

# Proliferation activity and apoptosis in granulation tissue and cholesteatoma in middle ear reoperations

Marcin Durko<sup>1</sup>, Dariusz Kaczmarczyk<sup>2</sup>

<sup>1</sup>Otosurgery Department, Medical University, Łódź, Poland

<sup>2</sup>Department of Cytophysiology, Histology and Embryology, Medical University, Łódź, Poland

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*The aim of the study was to find out the potential prognostic value of proliferation activity and apoptosis in cholesteatoma and granulation tissue removed during middle ear reoperation in recurrent middle ear inflammation.*

*Granulation tissues and recurrent cholesteatoma were analysed after being surgically removed from the middle ear in a group of 25 patients qualified for middle ear reoperation procedure. Paraffin sections were stained with haematoxylin and eosin according to Mallory's method. Immunohistochemical reaction Anti-PCNA was performed. Apoptosis was evaluated using the TUNEL method. The percentage of PCNA-positive cells was 42–95% in the matrix of the cholesteatoma and 29–81% in the perimatrix. In the granulation tissue it was 35–75%. The percentage of apoptotic cells was 12–73% in the matrix of the cholesteatoma, 5–72% in the perimatrix and 1–65% in the granulation tissue. The prognostic value of the parameters studied in the recurrent middle ear inflammatory process is questionable, probably due to the small number of cases under examination.*

**Key words:** immunohistochemistry, chronic otitis media, PCNA

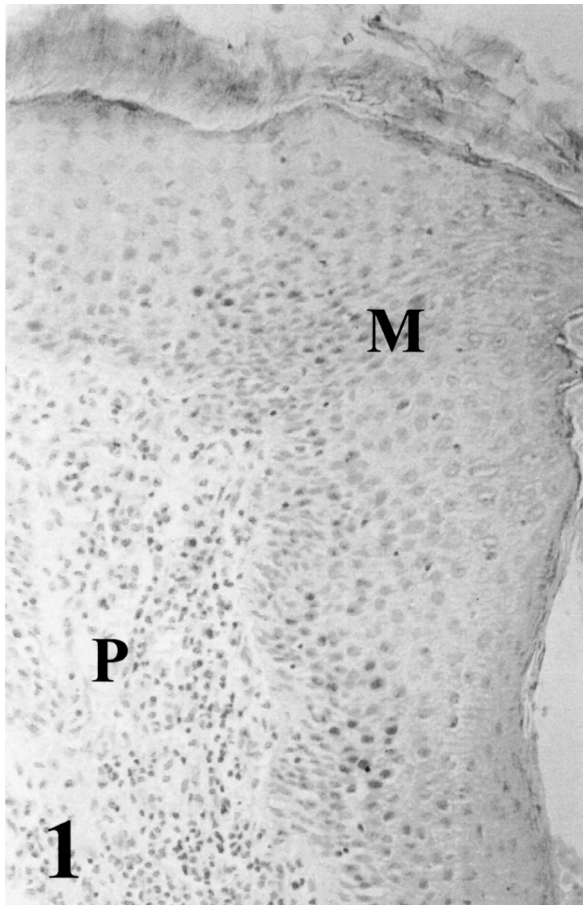
## INTRODUCTION

Recurrence of the inflammatory process following middle ear surgery is assessed as amounting to 5–33% of surgically treated cases. In most cases of recurrent inflammatory process in the middle ear the most frequent findings in the postoperative cavity are cholesteatoma and/or granulation tissue. The factors which contribute to failure in the surgical treatment of chronic otitis media appear to be the duration and aggressiveness of the inflammatory process and inadequate surgical technique. The aim of the study was to find out the potential prognostic value of proliferation activity and apoptosis in cholesteatoma and granulation tissue removed during middle ear reoperation in recurrent middle ear inflammation.

