Intratumoral hemorrhage and fibrosis in vestibular schwannoma: a possible mechanism for hearing loss

Clinical article

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Object. Vestibular schwannomas (VSs) are benign lesions with an unpredictable natural history. Perhaps the greatest barrier to predicting which patients need treatment is our poor understanding of how these tumors cause hearing loss in the first place. In this case-control study, the authors investigated the relationship between preoperative hearing loss and histological changes such as intratumoral microhemorrhage and extensive fibrosis.

Methods. From a prospectively collected database, the authors selected all patients with VS who had undergone microsurgical resection as their initial treatment for histopathologically confirmed VS. Histological specimens obtained in 274 of these patients were systematically reviewed by a blinded neuropathologist who graded the extent of microhemorrhage and fibrosis in these tumors. The effect of these variables on preoperative hearing loss was studied using binary logistic regression.

Results. On univariate analysis, patients with extensive intratumoral microhemorrhage or fibrosis (p < 0.0001), patients with larger tumors (p < 0.05), and patients 65 years of age or older (p < 0.05) were significantly more likely to have unserviceable hearing at the time of surgery. On multivariate analysis, only patients with extensive intratumoral microhemorrhage or fibrosis had an increased risk of having unserviceable hearing at the time of surgery (OR 3.72, 95% CI 1.3–10; p = 0.01). Older age and tumor size greater than 3 cm were not statistically significant risk factors for hearing loss, controlling for the effect of microhemorrhage and fibrosis.

Conclusions. In this study, the authors have demonstrated a correlation between the extent of nonneoplastic histological changes, such as microhemorrhage and fibrosis, and hearing loss. This alternate hypothesis has the potential to explain many of the exceptions to previously described mechanisms of hearing loss in patients with VS. The advent of high-resolution MR imaging technology to identify microhemorrhages may provide a method to screen for patients with VS at risk for hearing loss. (DOI: 10.3171/2010.5.JNS10256)

KEY WORDS • vestibular schwannoma • acoustic neuroma • microhemorrhage • hearing • hearing loss

Abbreviations used in this paper: AAO-HNS = American Academy of Otolaryngology—Head and Neck Surgery; IAC = internal auditory canal; NF2 = neurofibromatosis Type 2; VS = vestibular schwannoma.

This article contains some figures that are displayed in color online but in black and white in the print edition.
Hearing loss in vestibular schwannoma

Hemorrhage seems to be a common event in these lesions and is detectable on T2*-weighted gradient recalled echo MR imaging. In the present case-control study, we investigate the relationship between preoperative hearing loss and nonneoplastic histological changes such as intratumoral microhemorrhage and extensive fibrosis.

Methods

Patient Population

Clinical, radiographic, and audiometric data for all patients evaluated and/or treated for a known or presumed VS by the senior authors (L.H.P. and A.T.P.) at our institution over a 25-year period (1984–2009) were prospectively collected in a database. In this database we identified all patients with relevant data who underwent microsurgical resection of VS. This study was conducted with the approval of the UCSF Committee on Human Research (approval no. H41995–32911–01).

Microsurgical Resection

Tumors were exposed using 1 of 3 standard microsurgical approaches to the IAC: the subtemporal-middle cranial fossa approach (46 patients), the translabyrinthine approach (149 patients), or the retrosigmoid approach (79 patients). After appropriate exposure of the tumor, and identification of the facial nerve using electrical stimulation, the tumor capsule was incised, and a piece of the tumor was removed and sent to pathology prior to altering the tumor tissue with any significant electrocautery or dissection. After obtaining the pathological specimens analyzed in this study, the rest of the tumor was then removed using standard microsurgical techniques.

Pathological Analysis

Hematoxylin and eosin–stained deparaffinized sections of tumors were systematically reviewed by an experienced neuropathologist who was blinded to all patient data. Patients who did not have a tumor specimen that filled at least 75% of the grid were considered to have inadequate tissue samples and these patients were excluded from the study. In addition to confirming the diagnosis of schwannoma, 3 nonadjacent slides (that is, those taken from different tumor blocks) per patient were systematically analyzed for the presence and extent of additional histological features within the tumor, including the extent of microhemorrhage, extensive fibrosis, degenerative changes, inflammatory infiltrates, mitoses, and the presence of bizarre cells. Examples of these changes are displayed in Fig. 1.

