is conducted with industry-standard processes and systems whose 1) design qualifications have been tested and published in the extant scientific literature, 2) have a long history of factory acceptance testing, and 3) have been engineered to include startup testing and calibration each time the software is engaged. Most systems in use within preclinical testing environments do not fully comply with the FDA's equipment validation process regulations. Therefore, in most institutions, this portion of the ototoxicity study is exempted from compliance to Good Laboratory Practice Guidelines.

The reliability of system integrity, sensitivity and compliance can be assessed within each testing site by demonstrating Test 1 *vs.* Test 2, or Rater 1 *vs.* Rater 2 reliabilities of the ABR data sets. Figure 1, below, show comparative evaluations of ABR data from a proof-of-concept comparison scored by a professional, doctoral-degreed, university electrophysiology professor, with a 30-year record of active auditory research that was compared to threshold determinations of well-trained undergraduate ABR technicians assessing the relative auditory thresholds of three different frequencies in guinea pigs using industry standard software computer systems.

These data demonstrate the comparative auditory thresholds determined by ABR evaluations conducted by a qualified, industry expert-in-the field with three decades of

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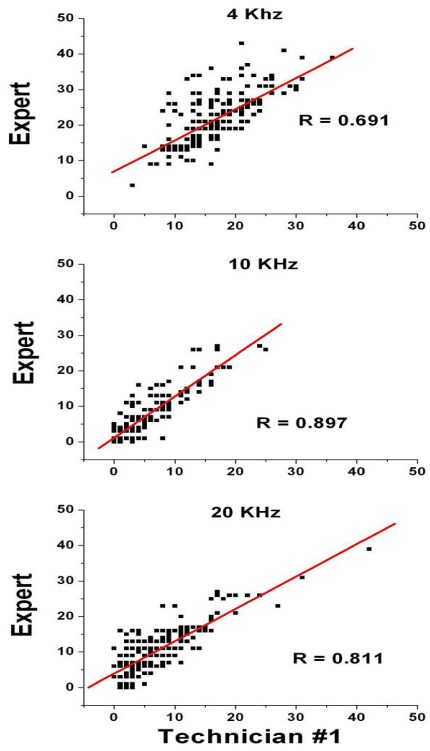

Figure 1: Inter-rater reliability for Sensory Threshold determinations using ABR Wave I functions generated at 4, 10, and 20 kHz pure sound wave stimuli in male guinea pigs. Correlation coefficients were generated by linear fit functions between a well-experienced electrophysiologist and a trained ABR technician for the subjective determination of sound thresholds (limens). All correlations representing Rater 1 *vs.* Rater 2 reliabilities were best fitted using straight line functions that were statistically significant, showing excellent agreement between the two raters.

electrophysiological monitoring of biological systems as a tenured professor in hearing research at a major U.S. University compared to a trained contract research industry technician using the same proprietary software data collection systems. Significant inter-rater reliabilities were demonstrated.

Thresholds for Wave I in standard ABRs measured with short tone "pips" as a function of frequency; defined as a short duration (15 ms) stimulus presented with a short rise/fall (0.1 ms) were presented to both ears individually and serially (left ear first, out of convention). Stimulus presentations with the lowest frequency (4 kHz) were conducted first and then in ascending order up to 32 kHz. The initial default intensity for each frequency was set at 80 dB and the intensity was lowered in 10 dB units until flattening of the Wave I signal was diminished. The 10 dB default value can be adjusted with experience. The frequency presentations were then tested in 5 dB increments to achieve the best sensory threshold (limen) for each animal. Each test session is scheduled to present a stimulus event (specific frequency and intensity) a minimum of 500 times to each animal (we generally use 1,000 to 2,000 presentations to average). Common practice on auditory function studies is to select three to four frequencies from the species-specific audiogram representing low, mid, and high frequencies within the sensitive hearing range. It is important to select a range of low to high frequency tones to examine function in various areas of the cochlea, and because the ABR evaluation can be used as a diagnostic tool when the appropriate frequencies are selected for use on study. A tone-pip that is greater than 2 wavelengths in duration at the lowest frequency tested (4 kHz: 0.00025 seconds) requires a minimum of 0.5 msec to complete. This 4 kHz signal has four cycles within 1 msec rise-time. Figure 2**,** below, shows the Wave I ABR thresholds conducted in over 2500 guinea pigs in our laboratory. Group mean thresholds and S.E.M.s are shown for the guinea pig auditory spectrum in over 2500 animals.

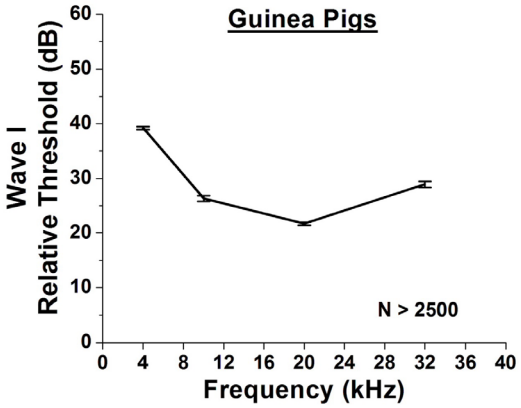

Figure 2: Pure tone audiograms for guinea pigs. Auditory threshold estimates for over 2,500 male guinea-pigs using Wave 1 ABR analysis. Each sound intensity from 80 dB to 0 dB were presented 512 times for 4, 10, 20, and 32 kHz pure sound tones in 10 dB steps. Each symbol represents the group mean Wave 1 threshold +/- 1 S.E.M.

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Upward shifts in the Wave I thresholds following drug administration suggests hair cell loss or conductive hearing loss as a result of inflammation, or impedance of inner ear ossicle movements. In contrast, downward shifts in the ABR functions would suggest hyperaccusis or increased sensitivity to a specific frequency range. Shifts in auditory thresholds can be reliably assessed under standard laboratory conditions within research environments that also conduct preclinical safety evaluation screening for NDA submissions.

