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Original Article

The Effect of the Status of the Ossicular Chain and Choice of Graft Material on Hearing Outcomes in Pediatric Cholesteatoma Surgery

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OBJECTIVE: Compare hearing benefit of incus preservation in primary cholesteatoma surgery versus cartilage-myringostapediopexy.

METHODS: Prospective cohort study in a tertiary referral center. Tympanoplasty utilizing cartilage or other grafts, with or without intact incus was performed in 195 ears (187 children) with intact stapes. Outcome measures were pre and post-operative four-tone air conduction (AC) threshold (0.5, 1, 2, 4 kHz) and proportion with normal hearing (AC $\leq$ 30 dB HL) at 12 months.

RESULTS: Ears with intact ossicles had better post-operative AC thresholds than those with incus eroded or removed (median 20 dB HL vs. 30 dB HL, Mann–Whitney $P < .001$). The normal hearing rate was 81/106 (74%) with intact incus and 46/89 (52%) without (Fisher's exact $P = .001$). Ears without intact incus and a cartilage-myringostapediopexy had better post-operative thresholds than those with a non-cartilage graft (28.8 dB HL vs. 36.3 dB HL, Mann–Whitney $P = .005$). Of ears without intact incus, 37/59 (63%) with a cartilage-myringostapediopexy and 9/30 (30%) with a non-cartilage graft had normal hearing post-operatively (Fisher's exact $P = .007$). By preserving the incus in 12 ears, 1 more ear would have normal hearing than with incus removal plus cartilage-myringostapediopexy (NNT = 12 (CI 3.6-); Fisher's exact = 0.1).

CONCLUSION: Preserving an intact ossicular chain conveys a small but significant hearing benefit in cholesteatoma surgery, the magnitude of which should be considered before deciding to remove the intact incus. Cartilage-myringostapediopexy provides a significant gain in hearing when the incus is absent, even without a partial ossicular replacement prosthesis.

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KEYWORDS: Cholesteatoma, ossiculoplasty, hearing outcomes, otology outcomes, middle Ear

INTRODUCTION

The adage that the object of surgery for cholesteatoma is to attain a "safe, dry ear," sets the minimum standards by which surgeons may define an acceptable outcome. Whilst such a technical standard is suitable for the surgeon, the patient will place greater emphasis on symptom management.[1] The ideal aim would be permanent elimination of cholesteatoma with the restoration of the ear to a state of normality: a disease-free, self-cleaning, normal-looking ear with normal hearing. These ideals remain elusive for many patients and balance must be found between more radical surgery to ensure disease clearance and a more conservative approach with the intention of limiting post-operative symptoms. In those presenting with more extensive disease, some degree of hearing loss from ossicular erosion is almost inevitable and there is a higher risk of residual and recurrent disease.[2]

In order to reduce the risk of leaving residual cholesteatoma in the medial epitympanum removal of the body of incus and head of malleus is often performed, and to reduce the risk of recurrence, a cartilage graft is often used to reinforce the pars tensa.[3] The question then arises, when considering other objectives of surgery, is: what impact do these 2 interventions have on hearing? If removal of the incus from an intact ossicular chain causes a significantly large increase in hearing threshold, then perhaps the slightly increased morbidity of a trans-mastoid approach, rather than the simpler trans-canal approach to the medial epitympanum is justifiable if it allows removal of disease while preserving the intact chain.[4] When the incus is absent, cartilage myringostapediopexy can produce favorable hearing thresholds.[5,6] Marginal audiological differences (6.6 dB) in hearing thresholds between ears with an intact ossicular chain and those with myringostapedopexy (either cartilage cap or non-cartilage graft) following removal

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of the incus have been reported previously at a duration of less than 6 months post-operatively.[7] How then do audiological outcomes compare between an intact ossicular chain, and an ear where the incus is absent either with or without cartilage grafting? This study investigates these questions by comparing hearing outcomes after cholesteatoma surgery in a cohort of children using prospectively acquired data.