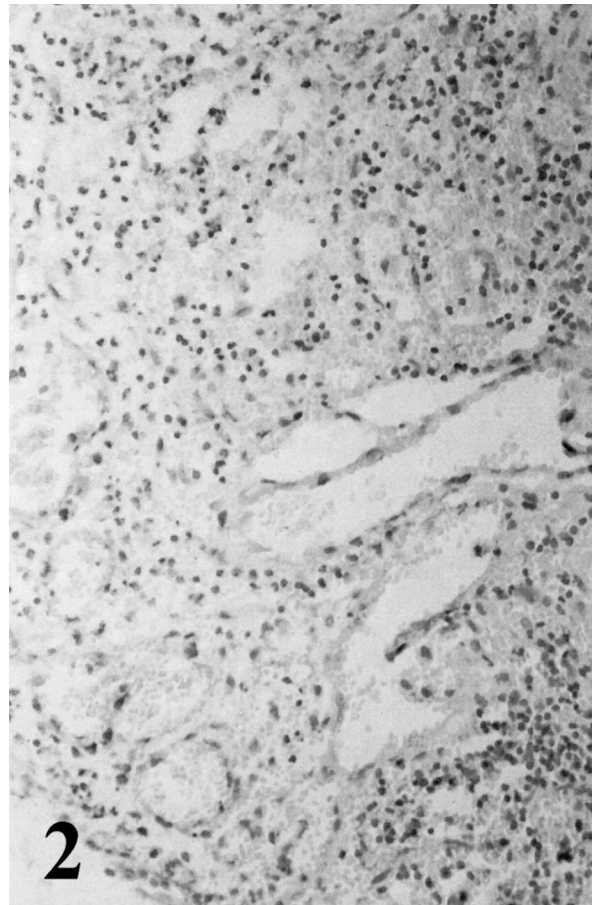
## MATERIAL AND METHODS

Granulation tissues and recurrent cholesteatoma were analysed after being surgically removed from the middle ear in a group of 25 patients qualified for reoperation procedure.

The group of patients studied was composed of 14 females and 11 males aged 25–71 years (mean: 43.6 years). The duration of the chronic otitis media symptoms (based on past otologic history) was in the range 4–52 years (mean: 22.7 years). The interval after the previous surgery ranged from 2 to 40 years (mean: 11.7 years). The number of previous otosurgical procedures on the ear before inclusion in the present study ranged from 1 to 4. Paraffin sections were stained with haematoxylin and eosin



**Figure 1.** PCNA, cholesteatoma; M — matrix, P — perimatrix. Enl. 150 ×.



**Figure 2.** PCNA, granulation tissue. Enl. 150 ×.

according to Mallory's method. For the immuno-histochemical reaction Anti-Proliferating Cell Nuclear Antigen-PCNA Clone PC10 (Dako) was used. Apoptosis evaluation was performed using the TUNEL method with *In Situ* Cell Death detection Kit, POD (Roche).

## RESULTS

Granulation tissue was observed in 14 patients (56%) and recurrent cholesteatoma in 11 cases (44%). In both granulation tissue and cholesteatoma positive PCNA cells were observed. The percentage of PCNA positive cells in the matrix of the cholesteatoma was 42–95%, while in the perimatrix of the cholesteatoma it was 29–81% (Fig. 1). A positive reaction for PCNA cells in granulation tissue was observed in 35–75% of the cells studied (Fig. 2).

Apoptotic cells in the matrix of the cholesteatoma varied from 12 to 73%, while in the perimatrix it was 5–72% (Fig. 3).

The percentage of apoptotic cells in the granulation tissue ranged from 1 to 65% (Fig. 4).