Cytocochleogram Parameters for Regulatory Review

A review of the published literature leads back to the seminal book published in 1966 from members of the Department of Otolaryngology, University Hospital, in Uppsala, Sweden.[27] The book gave detailed descriptions of tissue surface preparations to produce a map, which they were the first to call a "cytocochleogram", showing hair cell damage along the length of the uncoiled basilar membrane. The standard procedure in the Uppsala laboratory was to examine cochlear segments sequentially from the four turns of the organ of Corti, for example base to one-half turn, 0.5 to 1.5 turns, 1.5 to 2.5 turns, and 2.5 to 3.5 turns. For "standard" species, they prepared continuous, complete cochlea grams from the base to the apex. In their original treatise, Engström, Ades, and Andersson[26] demonstrated excellent inter-rater reliability (~0.95) for cell counts of inner and outer hair cells in their standard configuration of base to 0.5 turns, 0.5 to 1.5, 1.5 to 2.5, and 2.5 to 3.5 cochlear turns.

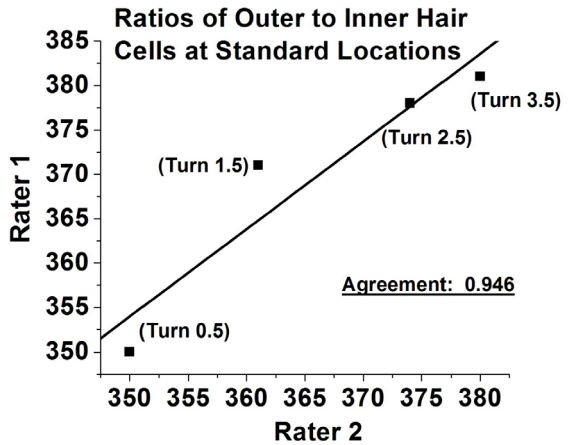

Figure 3: Inter-rater reliability for the ratio of outer to inner hair cell counts by two microscopy technicians at 0 to 0.5, 0.5 to 1.5, 1.5 to 2.5, and 2.5 to 3.5 mm of the guinea pig cochlea. Correlation coefficients were generated by linear fit functions between two readers in the Engström, Ades, & Andersson mapping of the cochlea (cf page 62). Correlations representing Rater 1 *vs.* Rater 2 reliabilities were best fitted using straight line functions that were statistically significant, showing excellent agreement between the two raters.

As reviewed by Santi,[28] with the advent of the electron microscope and the development of tissue fixation and embedding processes, Bohne[29] and Spoendlin & Brun[30] improved the methods for producing surface preparations of the whole organ of Corti so that they could be examined by both light and transmission electron microscopy. In 1986 Santi[28] published a relatively rapid method for fixation, embedding, trimming and mounting of the complete length of the organ of Corti that could be placed on a single slide that would be well-suited for light microscopy computer-assisted analysis to determine the number of "normal" and damaged hair cells. In spite of these pioneers in the field of cytopathology, in 2016 the harvesting, decalcification of the protective bone encasement of the tissue, as well as the surface preparation of the organ of Corti remains a meticulous, time-consuming, and challenging task.

Over the last five decades of examination and processing of the auditory sensory system there have been some generalized findings that may support the development of a standard process of neuropathological analyses that will provide convincing and compelling, scientifically-sound, and legally defensible data regarding the relative safety of any new NCE that will support NDA review by the FDA. Hawkins & Johnsson[31] reported that:

1) For reasons still not adequately understood, the hair cells at the extreme basal end of the cochlea are most often affected by the cytotoxic actions of the test agent. While scattered cell losses have been documented elsewhere along the continuum from base to apex, the early signs of ototoxicity of an NCE are identified by complete loss of outer hair and inner hair cells in the first 2 to 3 mm of the basilar membrane. In the Hawkins & Johnsson study,[31(p.176)] shortly following amino glycoside exposures a great majority of hair cells were still present above 5 mm. They concluded that it is not uncommon to find scattered losses of outer hair cells from the first to third row in untreated control animals – especially guinea pigs and cats.

2) With respect to chemical cytotoxicity from cisplatin and/or the aminoglycosides, the loss of hair cells typically starts at the first row of outer hair cells. The progression of damage usually shows greater loss of outer hair cells from the first row when compared to the second cell row. As the cellular signs of damage progresses, first row cells are lost followed in time by swollen and distorted second row cells; the third row of outer hair cells and the row of inner hair cells may still show intact and functioning cell integrity.[32]

3) In acoustic trauma (noise-induce deafness) the progression of functional changes and cell death is slightly different from chemical induced damage. The damage is initially noted in the first row of outer hair cells then the inner hair cell, followed by the second and third rows of outer hair cells. The spatial distribution of damage to the outer hair cells in row 1 and its associated inner hair cells differed in that the inner hair cells show damage mainly in the vicinity of the tonotopic exposure location and the outer hair cells damage spreads from the base (ear drum section) of the membrane up to the tonotopic area of maximal wave deflection.[33]

4) As the full expression of a NCEs cellular toxicity is identified within the cochlea, the parade of progression of

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cellular death from base to apex on the basilar membrane seems to be both dose- and time-dependent. With increasing doses or prolonged administration, cell death progressively increases.[32] In most cases, cochlear neurons degenerate secondarily after the inner hair cell loss.[34] As the sensory neurons die-out, disordered scarring of the reticular lamina, including the head plates of the pillar cells follows. In severe ototoxicity, localized lesions of the organ of Corti may occur in which all of the sensory and supporting elements have disappeared. This helps to categorize the quantitative destruction of cochlea in conjunction with the pathologist's qualitative reads.

While these facts may serve as a "rule of thumb" for establishing standardized protocols for tissue processing in an ototoxicity study, the extent of outer hair cell loss should not be inferred simply from duration or dose level of exposures. The progression of damage from amino glycoside antibiotic and cisplatin toxicity appears similar in that the initial insult is seen at the tympanic end (base) of the basilar membrane and then progresses up the length of the cochlea to the apex. The cilia seem to be first affected in the death or injury cascade. The first row of outer hair cells seems to show toxicity before the second and third rows. Following outer hair cell death the cascade of cell death is then expressed in the inner hair cells. With hair cell integrity compromised, cell death evolves to include spiral ganglion cells. It remains a requirement to perform some cellular count of complete hair cell loss down some specified length of the uncoiled or processed organ of Corti (cytocochleogram).