MATERIALS AND METHODS

Study Design

Ethical approval for this study was granted by the hospital's Research Ethics Board. Patients with cholesteatoma were identified from a prospectively collected consecutive dataset of a single surgeon in a tertiary center. Inclusion criteria were set to maximize focus on the study objectives and minimize the effect of other variables on hearing outcome. Ears that underwent primary surgery for congenital or acquired cholesteatoma in which the stapes superstructure was intact were included in the analysis. All surgeries were completed by an intact canal wall technique and included reconstruction or reinforcement of the tympanic membrane but no ossiculoplasty. Other surgical approaches were excluded (e.g., tympanotomy for removal of a small congenital cholesteatoma or ossicular reconstruction with an incus interposition). Using IOOG nomenclature surgeries can be categorized as S: 1, A: any, M: 1a/b ± 2a, E: any, O: any, A: any, T: 1-3, O: n/x/sd.[8] Ears in which a sensorineural hearing loss was identified pre-operatively, any of the ossicles were fixed, or the stapes superstructure was eroded were excluded. Sensorineural hearing loss was defined as a four-tone average bone conduction (BC) threshold of >25 dB HL. Ears were also excluded if pure tone audiometric data could not be obtained, for example, because of insufficient follow-up, developmental delay, or the young age of the child.

Ears were categorized initially by 1 of the 2 variables, the status of the incus intraoperatively (intact/disrupted) and the choice of graft material (cartilage/non-cartilage, typically temporalis fascia). Ears were then categorized by both variables to produce four groups; group A were those ears with an intact ossicular chain and a cartilage graft, group B those ears with an intact ossicular chain and a non-cartilage graft, group C those ears with a disrupted ossicular chain and a cartilage graft and group D ears with a disrupted ossicular chain and a non-cartilage graft. Ears with an intact ossicular chain, but with partial erosion of the long or short process of the incus were categorized as functionally intact ($n = 6$) and categorized into groups A or B.

Hearing outcomes were assessed pre-operatively and at 2 and 12 months post-operatively. Hearing tests were performed by audiologists in line with local guidelines. Air conduction (AC) thresholds were tested at 0.5, 1, 2 and 4 kHz and where >30 dB HL AC, BC at these frequencies were also tested. Word recognition score was not routinely performed. Normal hearing was defined as an AC threshold of ≤30 dB HL at 12 months.

Data was collated in Prism (GraphPad Software, Inc. San Diego, CA, USA) version 8.1c for statistical analysis. As the behavioral tolerance of children is not always conducive to full and reliable bone conduction testing, an a priori plan was prepared for incomplete data. Three different strategies were prepared and the results of each compared to give a measure of the reliability of the available data: (1) data from ears without complete 4-PTA BC threshold were excluded, (2) missing data replaced with assumption 4-PTA BC threshold = 0 dB HL as post-op AC threshold = 0 dB HL and remainder excluded, (3) remaining missing pre-op BC data replaced with best post-op BC data (if lower than pre-op AC threshold) and remainder excluded.

Mann-Whitney U test was used to compare non-parametric continuous variables (hearing threshold) between groups. Fisher's exact test was used to determine the statistical differences in proportions between the groups.

RESULTS

In this study, 488 ears underwent primary surgery for cholesteatoma between January 2003 and November 2019. One hundred ninety-five ears in 187 children met the criteria for inclusion. Sixty ears were in group A, 46 in group B, 59 in group C, and 30 in group D. The study population had a median age at the time of surgery of 11.0 years (range 3.0-17.9 years), group A 11.3 years (range 5.7-17.9 years) (Mann–Whitney $P = .44$), group B 9.6 years (range 3.0-16.4 years) (Mann–Whitney $P = .01$), group C 11.3 years (range 5.0-17.2 years) (Mann–Whitney $P = .69$) and group D 11.8 years (range 5.2-17.5 years) (Mann–Whitney $P = .17$). Surgery was performed on 77 (41%) female and 110 (59%) male patients (4 in each undergoing bilateral surgery). Groups A-D had a similar gender distribution (Fisher's exact $P = .69$).

Seventy (89%) ears in which the ossicular chain was found to be disrupted and 51 (48%) ears in which the ossicular chain was intact were found to have advanced disease (EAONO/JOS Stage ≥ 2) at the time of surgery. Seventy-seven (65%) ears in which a cartilage graft was placed (group A + C) and 44 (58%) ears in which a non-cartilage graft (group B + D) was placed had advanced disease (Fisher's exact $P = .44$). Fifty-nine (66%) ears in which the ossicular chain was disrupted and 60 (57%) with an intact ossicular chain received a cartilage graft (Fisher's exact $P = .19$).

