Biochemical and bioimaging Evidence of Cholesterol in Acquired Cholesteatoma

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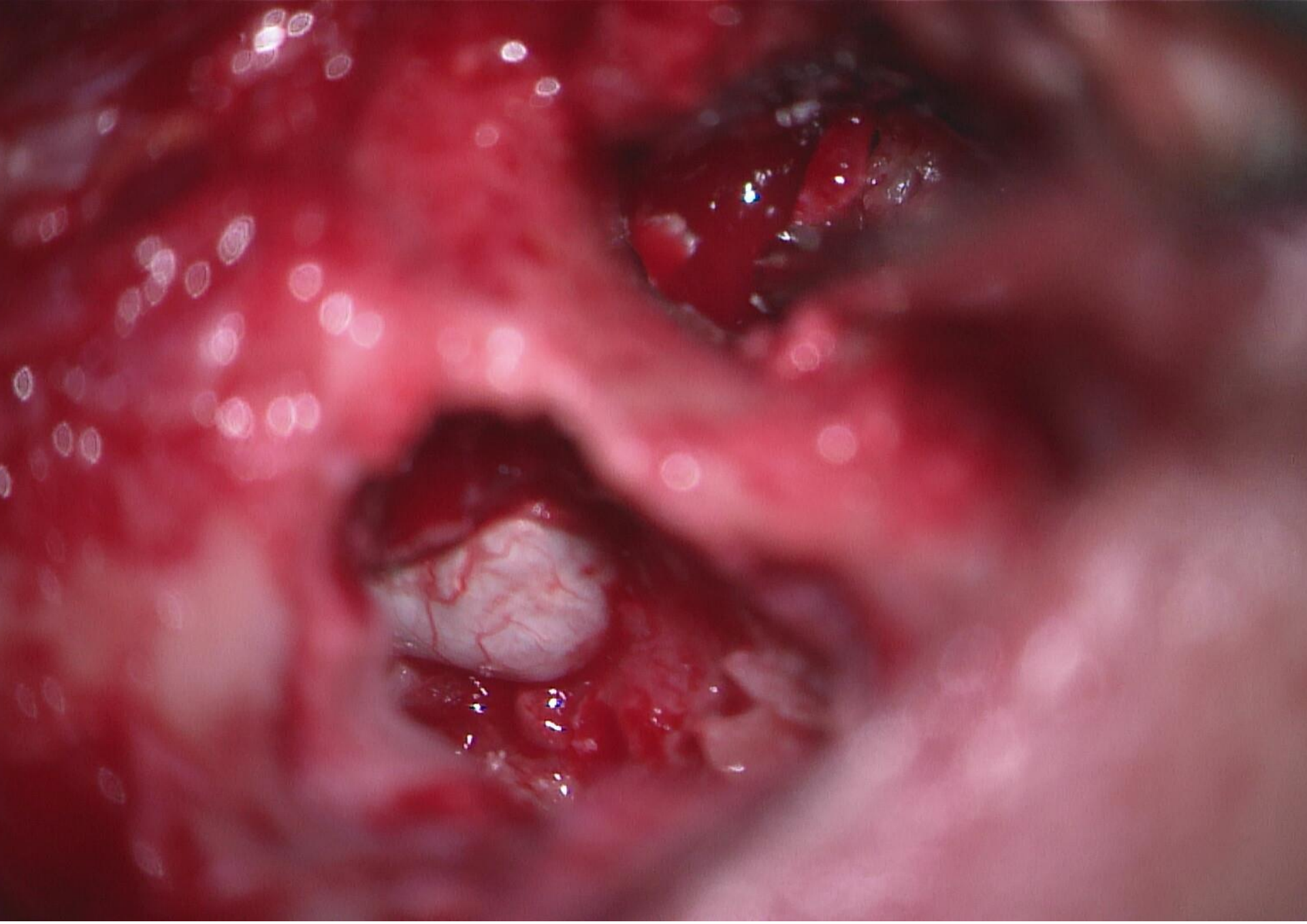


Right ear attic cholesteatoma, canal wall up (so far) mastoidectomy

Question

- Does Cholesteatoma contain cholesterol?