Information on the hair cell density of the guinea pig cochlea is scarce in the literature.[35,36] Detailed information was given by Burda[37] (ten cochleas processed, including the hook[1]), who demonstrated the hair cell density that is dependent on the relative distance from the apex and the averaged value of hair cell density for the whole cochlea. The averaged values of hair cell density can be compared directly, whereas, the spatial dependence can only be compared qualitatively due to the divergent presentation (sector-wise *vs.* stepped distances of the cochlea length). For the whole cochlea, Burda[37] found an average of 360.6 outer hair cells per millimeter and 100.4 inner hair cells per millimeter of basilar membrane. Linss, Linss, Emmerich & Richter[38] showed excellent agreement with these cell counts by reporting average values of 389 outer hair cells per millimeter (all three rows) and 101 inner hair cells per millimeter of basilar membrane. Qualitatively, Burda[37] found two segments of the basilar membrane showing maximal hair cell densities. One is located at the apex (helicotrema end) and the other about 30% of the cochlea length from the base. Linss, Linss, Emmerich & Richter[38] results also confirmed this spatial dispersion.

The table below lists the averaged group lengths of the guinea pigs organ of Corti reported in the published scientific literature appearing in peer-reviewed journals.

Basilar Membrane Length	Reference
20.8 mm	39
18.8 mm	40
20.32±0.69	41
18.17+/- 0.15 mm	42
21.79 mm	43
18.6 mm	44
20.5±0.6	37
18.5 mm	45
18.8+/-0.5 mm	46

According to Solntseva,[40] in the guinea pig (*Cavia porcellus*) the cochlea forms 4.5 turns. The average length of the total basilar membrane is approximately 18.8 mm and its average length for each of the turns is: 8.5, 4.8, 3.4, and 2.1 mm, respectively. The average number of hair cells in the guinea pig is 8,399, of which 1,899 are inner hair cells and the remaining 7,040 are outer hair cells. In the lower cochlear turn the number of inner hair cells is 874; in the second turn 437; in the third turn 403, and the fourth turn has 124; the remaining half turn of the apical end has 61 cells.

As described by Santi[28] and colleagues,[46-48] cell counting of the entire cochlea is extremely time-consuming. Identifying and counting all of the cochlear hair cells on serial sections of imbedded and processed slides under a light microscope can add weeks to months onto the study timeline depending on the number of trained technicians and available microscopes dedicated to the project. In addition to the sheer number of cells to be counted in a full cytocochleogram, the position of the slide must be repositioned to reflect the density changes along the length of the basilar membrane. Most of the analysis of minimal to moderate damaged cochlea is spent counting normal hair cells. The process may be more efficient by concentrating on hair cell damage rather than counting normal hair cells.

Proposed Process for Cytocochleogram Evidence of Ototoxicity Liability for NDA Submission

As stated above, current health agency administrative and regulatory policies provide little information on what defines a "cytocochleogram". While decades of ototoxicity research results have accumulated there is no clear definition of how to construct a standard cochleogram.[41] The need for these data are critical to the FDA's statutory responsibility for drug labelling prior to NDA approval, and for its regulatory responsibilities established by Congress to ensure the health-impact and safety of all new products entering the market place. The relative issue in regard to risk assessment pertains to the risk to benefit ratio of the new drug when compared to current treatments. The FDA may approve a new drug knowing that it has the potential to induce hearing loss, if the health benefit of the drug outweighs the deleterious influence of hearing loss in the targeted population, however, they must have a constant concern for "black box warning" or training material content to doctors who prescribe the drug if preclinical biomarkers suggest a potential

[1]The "hook" area of the cochlea was previously referred to as the "cochlear hamulus" and is located at its base by the tympanic membrane and oval window near the temporal bone where it bends or "hooks" downward. This specific region is critically important in placement of cochlear implants.

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for ototoxicity. The issue here is not so much on how much hearing loss is induced by a given drug because that will be an empirically-driven quantitative measure based on dose and duration of treatments. The focus for risk liability is simply, "Is there damage or not?"

So the goal in preclinical safety screening of new drugs for selective damage to the VIIIth cranial nerve compartment is simply to demonstrate a valid and reliable predictor of cell death in the cochlea following local or systemic exposure to the new drug entity. As described above, most cell damage whether by chemical (drug) or physical insult (loud noise) appears to originate near the basal end of the cochlea – not the apex. Demonstrative evidence of selective cell damage at the base of the cochlea is a relatively reliable measure of ototoxicity liability. If cell death is induced there, and shows dose-dependency within a study, or time-dependency between studies, then sufficient, credible evidence exists determinative of labelled warnings on the product advertising and packaging materials.

Ototoxicity Screening/Testing

Screening for ototoxic effects should be routinely carried out in short-term and subchronic toxicity studies with an appropriate species, preferably guinea pig, for 1) any new chemical entity that has the ability to penetrate an intact tympanic membrane; 2) is expected to reach the middle or inner ear during clinical use, 3) is introduced directly to those regions, or 4) has demonstrated renal toxicity during preclinical screening toxicology studies, or 5) is structurally similar to any other known ototoxicant and is administered systemically.

The ototoxicity screen should be age appropriate and would typically include: (1) specific histopathological examination of tissue samples representative of major areas of the VIIIth cranial nerve compartment, including cochlea, tympanic cavity, ossicles, semicircular canals, and Eustachian tubes.

Ototoxicity Screening Studies

The study reports should include an assessment of the potential for the test substance to adversely affect the structural or functional integrity of the sensory and transductive organs of the ear, adverse effects of the integrity of vestibular function or associated balance disorder syndromes. In regards to risk assessment analysis for regulatory review and product labelling, any cell damage or loss in the basilar end of the cochlea that is not demonstrated in vehicle control or aged match cohorts is sufficient evidence to warrant further testing or "Black Box" warnings on the label of the marketed product.