Seventy-nine (41%) ears had complete pre-operative BC recorded at all 4 frequencies to give 4-PTA. In the remaining ears, different methods were used to estimate pre-operative BC thresholds using available data. Nineteen (10%) of these ears had post-operative 4-PTA air conduction thresholds of 0dB HL so their pre-operative 4-PTA BC was considered as 0 dB HL. In 83 (43%) ears, complete 4-PTA BC 12 months after surgery had thresholds lower than their pre-operative 4-PTA air conduction thresholds, so this was considered for use *in lieu* of pre-operative BC threshold. Fourteen (6%) ears were excluded as having no usable BC data. Median 4-PTA BC threshold in ears where it was recorded pre-operatively (7.5 dB) and those in which a post-operative 4-PTA BC was substituted for pre-operative data (6.3 dB) did not differ (Mann–Whitney $P = .87$).

Ossicular Chain Disruption and Hearing Outcomes

As shown in Table 1 AC thresholds were lower and ABG narrower both pre-operatively and post-operatively in ears with intact incus (group A + B) than those where the incus has been disrupted (group C + D). AC thresholds improved and ABG closure occurred in the latter but not the former (Figures 1a and 2a).

The proportion of ears with normal pre-operative hearing was 67% (71/106) in group A + B with intact ossicles and 36% (32/89) group

Table 1. Pre-operative and Post-operative Median AC Thresholds, ABG, and ABG Closure in All Groups Analyzed

	Number	Pre-Op AC	Post-Op AC	*P*1	Pre-Op ABG	Post-Op ABG	*P*2	ABG Closure
A + B (Ossicular chain intact)	106	23 (13.5) [2.5-58.8]	21 (11.6) [5.0-66.3]	.08	20 (13.1) [−10.0 to 62.5]	15 (12.1) [−3.8 to 66.3]	.12	0 (12.5) [−37.9to 27.5]
C + D (Ossicular chain disrupted)	89	35 (14.1) [6.25-71.25]	30 (12.4) [8.8-75.0]	.04	29 (14.3) [−11.3 to 55.0]	24 (12.4) [−6.3 to 67.5]	.03	4 (14.5) [−26.3 to 40.0]
*P*3		<.001	<.001		<.001	<.001		.33
A + C (Cartilage graft)	119	31 (13.6) [2.5-51.3]	28 (10.7) [8.8-56.3]	.00	24 (14.0) [−11.3 to 62.5]	19 (11.3) [−6.3 to 55.0]	.00	4 (13.4)[−30.0 to 40.0]
B + D (Non-cartilage graft)	76	27 (16.0) [6.3-65.0]	24 (15.2) [5.0-75.0]	.51	21 (14.6) [−10.0 to 55.0]	19 (14.3) [2.5 to 67.5]	.81	−1 (12.9)[−38.0 to 33.8]
*P*4		.07	.82		.17	.58		.02
A (Ossicular chain intact, cartilage graft)	60	24 (13.1) [2.5-58.8]	21 (10.9) [8.8-51.3]	.10	20 (13.1) [−3.8 to 62.5]	15 (11.6) [−3.8 to 55.0]	.16	0 (13.3) [−30.0 to 27.5]
B (Ossicular chain intact, non-cartilage graft)	46	22 (13.5) [6.25-57.5]	19 (12.5) [5.0-66.3]	.69	20 (13.1) [−10.0 to 51.3]	15 (12.8) [2.5-66.3]	.49	0 (16.1) [−38.0 to 22.5]
*P*5		.05	.10		.46	.83		.45
C (Ossicular chain disrupted, cartilage graft)	59	35 (13.1) [6.3-71.3]	29 (10.2) [8.8-56.3]	.00	30 (13.8) [−11.3 to 53.8]	23 (10.8) [−6.25 to 47.5]	.00	6 (13.4) [−22.5 to 40.0]
D (Ossicular chain disrupted, non-cartilage graft)	30	36 (16.1) [7.5-65.0]	36 (14.4) [13.8-75.0]	.89	26 (15.3) [3.8-55.0]	26 (14.2) [8.8-67.5]	.77	−3 (15.3) [−26.3 to 33.8]
*P*6		.85	.01		.67	.05		.03

Median (SD) [range].
*P*1 = Mann–Whitney between pre-operative and post-operative AC threshold.
*P*2 = Mann–Whitney between pre-operative and post-operative ABG.
*P*3 = Mann–Whitney between ears with ossicular chain intact (group A + B) and ossicular chain disruption (group C + D).
*P*4 = Mann–Whitney between ears with cartilage grafts (group A + C) and non-cartilage grafts (group B + D).
*P*5 = Mann–Whitney between group A and group B.
*P*6 = Mann–Whitney between group C and group D.