Semi quantitative assessment of the extent of hemorrhage and fibrosis was performed using an overlying 2 × 2–mm grid (Edmond Scientific), which divided the tissue into a 10 × 10 matrix with each square corresponding to 0.04 mm². It is not possible to obtain a representative image with this grid on one image; however, for conceptual purposes, we have provided an image (Fig. 2) taken with a smaller matrix grid, which has thicker dividing lines, to depict the concept. The entire image was reviewed systematically at a magnification of 20 to count the number of squares completely filled with hemorrhage or fibrosis, as well as the number of squares filled by tissue, and this was used to estimate the percentage area of the tumor composed of these changes. If necessary, fractional filling of a square with hemorrhage or fibrosis was counted as one-half or one-quarter of a square, depending on the portion filled. We did not count hemorrhages found on the edges of tumor given the high possibility these were due to surgical trauma, and we instead focused on the extent of hemorrhage found deeper within in the tumor. Because these data were a semiquantitative estimate of area, the variables were analyzed as a binary variable, with extensive hemorrhage defined as greater than 25% of the total area filled with hemorrhage, and extensive fibrosis defined as greater than 50% of the total area filled with fibrosis. Extensive fibrosis was defined by an estimate of total area of fibrotic tissue, and defined as being greater than 50% of the total tumor area. These cutoffs were determined empirically, by first sub-analyzing hearing outcomes in smaller groups, and setting the cutoffs based on the apparent natural division in the data. In the case of hemorrhage and fibrosis, the 25% and 50% cutoffs, respectively, seemed to be the point where hearing outcomes worsen (Fig. 3). In some analyses, we combined these 2 variables because we believed that they are most likely manifestations of the same process. Figure 4 depicts a frequent finding in these hemorrhages, which is the intermixing of fibrotic tissue and microhemorrhage, which we believe supports the premise that repeated microhemorrhages cause scarring in these tumors. It should be noted that extensive hemorrhage is an independent univariate predictor of hearing loss in our analysis, without being combined with extensive fibrosis.

Other pathological findings were less common than hemorrhage or fibrosis. Accordingly, the presence of any degenerative changes, inflammatory infiltrates, mitoses, or bizarre cells was the criteria for scoring these positive.

Data Analysis

Because it is impossible to know precisely the duration of tumor growth prior to diagnosis, the last audiogram obtained prior to surgery was used to determine the hearing status of these patients as a surrogate for the cumulative burden of hearing loss in the natural history. Audiometric testing consisted of bilateral pure-tone audiometry performed at octave frequencies from 250 to 8 kHz with a maximum intensity of 110 dB hearing loss, and speech audiometry measurements performed to a maximum intensity of 110 dB sound pressure level. For data analysis, pure-tone audiometry thresholds were recorded as the average of pure-tone hearing thresholds by air conduction at 0.5, 1, 2, and 3 kHz, and speech discrimination scores were recorded with their corresponding presentation levels. Auditory function was classified according to the AAO-HNS Committee on Hearing and Equilibrium guidelines. “Serviceable hearing” was defined as AAO-HNS Class A or B hearing, which corresponds to having both a pure-tone audiometry threshold of less than 50 dB and speech discrimination scores greater than 50%, as determined by audiometric assessment. Hearing outcomes for patients with bilateral tumors in the setting of
NF2 were analyzed for the tumor being resected, and no patient in this cohort underwent bilateral surgeries.

**Statistical Analysis**

Univariate analysis was used to identify covariates that might affect the rate of serviceable hearing loss in these patients. Binary and categorical variables were compared using Pearson’s chi-square test or the chi-square test for trend, respectively. Continuous variables were compared using an independent samples t-test after statistical demonstration of the normality of the data.

Variables that impacted hearing outcome that had a p value of 0.2 or less on univariate analysis were included in stepwise binary logistic regression modeling. Given that the transition between Classes B and C hearing in the AAO-HNS system denotes the level of hearing when a patient is usually unable to use the telephone with the affected ear,\(^1\) we analyzed hearing outcomes as a binary variable, with Classes C and D hearing being termed “non-serviceable” and Classes A and B hearing being termed “serviceable.” All odds ratios on multivariate analysis reflect the risk of having non-serviceable hearing at the time of surgery. The goodness of fit of the regression model was confirmed by demonstrating a nonsignificant probability value on the Hosmer-Lemeshow test.\(^2\)

We tested interaction terms between each of the 3 variables, which were found to significantly affect hearing on univariate analysis. The statistical significance of the interactions was assessed using backward stepwise regression, in which statistical significance was estimated by means of the likelihood-ratio test to assess the effect of removing interaction terms for all strata of the given variable.\(^1\) After finding that none of the interaction terms would significantly alter the log likelihood of the regression model if removed (unadjusted p > 0.2 for all terms),
we calculated the adjusted odds ratios without adjusting for interactions.

Continuous variables are presented as the mean ± SEM. Statistical tests were considered significant when the 2-sided \( p \) value was less than 0.05 after correcting for multiple comparisons using the Bonferroni method. All statistical tests were performed using SPSS version 17.