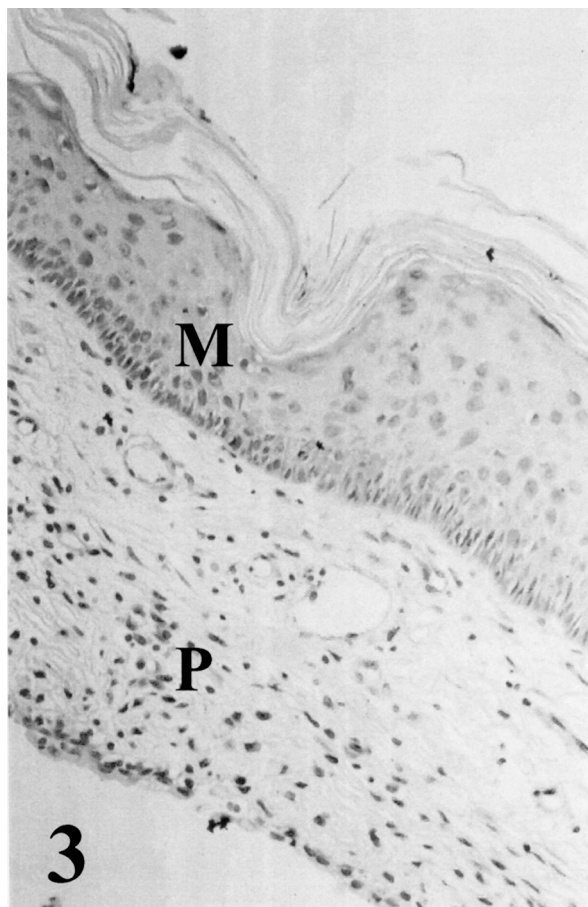
## DISCUSSION

The higher proliferation activity found in the matrix of the cholesteatoma confirms previous observations by other authors [1, 2, 5, 7]. It appears to be correlated with the natural proliferation activity of the cells in the parabasal layer. In comparison to the normal skin samples [5] proliferation activity in the epithelial layers analysed is significantly higher. The higher number of apoptotic cells in the matrix of the cholesteatoma may be explained by decreasing proliferating activity in a suprabasal direction starting from the parabasal epithelial layer and also by simultaneous cell ageing.

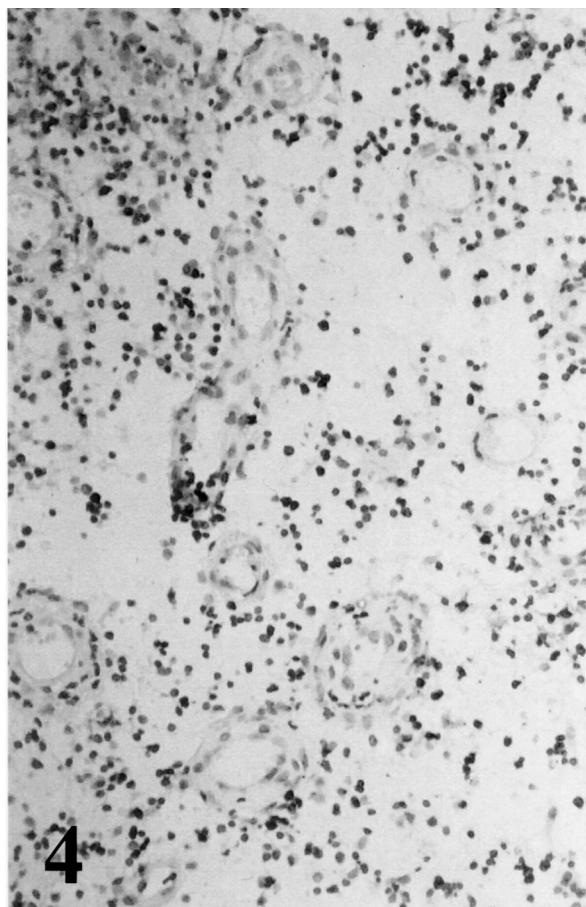
The similar values of the proliferation activity and apoptosis observed in the perimatrix of the cholesteatoma and granulation tissue may be due to the process of angiogenesis [3, 4, 6].

In our study no statistically significant differences were found between proliferation activity and apoptosis in cholesteatoma and granulation tissue.

The results obtained suggest that patient age and the duration of the inflammatory process may have no influence on proliferation activity and apoptosis



**Figure 3.** Apoptosis, cholesteatoma; M — matrix, P — perimatrix. Enl. 150 ×.



**Figure 4.** Apoptosis, granulation tissue. Enl. 150 ×.

in the middle ear qualified for reoperation. The prognostic value of the parameters studied in recurrent middle ear inflammatory process is questionable, probably due to a small number of cases studied.

### ACKNOWLEDGEMENTS

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