Neuropathology: Collection, Processing and Examination of Tissue Samples

A gross middle ear assessment should be performed using a dissecting microscope. During this assessment, the middle ear should be opened by removing regions of the temporal bone to reveal the ossicles. The presence of white or red discoloration, fluid, edema/swelling, or redness should be noted and rated. Integrity and appearance of the tympanic membrane should also be required. A instrument (i.e. forceps) should be used to gently apply pressure to the ossicles and their mobility is noted and rated in the pathology record. Photo-micrographs should be taken when necessary to demonstrate representative observations. Ratings, where required (i.e., for assessments of edema, erythema, and ossicle mobility), should be performed on a scale of (0) Not Present, (1), Minimal, (2) Moderate, and (3) Severe (or other suitable grading criteria). Ossicle mobility may be limited to simple rating as present or not present. Comments may be added to any of these ratings for further description of the finding at the discretion of the rater. The ossicles should be prepared for histological evaluation by light microscopy.

To provide for adequate sampling as well as optimal preservation of cellular integrity for the detection of neuropathological alterations, tissue should be prepared for histological analysis using *in situ* perfusion and paraffin and/or plastic embedding procedures. Paraffin embedding is acceptable for tissue samples from the central nervous system including cranial nerves. Plastic embedding of tissue samples from the central nervous system is encouraged, when feasible.

a. Histopathology: Histopathological evaluation should be conducted for the Organ of Corti (cytocochleogram) and middle and inner ear structures (Otic Microscopy) for all animals in ototoxicity screening studies. Our laboratory reserves one ear for cytocochleogram and the alternate ear for otic histopathology for every animal on study. We have also included non-treated age-, breeder-, gender-, and generational-matched cohorts for comparisons as well.

b. Fixation and processing of tissue: The neural tissue should be fixed by *in situ* perfusion with an appropriate aldehyde fixative. As described above, any gross abnormalities of tympanum, internal and external auditory canal, inner ear structures such as the ossicles, and vestibular labrynthine body should be noted.

i) **Fixation:** Following perfusion flush of saline and perfusion fixation with paraformaldehyde (or other applicable perfusion media), the middle and inner ears should be collected intact and fixed in paraformaldehyde for a predetermined functional time interval (i.e. 1 to hours). Tissues should then be transferred to a lower concentration of paraformaldehyde, and stored refrigerated until analyzed. The bullae should be subsequently dissected free of the remaining temporal bone, opened to allow observation, access to the middle ear, and to accommodate decalcification. The tissue block should than be placed in decalcification solution.

ii) **Decalcification:** The hard bony material must be decalcified

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using a gentle decalcification solution (for example, 10% EDTA) for tissue harvesting and processing. When the tip of a probe or fine tipped forceps encounters little resistance, the decalcification procedure will be considered complete.

iii) **Trimming:** Fine tipped rongeurs, scissors, forceps and blades are used to further trim the bone of the middle ear.

iv) **Embedding:** A modified version of ethanol fixation and paraffin embedding of otic tissue allows for clear visualization of natural fluorescence of reporter proteins needed for cellular differentiation and identification while maintaining excellent tissue morphology of inner ear tissues. Tissues can be dehydrated in step-wise processes using such agents as ethanol and then subsequently followed by exposures to xylenes. Specimens are generally allowed to come to room temperature prior to being permeated with paraffin by repeated immersions in 4 paraffin baths at 56 ˚C. Blocks of paraffin embedded tissue are then stored refrigerated in the dark until use. Sections of tissue 5 to 10 µm thick can be mounted and dried for review.

For plastic sectioning, the bullae need to be dehydrated through a graded series of alcohols and embedded in plastic, either Methacrylate or EPON-like resin (e.g., EMBED 812, or paraffin).

v) **Sectioning:** Prepared blocks should be trimmed and initial paraffin or plastic sections be used for orientation. Blocks should be aligned and several micron sections cut on the microtome. Periodic sections should be stained (see below) and assessed under a dissection or light microscope, to further align the block and determine when sections containing ossicles are observed, and then every third or fourth section should be mounted on a glass slide. This process should be continued until sections containing all the ossicles are obtained. Sections should then be stained with appropriate stains (i.e. H&E, Richardson's and/or Paragon) and cover slipped.

c. Otic Microscopy: Many systemically administered drugs will have access to the functional organization and operations of middle and inner ear systems. The FDA's guidance document does suggest direct dose administrations into the middle ear as a method to ensure adequate concentration delivery required for the full assessment of toxicological effects. Therefore, it is essential to include an assessment of the direct and inflammatory effects of such administrations on the mucosa and structures of the middle ear following the direct intra tympanic administration of the drug. Our standard protocol includes otic microscopy on one ear and cytocochleogram analysis of the alternate ear from both control and drug treated cohorts.

The result of otic microscopy may be employed to confirm or refute any ABR findings suggestive of conductive hearing loss. When approaching the evaluation of the middle ear, it is important to identify the structures intrinsic to sound conduction. If the temporal bone is oriented precisely, it is possible to collect sections of the tympanic membrane, ossicular chain, tensor tympani, round window, cochlea, and middle ear mucosa. Evaluation of these structures will provide insight into how the drug is interacting with the milieu of the middle ear. Signs of inflammation, fibrosis, and necrosis can result from direct administration of drugs into the middle ear, either by intra tympanic injections or, in the laboratory setting, by inner ear cannula injections. Detailed examination of the ossicles, round window, cochlea (bone, stria, organ of corti), auditory nerve, spiral ganglion, middle ear, and Eustachian tubes are essential in this analysis. Close and detailed examination of this portion of the small compartment of the auditory structures is critical in the identification of conductive disturbances that are reflected in augmented hearing thresholds in the ABR but are due to mechanical, inflammatory, or direct toxic effects on the conductive portions of transducing air pressure waves to nerve impulses.

The bone surrounding the structures of the inner ear is unique and embryologically distinct.[49] This bone is called the endochondral bone and forms the otic capsule. It is a primitive form of bone with dense extracellular matrix components. The footplate of the stapes is derived from otic capsule material and in pathological states the footplate may be exceptionally dense, thickened, and fixed to the surrounding oval window. The remaining bone tissues of the inner ear (ossicles) and temporal bone (mastoid cavity) are more similar to cranial and lamellar bone histologically. The otic capsule is extremely dense and with little porosity in terms of its micro architecture. The surface of this bone should be smooth and marble-like. In contrast, cortical bone (substantia compacta) is punctuated by pockets of fluid and cellular materials. Cortical bones and lamellar bones (substantia spongiosa) are the two bone types making up the calvarial vault. Observation and evaluation of the calvarial vault is essential in determining the role of conductive disturbances to changes in ABR thresholds. These evaluations are limited to the otic microscopy section of the post life tissue processing protocol.