**Results**

**Patient Population**

Over the study period, there were 772 patients who underwent microsurgical resection in the entire database; accordingly, the study population of 274 patients represents slightly more than one-third of the total database. The 274 patients underwent microsurgical resection for VS and had an available, adequately sized histopathological specimen during the study period. The remaining patients were excluded due to the lack of multiple available H & E–stained sections (sometimes the tissue samples provided from surgery were very small, and, while adequate to establish a diagnosis, were too small to meaningfully systematically quantify internal intratumoral microhemorrhage, fibrosis, and other findings). Table 1 provides a comparison of data obtained in patients studied for the role of hemorrhage in hearing loss and the overall cohort in the database. Statistical comparisons demonstrated that the age and sex distributions did not differ between the groups, the initial tumor sizes were almost identical, and NF2 was neither over- nor underrepresented in this cohort. Although we cannot definitively rule out a selection bias, we also cannot find any obvious intergroup differences that could better explain these data.

Not surprisingly patients in the cohort undergoing resection were relatively young (50 ± 2.5 years at time of surgery). Of note, 77% of patients had serviceable hearing at the time of surgery. Sixty-two percent of patients had tumors greater than 2 cm in largest diameter, and 25% of patients had tumors greater than 3 cm.

**Frequency of Unusual Histological Features in VS**

Examples of our definition of varying degrees of intratumoral microhemorrhage and extensive fibrosis are shown in Fig. 1. Some degree of hemorrhage was present in most specimens; however, in most cases (77%) this hemorrhage comprised less than 25% of the total area of the image. In many of these cases, it was less than 10%. In other cases, this hemorrhage was more extensive and comprised over 50% of the slide (Fig. 1A).

Some intratumoral fibrosis was present in nearly all tumors; however, in 13% of tumors fibrosis was extensive and comprised a large portion of the slide (Fig. 1B). Given the known pathophysiological link between hemorrhage and subsequent fibrosis, we hypothesize that these regions of fibrosis represent areas of previous hemorrhage. This is supported by the frequent presence of regions of hemorrhage immediately adjacent to regions of extensive fibrosis. Often areas of hemorrhage contained areas of fibrosis interspersed within the hemorrhage (Fig. 4), again suggesting that old hemorrhage organizes into fibrotic tissue over time.

Other histological changes were noted in our systematic review of images at higher power (Table 2). Most
Fibrosis and Hearing Loss

Relationship Between Intratumoral Microhemorrhage or Fibrosis, Mitoses, and Hearing Loss

On univariate analysis, patients in whom extensive intratumoral microhemorrhage or fibrosis was documented on histopathology performed at the time of surgery were significantly more likely to have nonserviceable hearing than those without these degenerative changes (35% vs 17%, respectively; p < 0.0001) (Table 3). Additionally, tumor size (p < 0.05) and age exceeding 65 years (p < 0.05) predicted worse hearing outcomes on univariate analysis. Sex and NF2 status did not predict worse preoperative hearing status on univariate analysis (Table 3).

On multivariate analysis, only extensive intratumoral microhemorrhage or fibrosis remained a significant risk factor for having nonserviceable hearing at the time of surgery (OR 3.72, 95% CI 1.3–10, p = 0.01) (Table 4). Older age and tumors with greater than 20-mm extension into the cisternal space did not confer a statistically significant increase in risk of hearing loss.

Discussion

When left untreated, VSSs most commonly cause problems related to hearing loss in the ipsilateral ear. For some time, this has largely been assumed to result from tumor growth, with resultant compression of the cochlear nerve and/or the acoustic branch of the anterior inferior cerebellar artery. In this study, we have demonstrated a correlation between the extent of nonneoplastic histological changes, such as microhemorrhage and fibrosis, and hearing loss. While the present study is correlative, and does not exclude the possibility that slow tumor growth causes hearing loss in some patients with VS, it suggests that in many cases this process might result from intratumoral microhemorrhage, with a rapid increase in intracanalicular pressure in the IAC. This finding must be interpreted in light of the inherent selection bias of a study with surgery as a requisite entry criterion, as patients with more indolent tumors are probably less likely to experience hearing loss, probably less likely to receive an imaging study of the IAC, and probably less likely to undergo surgery if their tumors are discovered. Our hypothesis has the potential to explain many of the exceptions to the historical explanation of the mechanisms of hearing loss in VS, such as the occurrence of sudden hearing loss in patients with small tumors, as well as the poor correlation between tumor size and rates of hearing loss.