C + D with incus disruption (Fisher's exact $P < .001$). Of those with normal pre-operative hearing, hearing became abnormal after surgery in only 9 (13%) group A + B ears but 10 (31%) group C + D ears (Fisher's exact $P = .06$). Of those with an abnormal hearing before surgery 19 (54%) group A + B ears and 24 (42%) group C + D became normal following surgery (Fisher's exact $P = .29$). Improvement in the proportion of ears with normal hearing was not seen in group A + B (Fisher's exact $P = .09$) after surgery but was in group C + D (Fisher's exact $P = .05$). Eighty-one (76%) group A + B ears and 46 (52%) group C + D ears had normal hearing thresholds post-operatively (Fisher's exact $P < .001$). By preserving the incus in around 4 ears, 1 more ear would have normal hearing post-operatively (NNT = 4.2 (CI 2.6-8.6)).

Graft Material and Hearing Outcomes

As shown in Table 1 AC thresholds and ABG did not differ, pre-operatively or post-operatively, between ears in which a cartilage graft was placed (group A + C) and ears in which a non-cartilage graft was placed (group B + D). AC improved and ABG closure was achieved in group A + C but not in group B + D (Figures 1b and 2b).

The proportion of ears with normal pre-operative hearing was 47% (56/119) in group A + C with cartilage myringostapediopexy and 62% (47/76) group B + D with no cartilage graft (Fisher's exact $P = .06$). Of those with normal pre-operative hearing, hearing became abnormal after surgery in 10 (18%) group A + C ears and 9 (21%) group B + D ears (Fisher's exact $P = 1.00$). Of those with an abnormal hearing before surgery 33 (53%) group A + C ears and 10 (34%) group B + D became normal following surgery (Fisher's exact $P = .12$). The proportion of ears with normal hearing as a result of surgery in group A + C (Fisher's exact $P = .004$) improved but did not in group B + D (Fisher's exact $P = .74$). Seventy-nine (66%) group A + C ears and 48 (63%) group B + D ears had normal hearing thresholds post-operatively (Fisher's exact $P = .65$). In utilizing a cartilage graft in 31 ears, 1 more ear would have normal hearing post-operatively (NNT = 31 (−5.9 to 9.5)).

Impact of Graft Choice Upon Hearing Outcomes in Which the Ossicular Chain Is Intact

Ears in which the incus was intact and a cartilage graft placed (group A) had worse pre-operative median AC threshold than those with intact incus who received a non-cartilage graft (group B), but post-operatively no difference was present. ABG did not differ between group A and group B pre-operatively or post-operatively. There was no improvement in AC or ABG in either group following surgery (Table 1, Figures 1c and 2c). Thirty-three (55%) group A ears and 23 (50%) group B ears had an improvement in AC threshold following surgery (Figure 3a).

Thirty-seven (62%) group A ears and 34 (74%) group B ears had normal hearing threshold pre-operatively (Fisher's exact $P = .22$). Seven (19%) group A ears and 2 (7%) group B ears with normal hearing thresholds pre-operatively had abnormal hearing thresholds