Representative sections should be assessed using a light microscope under low and high magnifications for each bullae. Based on at least three sections from each animal, ossicles should be graded as normal or pathology noted. Photos should be taken when necessary to demonstrate representative lesions.

Detailed dissection procedures should be conducted per standard operating procedure guidelines of the institution. The tissue samples should be post-fixed and processed according to standardized published histological protocols. Tissue blocks and slides should be appropriately identified when stored. Histological sections should be stained for hematoxylin and eosin (H&E), phalloidin, or a comparable stain according to standard published protocols.

d. Cytocochleogram Microscopic Review: This level of analysis

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is specifically called out in the FDA guidance document. As stated above, our laboratory generally conducts cytocochleograms on one ear and otic microscopy on the other. The details of the cytocochleogram are based on previous work by Viberg & Canlon.[41]

The Organ of Corti should be fixed and stained with phalloidin (actin staining), or equivalent fluorescent stain. The cochlea should be uncoiled, trimmed, and placed on slides for reading. The length of the cochlea should be measured and documented in the study record. Tissues from all animals in the control and a minimum of 3 dose groups should be examined. Based on the etiology of chemical induced ototoxicity, quantitative screening procedures should include a complete total inner and outer hair cell count for a minimum cochlear distance of 40% of the full membrane as measured from basal end. The total number of hair cells and/or the total number of lost cells per unit area of the basilar membrane can be plotted as a function of percent distance from the cochlea apex and graphically plotted to establish the cytocochleogram.

e. Otic Histological Qualitative examination: Representative histological sections from the otic microscopy designated ear tissue samples should be examined microscopically by an appropriately trained pathologist for evidence of any treatment-related alterations. Particular attention should be paid to regions known to be sensitive to neurotoxic insult or those regions likely to be affected based on the results of functional tests. Such treatment-related alterations should be clearly distinguished from artifacts resulting from influences other than exposure to the test substance (age, mechanical damage related to high noise exposure, dosing procedure, etc.). A stepwise examination of tissue samples is recommended. In such a stepwise examination, sections from the high dose group are first compared with those of the control group. It is good practice to include tissues from vehicle control injection cohorts, sham (untreated) controls, and drug treated cohorts in each study, especially in those that utilize intratympanic administration of test article.

If no neuropathological alterations are observed in samples from the high dose group, subsequent analysis of lower dose groups is not required. If alterations are observed in samples from the high dose group, samples from the intermediate and low dose groups are then examined sequentially. If treatment related effects are noted in any dose group an ototoxic risk should be assumed.

f. Description of the Process for Review of Pathology Data: Detailed descriptions and diagnostic criteria of pathology data should be standard operating procedure. Occasionally, a reviewing pathologist is asked to examine all of the pathology findings in a study. All information available to the reviewing pathologist should be made available to the peer review pathologist.

The report should include a narrative that provides an overview of the pathology findings from the study pathologist's perspective as well as other observations documented during the study. A discussion that includes qualitative description of lesions and that highlights differences among treated and control groups are an essential part of the interpretation and evaluation of ototoxicity data. Remarks about possible pathogenesis, strengthened by references to the scientific literature, could be an important part of the pathologists or Study Director's narrative. Significant events, such as incidences of stereotypic behaviors associated with vestibular and balance disorders, and the impact of such events on the study outcome should be discussed. Differences in the incidence of key histopathologic findings among groups should be discussed; if observed differences are not regarded as treatment-related, then the basis for this conclusion should be provided.

It should be remembered that ototoxic events never occur in isolation. There is ample evidence that cytotoxic drugs may also have angiotoxic effects in the same tissue.[31] Affected damaged capillaries may collapse upon themselves and give the appearance of an intravascular strand linking two normal-appearing vessels, or it may disappear completely, and leaving an avascular channel (AVC). The AVCs are also found in the spiral ligament and in certain other tissues of normal guinea pigs, presumably as the result of the same process of gradual devascularization that occurs in human labyrinthine tissues with aging. Hair cell loss is also accompanied by protrusion of the supporting cells into Nuel's space and the tunnel of Corti, resulting in a disturbed micro architecture of the organs of Corti and eventually in complete replacement of the sensory epithelium by a single layer of epithelial cells.[50-57]

g. Evaluation of data: The findings from the ototoxicity screening battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological findings (i.e. renal toxicity). The evaluation should include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any sensorineuronal or conductive hearing loss effects. The evaluation should include appropriate statistical analyses, for example, parametric tests for continuous data and nonparametric tests for the remainder. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze data. Statistical analysis comparing total cellular counts of treatment *vs*. control animals at each turn of the cochlea or distance from apex or tympanum must be made and supplied. The report must include pre *vs.* post exposure ABR threshold evaluations (i.e., Wave 1 thresholds) of intact animals, cytocochleograms of a minimum of 40% of cochlea distance, with respect to basal end of the membrane, and any gross necropsy findings and lesions observed.

CONCLUSION

Far too many drugs are being approved based on risk assessment

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platforms that do not include the requirements of processing and evaluation of structures associated with the VIIIth cranial nerve. Drug-induced hearing loss, tinnitus, or balance disorders pose a significant impact on the quality of life and dose compliance of those prescribed a drug by their physician with the assumption that the drug is safe. Preclinical auditory safety assessments are not part of the core battery of regulatory-required tests conducted during product development and approval. We have proposed a systems-approach and protocol criteria for the valid and reliable assessment of auditory function that can be easily included as a screening tool for ototoxicity within the Tiered Structure of preclinical assays required for approval of any chemical entity targeted for human use.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

All contributors assisted in literature review, consolidation and preparation of the manuscript.

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Case Report

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Bilateral Internal Jugular Vein Thrombosis: An Unusual Presentation of Lemierre Syndrome

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ABSTRACT

Background: Lemierre syndrome is a rare disease characterized by internal jugular vein thrombosis and septic emboli. These symptoms typically develop after acute oropharyngeal infection by *Fusobacterium necrophorum*.
Case Report: We present an unusual case of 54 years old lady suffering from Lemierre syndrome with bilateral internal jugular vein and carotid artery thrombosis with fatal outcome.
Conclusion: Lemierre syndrome is a rare disease. Thrombosis of bilateral Internal Jugular Vein associated with Lemierre syndrome has never been reported.