A number of studies have suggested that rapid tumor growth documented on serial MR images is a strong predictor of hearing loss in untreated VS. In light of these data, it is unclear, however, if rapid tumor growth on imaging studies results from an increase in the number of cells or, rather, from a series of microhemorrhages into the tumor, with subsequent scar formation. The well-documented ability of nerve tissue to accommodate relatively large mass lesions without symptoms when these changes occur slowly would suggest that the rapid increase in intracanalicular IAC pressure caused by intratumoral hemorrhage would be less well tolerated than the slower increase in pressure caused by growth of a benign neoplasm, which is often able to remodel the surrounding bone of the IAC. Alternately, sudden increases in intracanalicular pressure could lead to compressive compromise of the cochlear blood supply, which has been proposed as a possible mechanism by which progressive hearing loss occurs in these patients. Pressure within the IAC in patients with VS has been studied by a number of groups; it is elevated in a variable fraction of cases but can be normal in very large tumors.

We hypothesize...
Hearing loss in vestibular schwannoma

TABLE 3: Results of the univariate analyses pertaining to rates of preoperative hearing loss*

<table>
<thead>
<tr>
<th>Variable</th>
<th>AAO-HNS Class (% of patients)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>female</td>
<td>6</td>
<td>72</td>
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<tr>
<td>male</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>age &lt;65 yrs</td>
<td>6</td>
<td>74</td>
</tr>
<tr>
<td>age ≥65 yrs</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>non-NF2</td>
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<td>74</td>
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<tr>
<td>NF2</td>
<td>8</td>
<td>71</td>
</tr>
<tr>
<td>intracanalicular extension</td>
<td>7</td>
<td>79</td>
</tr>
<tr>
<td>&lt;10 mm in cistern</td>
<td>7</td>
<td>79</td>
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<tr>
<td>10–20 mm in cistern</td>
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<td>78</td>
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<tr>
<td>&gt;20 mm in cistern</td>
<td>5</td>
<td>64</td>
</tr>
<tr>
<td>≤25% hemorrhage</td>
<td>3</td>
<td>76</td>
</tr>
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<td>&gt;25% hemorrhage</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>≤25% &amp; minimal scarring</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>&gt;25% or extensive scarring</td>
<td>9</td>
<td>57</td>
</tr>
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<td>6</td>
<td>70</td>
</tr>
<tr>
<td>degenerative changes present</td>
<td>0</td>
<td>86</td>
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<tr>
<td>inflammatory infiltrates absent</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>inflammatory infiltrates present</td>
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<td>71</td>
</tr>
<tr>
<td>bizarre cells absent</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>bizarre cells present</td>
<td>9</td>
<td>73</td>
</tr>
</tbody>
</table>

*NS = not significant.

this variability might result from the presence or absence of intratumoral microhemorrhage, but further work is necessary to test this hypothesis. Notably, due to the relatively small number of events, we do not have enough patients to provide an internal statistical validation by dividing the cohort and internally test the predictive value of our regression model in one half of the cohort, as the data are not powered to detect a difference in a small cohort.

The natural history of VSs has proven difficult to accurately elaborate, largely because the tumor’s slowly progressing insidious course prevents observation of these tumors from their onset. For example, almost all patients in this study opted for treatment of their tumors shortly after diagnosis, preventing meaningful analysis of the preoperative clinical course of the untreated tumor in most patients. Furthermore, if episodic microhemorrhages are the cause of hearing loss in some patients, it is quite plausible that many of these microhemorrhages would have resolved by the time of resection. For this reason, we hypothesized that intratumoral fibrosis might represent chronic inflammatory changes due to a series of prior microhemorrhages and that tumors with extensive fibrosis might have been tumors that hemorrhaged in the past more often than other tumors.7,9,15

Conclusions

It is important to note that, the extent of an intratumoral microhemorrhage could potentially be quantifiable preoperatively by using existing imaging technology,32 making these findings immediately relevant to current practice. In this hypothetical management strategy, patients with VS with significant intratumoral hemorrhage at presentation could be identified as having an aggressive variant tumor and directed to earlier microsurgical or radiosurgical treatment, while patients with a less aggressive variant could potentially be closely observed, with less risk of hearing loss in the immediate future. Regardless of the exact pathophysiological link between these microhemorrhages and hearing loss, based on our results these histopathological findings seem to portend a more aggressive phenotype and a worse natural history. While more investigation is necessary to determine the wisdom of imaging-directed therapy for VS, it seems to be a goal worthy of further attention.

Disclosure

The authors report no conflict of interest concerning the mater-
ials or methods used in this study or the findings specified in this paper.

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Author contributions to the study and manuscript preparation include the following. Conception and design: Parsa, Sughrue, Pitts. Acquisition of data: Sughrue, Kaur, Yang, Pitts, Tihan. Analysis and interpretation of data: Sughrue, Kaur, Rutkowski, Yang. Critically revising the article: Parsa, Sughrue. Drafting the article: Par­


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