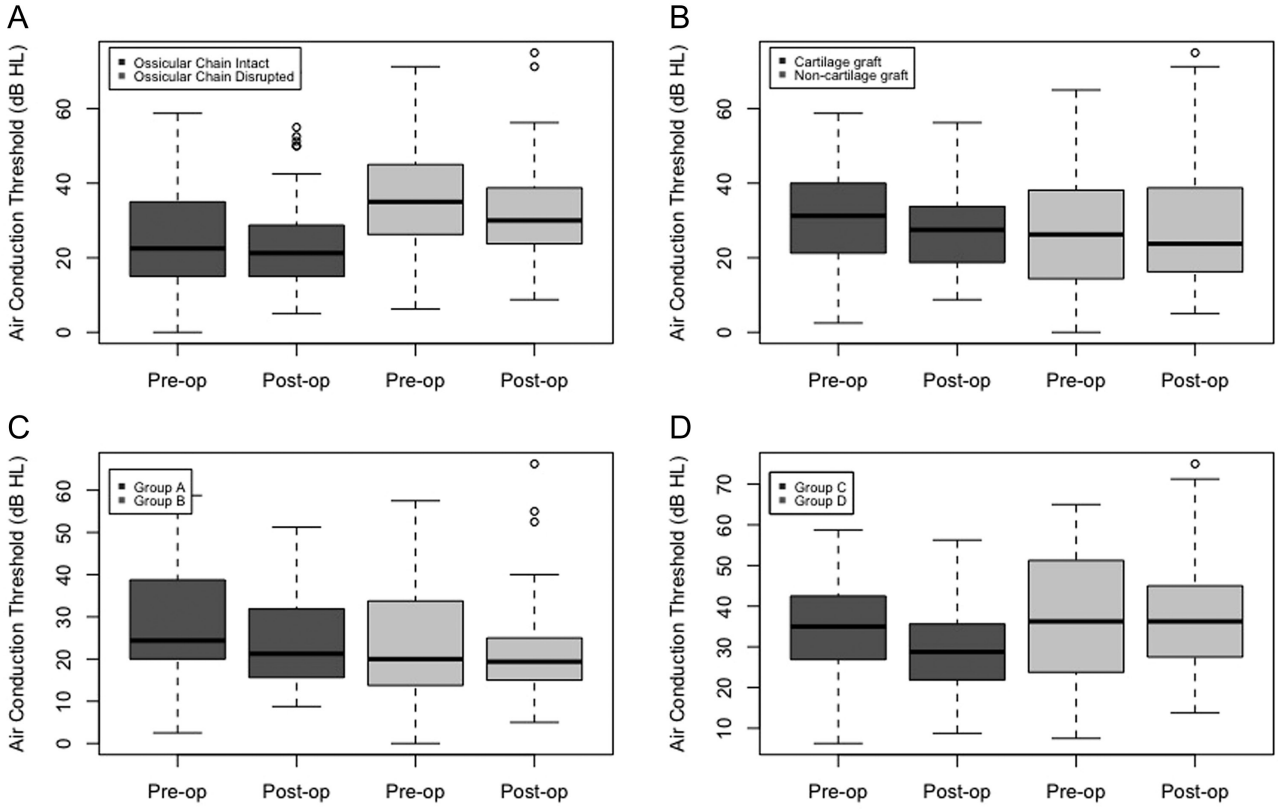

Figure 1. Boxplot showing pre-operative and post-operative median AC hearing threshold in ears with; (a) Intact or disrupted ossicular chain, (b) Cartilage or non-cartilage graft, (c) Group A and B (Incus intact), (d) Group C and D (Incus disrupted).