KEYWORDS: Bilateral internal jugular vein thrombosis; Fusobacterium necrophorum; Lemierre Syndrome.

INTRODUCTION

Lemierre syndrome is acute anaerobic oropharyngeal infection with secondary septic emboli causing internal jugular vein thrombophlebitis and other metastatic infections. Septic emboli may also cause infections in other organs like lungs, bones, joints and brain. The most commonly isolated pathogen is Fusobacterium necrophorum.[1] Lemierre syndrome is often described as the "forgotten disease".[2] An uncommon disease, its mortality rates are still high.

CASE REPORT

A 54 years old obese woman presented to Otolaryngology department with complaints of dysphagia and progressive swelling in neck since two months but had deteriorated in past 15 days. There was no known comorbidity. The patient had taken treatment at another hospital. On presentation, the patient was a febrile, oriented and vitals were stable. There was a diffuse swelling around neck. Video laryngoscopy revealed a swelling in posterior pharyngeal wall with normal larynx. Magnetic resonance imaging done in previous hospital revealed a retropharyngeal abscess (Figure 1) following which incision and drainage of posterior pharyngeal wall was done in emergency operation theatre. Drainage revealed no pus and patient was admitted in intensive care unit. Haematological profile revealed Total Leukocyte Count of 18400/mm^3 with predominant polymorphs and serum creatinine of 2.4 mg%. Ultrasound abdomen showed bilateral hydronephrosis with small left uretric calculus. Radiograph chest showed bilateral pleural effusion, right more than left with mediastinal widening (Figure 2). Serum cortisol was 715 IU/L ruling out Cushing Syndrome. Contrast enhanced computed tomography neck showed soft tissue edema extending from scalp to neck with bilateral Internal Jugular Vein (IJV) thrombosis (Figure 3). Throat swab and blood culture did not reveal any pathogens. She was started on broad spectrum intravenous antibiotics consisting of Levofloxacin, Teicoplannin and Clindamycin. Low molecular weight heparin was added to prevent further thromboembolism. Cardiothoracic unit consultation denied for any surgical intervention as ligation of bilateral IJV will lead to cerebral edema. Next day she developed dyspnoea and had to be intubated. The swelling of the neck increased progressively and inspite of adequate anaerobic antibiotic cover patient succumbed to her illness after five days.

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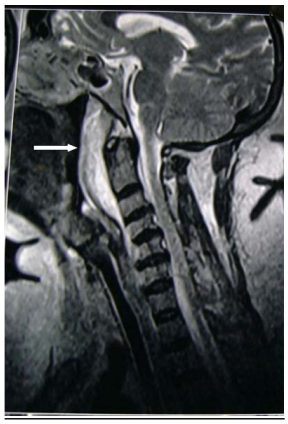

Figure 1: Retropharyngeal abscess in MRI neck (white arrow).

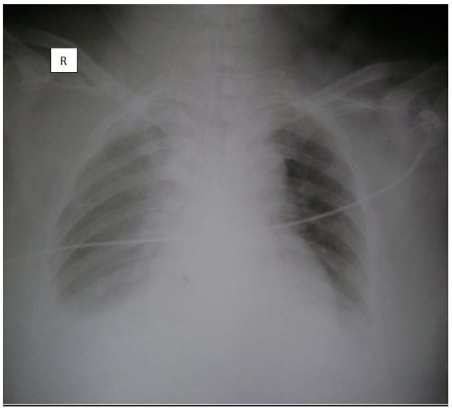

Figure 2: Radiograph showing bilateral pleural effusion.

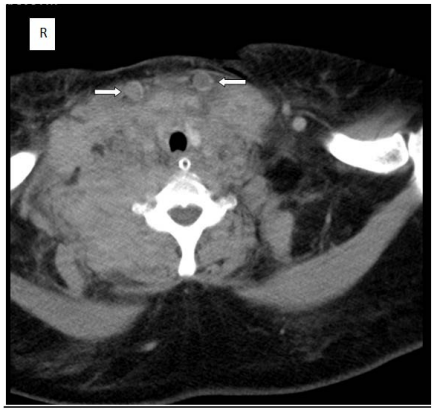

Figure 3: CECT neck showing bilateral internal jugular vein thrombosis (white arrow indicating bilateral IJV).

DISCUSSION

Courmant and Cade in 1900 were first to describe the correlation between oropharyngeal infection and sepsis.[3] Lemierre in 1936 published a series of 20 cases, 18 of whom died.[4] Karkos PD et al conducted a systematic review of 84 research articles. The median age of the patients was 22 years. Most of the patients were young non immune compromised individuals who presented with sore throat, neck mass or neck pain.[5] The oropharyngeal infection ranged from mild to fulminant in severity. The septic emboli from oropharyngeal infection caused ipsilateral internal jugular vein thrombosis and infection of the lungs. Fusobacterium is unique among non spore forming anaerobes, first of its virulence and association with Lemierre's syndrome as a monomicrobial infection and second because it seems probable that it is an exogenously acquired infection.[6] In addition to Fusobacterium, various anaerobes isolated from blood include Streptococcus, Bacteroides and Peptostreptococcus.[7]

The septic emboli could be thrown by infection from other extra pharyngeal sites like middle ear, female urogenital tract and gastrointestinal tract. The septic emboli have been identified in organs such as bone, meninges, abdominal viscera, peritoneum and soft tissue.[8]

X-Ray Chest was the first investigation asked in 92% of cases. IJV thrombosis has been diagnosed with Ultrasonography. PP Suthar et al have recommended Colour Doppler as the preferred modality to diagnose IJV thrombosis.[9] Broad spectrum antibiotic therapy with potent anaerobic activity for e.g. either clindamycin or a combination of a beta-lactam and metronidazole is the cornerstone of management. Improvement with symptoms, resolution of leucocytosis and falling inflammatory indices are signs of response to treatment. The treatment is continued for 2-6 weeks. The intravenous antibiotics may be changed to oral based on response. Following regression of symptoms, patients can be kept over oral clindamycin therapy for 04 weeks.[10] Availability of appropriate antibiotics has significantly reduced the mortality rate to 17 %.[11]

The use of anticoagulants for septic emboli is debatable. Lustig et al reported similar outcome irrespective of whether anticoagulants were used or not.[12] Some have recommended anticoagulation cavernous sinus thrombosis only. While the use of anticoagulants seems logical, there are no randomised controlled trials to support their use.[11] In this case, patient had thrombosis of both internal jugular veins and the common carotid arteries. The patient was not responding to the antibiotics. Hence, low molecular weight heparin was used in standard doses.