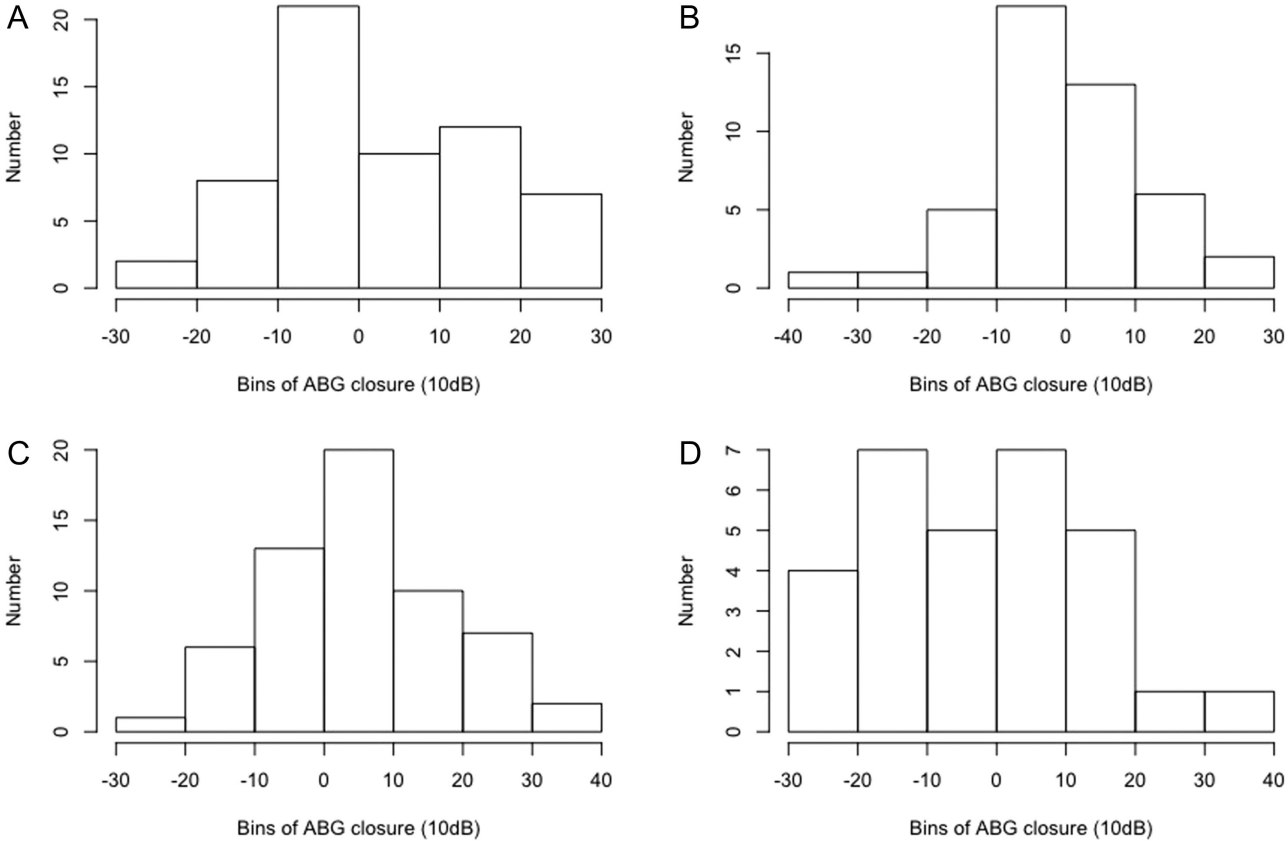

Figure 2. Histogram showing frequency of ABG closure in bins of 10 dB in (a) Group A (Incus intact and cartilage graft), (b) Group B (Incus and non-cartilage graft), (c) Group C (Incus disrupted and cartilage graft), (d) Group D (Incus disrupted and non-cartilage graft).

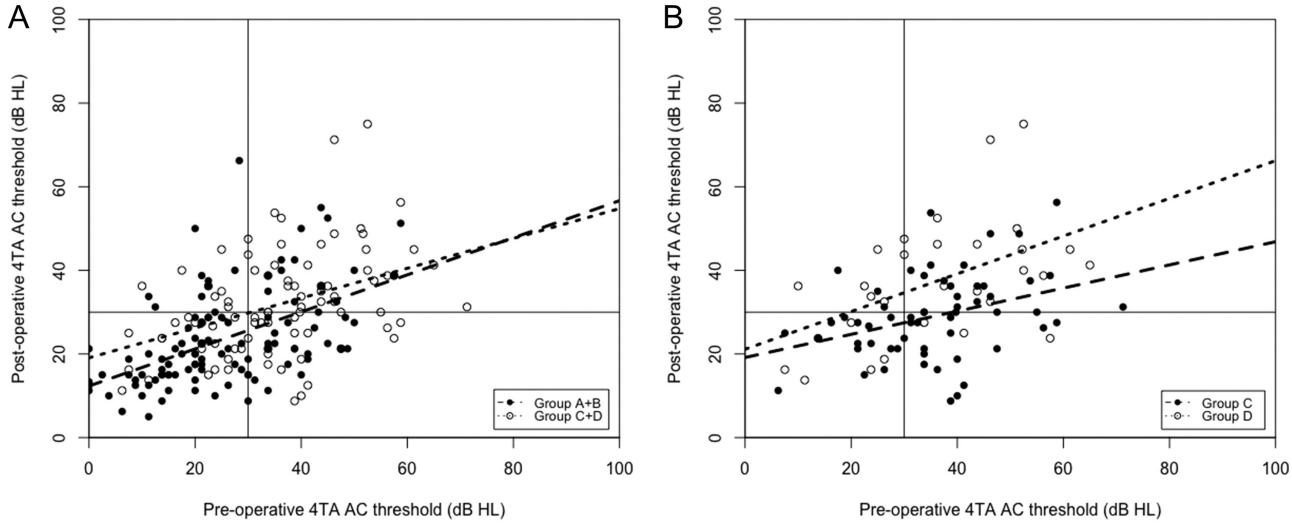

Figure 3. (a) Pre-operative and post-operative air conduction hearing thresholds in group A + B (Incus intact), and group C + D (Incus disrupted). Points to the left of the vertical line at 30dB have normal hearing pre-operatively and those below the horizontal line at 30dB have normal hearing post-operatively. (b) Pre-operative and post-operative air conduction hearing thresholds in group C (Incus disrupted and cartilage graft) and group D (Incus disrupted and non-cartilage graft). The latter having a worse pre-operative hearing. Points to the left of the vertical line at 30dB have normal hearing pre-operatively and those below the horizontal line at 30dB have normal hearing post-operatively.

following surgery (Fisher's exact $P = .17$). Twelve (52%) group A ears and 7 (58%) group B with abnormal hearing thresholds pre-operatively had normal thresholds following surgery (Fisher's exact $P = 1.00$). As shown in Table 2 group A ears were no more likely than those in group B to have normal hearing thresholds post-operatively (Figure 4).

Impact of Graft Choice Upon Hearing Outcomes in Which the Ossicular Chain Has Been Disrupted

As Table 1 shows, median AC thresholds pre-operatively do not differ between ears with disrupted incus which receive cartilage (group C) or non-cartilage graft (group D). Post-operatively those with a cartilage graft have a lower median AC threshold. Group C also shows a narrower ABG post-operatively. Accordingly, group C also shows improvement in median AC threshold and ABG closure while group D does not (Table 1, Figures 1d and 2d). Forty-two (71%) group C ears and 14 (47%) group D ears had an improvement in AC threshold following surgery (Figure 3b).

Nineteen (32%) group C ears and 13 (43%) group D ears had normal hearing threshold pre-operatively (Fisher's exact $P = .35$). Three (16%) group C ears and 7 (54%) group D ears with normal hearing thresholds pre-operatively had abnormal hearing thresholds following surgery (Fisher's exact $P = .04$). Twenty-one (53%) group C ears and 3 (18%) group D with abnormal hearing thresholds pre-operatively had normal thresholds following surgery (Fisher's exact $P = .02$). The frequency of normal hearing as a result of surgery improved in group C (Fisher's exact $P = .001$) but did not in group D (Fisher's exact $P = .42$). As shown in Table 2 ears in group C are more likely to have normal hearing thresholds following surgery than those in group D (Figure 4). In placing a cartilage graft in around 3 ears where the incus has been disrupted 1 more ear will have normal hearing post-operatively (NNT = 3.1 (CI 1.9-8.2)).

Table 2. 2 × 2 Table Showing Rates of Normal Hearing Following Surgery in All Groups

		Graft		Fisher's exact
		Cartilage	Non-Cartilage	
Ossicular chain	Intact	42/60 (70%)	39/46 (85%)	0.11
	Disrupted	37/59 (63%)	9/30 (30%)	0.007
	Fisher's exact	0.44	<0.001	

Comparison of Ears With an Intact Incus and Ears in Which a Cartilage Graft Has Been Placed Following Disruption of the Incus

Ears with intact incus (group A + B) had lower AC thresholds than ears with a cartilage-myringostapediopexy (group C) both pre-operatively (Mann–Whitney $P < .001$) and post-operatively (Mann–Whitney $P = .002$). ABG is also narrower in group A + B both pre-operatively

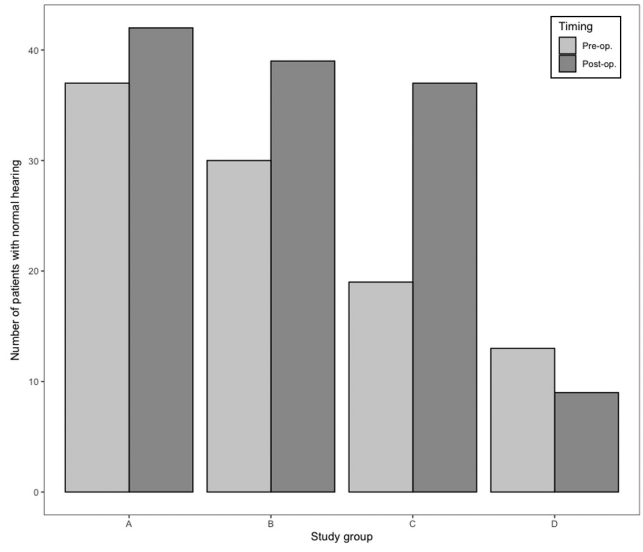

Figure 4. Frequency of normal hearing thresholds pre-operatively and post-operatively in groups A (Incus intact and cartilage graft), B (Incus and non-cartilage graft), C (Incus disrupted and cartilage graft), D (Incus disrupted and non-cartilage graft).

(Mann–Whitney $P < .001$) and post-operatively (Mann–Whitney $P < .001$) (Table 1).

Group A+B ears have a higher frequency of normal hearing pre-operatively than group C pre-operatively (Fisher's exact $P < .001$) but not post-operatively (Fisher's exact $P = .07$). In cholesteatoma surgery without cartilage for tympanic membrane reconstruction, preservation of the incus in around 2 ears would result in 1 more with normal hearing (NNT = 1.8 (CI 1.3-2.8). In contrast, when using cartilage for tympanic membrane reconstruction, the incus would have to be preserved in more than 12 ears, to gain 1 more ear with normal hearing than with cartilage-myringostapediopexy (NNT = 12.2 (CI 3.6-∞); Fisher's exact = 0.1).