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CONCLUSION

Most cases of Lemierre syndrome have been described in young males with ipsilateral Internal Jugular Vein thrombosis. Present case was a middle aged woman who had retropharyngeal abscess followed by bilateral internal jugular vein thrombosis which has never been reported in literature. The prognosis in bilateral IJV thrombosis remains poor.

CONFLICTS OF INTEREST: None.

CONSENT

Authors obtain written informed consent from the patient for submission of this manuscript for publication

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Case Report

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The First Case of Endoscopic Transnasal Removal of an Ectopic Molar Tooth from the Pterygomaxillary Fossa: A Low Morbidity Approach

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ABSTRACT

Ectopic teeth erupted in the maxillary sinus (MS) or Pterygomaxillary Fossa (PF) are rarely reported. Though often asymptomatic, patients with ectopic teeth in the MS or PF may suffer from facial pain/numbness, purulent nasal discharge, facial edema, epiphora, and haemoptysis. Caldwell-Luc procedure is traditionally performed to remove ectopic teeth from the sinus, though several side effects and complications have been reported. The maxillary facial pain and numbness following such procedure can be extremely bothersome. This paper reports the case of a young woman suffering from maxillary facial pain and swelling due to an ectopic molar tooth in the PF and related maxillary sinusitis. Tooth removal and MS cleaning were carried out through a transnasal endoscopic approach. The postoperative course was uneventful. The patient did not complain any facial pain or numbness. We conclude that transnasal endoscopy is a painless and easy approach for the removal of ectopic teeth from the PF thanks to the low morbidity of intranasal antrotomy and advantages of endoscopic vision.

KEYWORDS: Endoscopic extraction; Transnasal removal; Ectopic tooth; Pterygomaxillary fossa.

INTRODUCTION

Ectopic teeth erupted in the maxillary sinus (MS) or pterygomaxillary fossa (PF) are rarely reported.[1-4] The causes of eruption of a tooth into the maxillary sinus are still unclear. However, some clinical conditions are suspected to be involved, such as developmental disturbances (cleft palate), displacement of teeth by trauma, interventions or cyst, infection, genetic factors, crowding, and dense bone.[1-3]

Caldwell-Luc approach, traditionally performed to remove foreign bodies and ectopic teeth from the sinus, may have several side effects and complications (facial pain, teeth sensory impairment or injury, cheek edema, infraorbital nerve numbness and neuralgias, maxillary hematoma/sinusitis, maxillary wall weakness, etc).[4,5]

In this paper, we report the case of a young woman suffering from maxillary facial pain and swelling due to an ectopic molar tooth in the PF and related maxillary sinusitis. Tooth removal and MS cleaning were carried out through a transnasal endoscopic approach, thus preventing the side effects of Caldwell-Luc "external" approach. To our knowledge, this is the first case of endoscopic extraction of an ectopic molar from the PF in the literature. The surgical details and advantages of our approach are discussed, together with the etiopathogenesis of our findings.

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CLINICAL REPORT

A 23-year-old woman was referred by her dentist to the Department of Otolaryngology–Head & Neck Surgery of "Carlo Poma" Civil Hospital of Mantova (Italy) for the management of an ectopic molar in her left PF. She suffered from recurrent episodes of left maxillary pain and swelling. Her medical history was not significant. She denied any previous dental/maxillary trauma.

Panoramic radiograph and CT scans of the maxillofacial region showed an ectopic molar tooth occupying the left PF and MS posterior portion, together with a complete erosion of the maxillary sinus posterior wall. A follicular cyst surrounded the tooth (Figures 1 and 2).

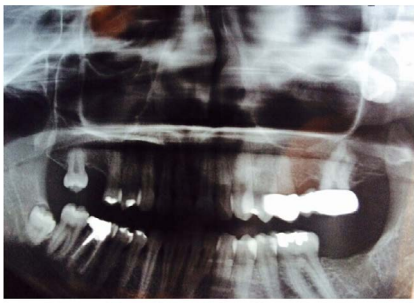

Figure 1: Ortopantomography showing the ectopic molar tooth in the left pterygomaxillary fossa.

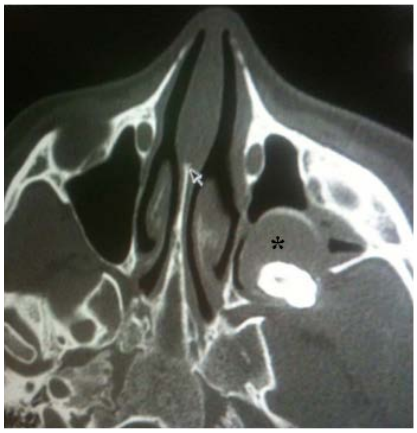

Figure 2: Axial CT scan, showing the ectopic molar tooth occupying the left posterior maxillary sinus and pterygo-maxillary fossa. Notice the erosion of the maxillary sinus posterior wall likely caused by the tooth-related follicular cyst (*).

A decision was taken, together with the patient, to endoscopically remove the tooth through a transnasal approach, in order to prevent the facial pain/numbness and teeth sensory impairment secondary to Caldwell-Luc approach.