DISCUSSION

The presence of an intact ossicular chain improves both the AC threshold and the probability of that threshold being within the normal range pre- and post-operatively. Ears with an intact ossicular chain had a median AC threshold gain post-operatively of 9 dB over those ears in which the incus had been disrupted. Ears in which the incus was disrupted and a cartilage graft placed had an improvement in their hearing following surgery of 6 dB but continue to have worse AC thresholds than those in which the ossicular chain is intact. Despite this, as previously reported at less than 6 months follow up, the use of a cartilage graft in ears with disrupted incus provides the same probability of normal hearing after surgery as if the ossicular chain were intact.[7]

The status of the ossicular chain and graft choice may only be determined at the time of surgery, however when consenting patients, they and their parents may wish to know what probability of normal hearing they have following surgery. Normal pre-operative AC thresholds provide a high (82% (84/103)) chance of normal post-operative hearing independent of the status of the incus. In this cohort, in which the stapes superstructure was always intact, hearing improved from abnormal to normal in 43/92 (46%) of ears, regardless of intra-operative findings or intervention.

Ears with more advanced cholesteatoma were found to have been more likely to have had the ossicular chain disrupted and to have had a cartilage graft. The former is perhaps unsurprising as the more advanced disease spread further throughout the middle ear and mastoid will have had either greater time or been more aggressive in causing damage to the middle ear. The increased prevalence of cartilage grafts in more advanced diseases is due to a surgical preference to use cartilage grafts in cases in which the incus is absent. The intention is to improve hearing and perhaps reduce the chances of recurrence through re-retraction of the tympanic membrane. As a result of this preference the number of children in group D (ossicular chain disrupted and a non-cartilage graft) is low, likely arising from earlier in the series when cartilage was used less often. We were able to determine the significance of the benefit of an intact ossicular chain to both AC threshold and rate of normal hearing. Cartilage grafts help the coupling of the ear drum to the stapes for transmission of acoustic energy when the incus is disrupted and appears to cause no significant disruption to hear when the incus is intact. The distribution of ears with the more advanced disease also provides challenges for our analysis of those ears in which the ossicular chain has been disrupted. We again see improvements in both hearing outcomes with a cartilage graft but none with non-cartilage grafts. The absence of difference in hearing outcomes after surgery between these 2 groups despite this improvement may be a result of small numbers in the latter group.

When the incus is not intact, interposition ossiculoplasty is often completed with a graft or prosthesis (PORP) placed between the stapes capitulum and tympanic membrane. Having previously achieved equivalent hearing thresholds with cartilage myringostapediopexy our practice has been to rely on this technique without interposition ossiculoplasty.[5] ABG closure in PORP has been reported elsewhere with values ranging between 8.5 dB and 14.2 dB.[9-12] Hearing outcomes have also been reported as a categorical "normal" using either a post-operative ABG ≤ 20 dB or as we have, post-operative AC threshold ≤ 30 dB HL. Meta-analysis of PORP using the former category showed 1627/2344 (69%) with a "good outcome."[13] This meta-analysis is not directly comparable with our population as it uses a different normative measure, includes adults as well as children, and follow up ranged from 1 month to 14 years. However, there is no difference in the rate of good outcome between this meta-analysis and our group C (Fisher's exact $P = .32$). AC ≤ 30 dB was utilized as a normal value in 22 ears who underwent PORP of which 68% reported normal hearing threshold at 12-18 months following surgery.[14] When these data are compared with group C, no significant difference is identified (Fisher's exact $P = .80$). We continue to think that the additional cost of PORP and the risk of extrusion do not justify its use over cartilage-myringostapediopexy for disrupted incus.

Surgeons should aim, where possible and without undue risk of residual disease within the epitympanum, to preserve the continuity of the ossicular chain as this will provide patients with the lowest AC threshold in that ear. When the incus has been eroded, or has to be removed, a cartilage-myringostapediopexy will provide patients with lower AC thresholds and a narrower ABG than if a non-cartilage graft is used. It is reassuring to note that when the incus does have to be removed, the use of a cartilage graft will give a similar chance of having a normal hearing threshold (i.e., AC 4-PTA <30 dB HL) as if the incus had remained intact.

Ethics Committee Approval: Ethics committee approval was granted.

Informed Consent: Informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.K., C.G.L., A.L.J.; Data Collection and/or Processing - C.G.L., E.K.; Analysis and/or Interpretation - C.G.L., E.K.; Literature Search - C.G.L.; Writing - E.K., C.G.L., A.L.J.

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