Under general anesthesia with orotracheal intubation, the ectopic tooth was removed by transnasal endoscopic sinus surgery without any bony window through the canine fossa: complete left uncinectomy and extended intranasal antrotomy were carried out with a 0° endoscope. The MS was examined with a 45° endoscope: a curved ostium seeker was used to confirm the erosion of MS posterior wall and identify the tooth, which extended from the posterior portion of the MS, through the sinus posterior wall, into the PF. The tooth crown was oriented antero-medially, while the root was placed postero-laterally. The ectopic molar was surrounded by soft connective tissue compatible with a follicular cyst. The tooth was delicately luxated anteriorly with a "frontal sinus type" curved hook. The cystic tissue connecting the tooth to the MS floor was cautiously detached with a hook and Weil forceps (Figure 3). The tooth was extracted from the maxillary sinus into the middle meatus through the antrotomy window and then removed through the nasal fossa (Figure 4). The specimen was then sent for histopathological examination, which turned out to be a dentigerous cyst.[2]

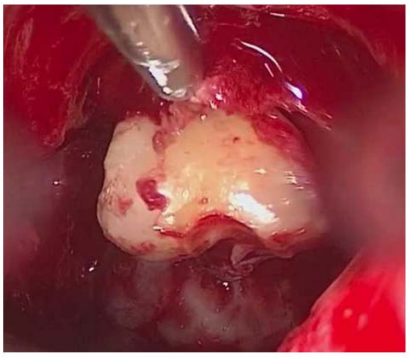

Figure 3. Vision of the left maxillary sinus through a medial antrotomy (70° endoscope): the ectopic tooth is detached from the surrounding follicular cyst and removed from the pterigomaxillary fossa.

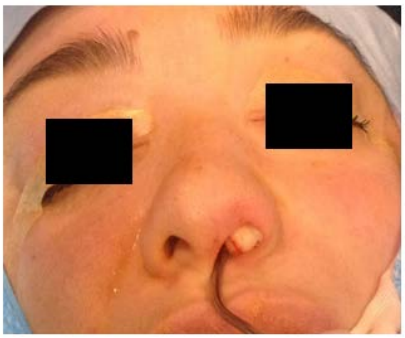

Figure 4: A blunt curved aspirator is used to extract the ectopic molar tooth through the left nasal fossa.

Preoperative antibiotic (amoxicillin/clavulanate 2.2 g iv) prophylaxis was administered. No nasal packing was placed. The postoperative course was uneventful. The patient did not complain any facial pain or numbness and no analgesic therapy was required. No sign of infection or fistula was observed. The patient has been on a regular follow up for more than a year with no evidence of recurrence.

DISCUSSION

The etiology of ectopic eruption of teeth in the MS remains unknown, though a role of trauma, infection, pathological conditions (tumors or dentigerous cysts), crowding, and developmental anomalies has been suggested. In particular, abnormal interactions between the oral epithelium and the

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underlying mesenchymal tissue during development may potentially result in ectopic tooth development and eruption.[1-4,6,7] Ectopic teeth in the MS may be permanent, deciduous, or supernumerary, with third molars being the most common ectopic dental elements in the MS.[6] As to ectopic molar tooth in the PF, to our knowledge, no other case has been reported so far in the literature.

In our case, no history of trauma, infection, or pathological condition was referred. Therefore, the etiology of the ectopic tooth was considered idiopathic. Basing on radiological and intra operative findings, we assumed the ectopic tooth initially developed in the posterior part of the MS and then migrated thorough the MS posterior wall into the PF. Such migration was likely enabled by the erosive/compressive effect of the tooth-related follicular cyst, which caused the interruption of the MS posterior wall and pushed the tooth into the PF. Our patient reported episodic left maxillary pain and swelling. In the literature, ectopic teeth in the MS have been associated with a variety of clinical manifestations, such as facial pain/headache, purulent nasal discharge, cheek edema/numbness, nasolacrimal duct obstruction/epiphora, and haemoptysis.[1-4,6,7] However, most patients are asymptomatic and ectopic teeth are discovered on routine dental radiographic examinations.[1-4] Further imaging techniques, such as maxillo-facial CT scan without contrast, are usually required to confirm the exact localization of the ectopic tooth and perform an appropriate treatment planning.[3,6,7]

Caldwell-Luc approach is the traditional procedure performed to attain direct view into the MS and remove ectopic teeth from the sinus.[3] In those cases, an extended (depending on tooth size) bony window of the MS anterior wall is removed, with consequent morbidity for the patient.[3,5,6] In particular, teeth sensory impairment or injury, cheek edema, infra orbital nerve numbness and neuralgias, maxillary hematoma/sinusitis, and maxillary wall weakness have been reported as possible side effects/complications of "external" Caldwell-Luc procedure.[3,5] Our decision to remove the tooth through a transnasal endoscopic approach was due to the request of the patient who asked to avoid any risk of postoperative facial pain/numbness and required a "minimally" invasive surgical approach. Endoscopic surgery is associated with lesser operative and postoperative morbidity that Caldwell-Luc approach.[3,5-7] Indeed, in our case we did not notice any complication of endoscopic surgery (i.e. orbital injury, CSF leak, loss of vision, diplopia, meningitis, nasolacrimal duct stenosis and epiphora) or oro-antral fistula.[6,7] In addition, intranasal antrotomy favors blood/mucous drainage from the maxillary sinus into the middle meatus and nose, which reduces the risks of local infections.[5] Finally, endoscopic magnification and "behind-the-corner" vision enables a relatively easy removal of the ectopic molar from the pterygo-maxillary fossa, which would be extremely more complicated in case of "external" approach.[6,7] In fact, while foreign bodies within the anterior part of the MS can be easily approached through a "minimally invasive" (i.e. tiny opening of MS anterior wall) Caldwell-Luc Procedure, an ectopic tooth in the PF would require a more extended opening through the MS anterior-lateral wall, which would increase the risk of postoperative facial pain/numbness and teeth sensory impairment. On the contrary, the endoscopic intranasal approach allows an optimal access to the PF and posterior MS with no risk of facial pain and teeth involvement.

CONCLUSIONS

Transnasal endoscopy is an easy and safe approach for the removal of ectopic teeth in the PF through MS thanks to the low morbidity of intranasal antrotomy and advantages of endoscopic vision. In particular, endoscopic approach should be considered in young patients who request a "minimally" invasive surgical approach and want to avoid the postoperative facial pain/numbness secondary to Caldwell-Luc procedure. An accurate imaging planning is mandatory for the correct selection of the surgical approach.

CONFLICTS OF INTEREST: None**.**

CONSENT

The authors obtain written informed consent from the patient for submission of this manuscript for publication.

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