Purpose

To visualize and quantify the cholesterol amount in cholesteatoma and normal skin

- image the lipid structures in the matrix of acquired cholesteatoma
- quantify the barrier sterols
- compare the distribution with that found in stratum corneum from normal skin

Methods

- Peroperative collection of cholesteatoma tissue, as complete and intact as possible
- Peroperative collection of retroauricular skin or abdominal skin from plastic surgery

Methods

- High-performance thin-layer chromatography
  - Lipid extraction 90-100% (Bligh-Dyer protocol)
  - 30microg total lipid per sample
  - Standards for cholesterol, cholesterol Sulphate, cholesterol esther on each plate
  - 19 cholesteatomas, and 19 non-matching abdominal skin samples

Methods

- Coherent Anti-Stokes Raman scattering (CARS) microscopy
- Label-free imaging of specific molecules based on vibrational energy of the sample
- Real-time, noninvasive examination also on living tissue
- 3 cholesteatomas and 3 matching retroauricular skin biopsies

Ethics

- The study was approved by the Ethical Committee of Region Syddanmark
- Followed the Declaration of Helsinki

Results

Total lipid content

HPTLC: Cholesterols per total dried tissue weight

HPTLC: Cholesterols per total lipid weight

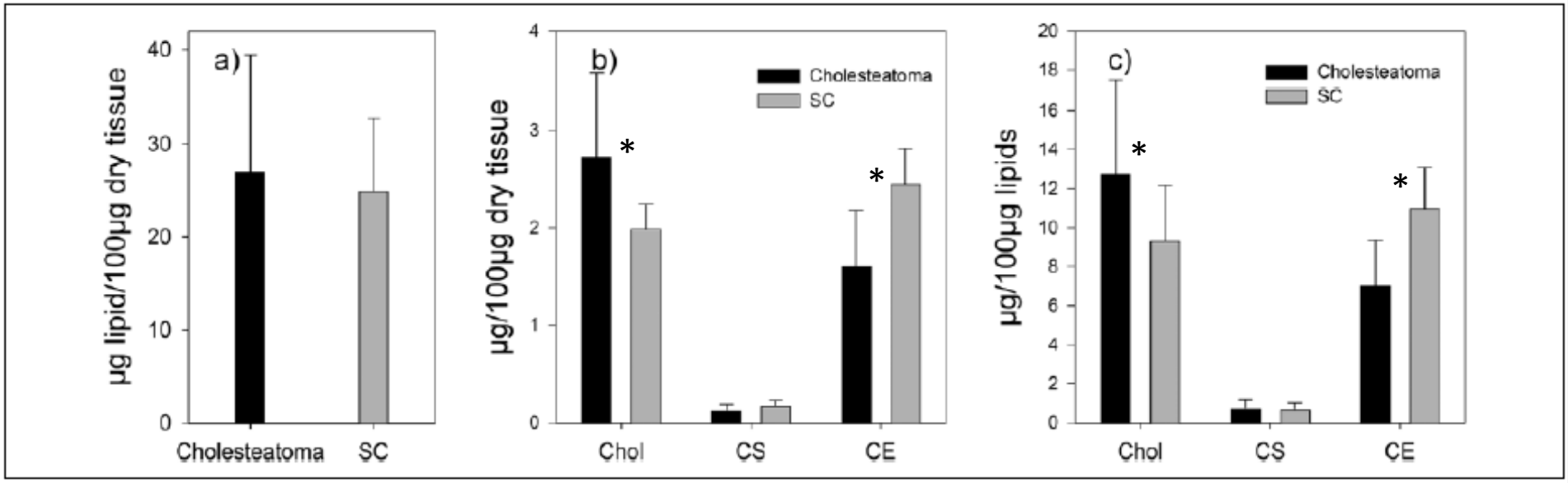
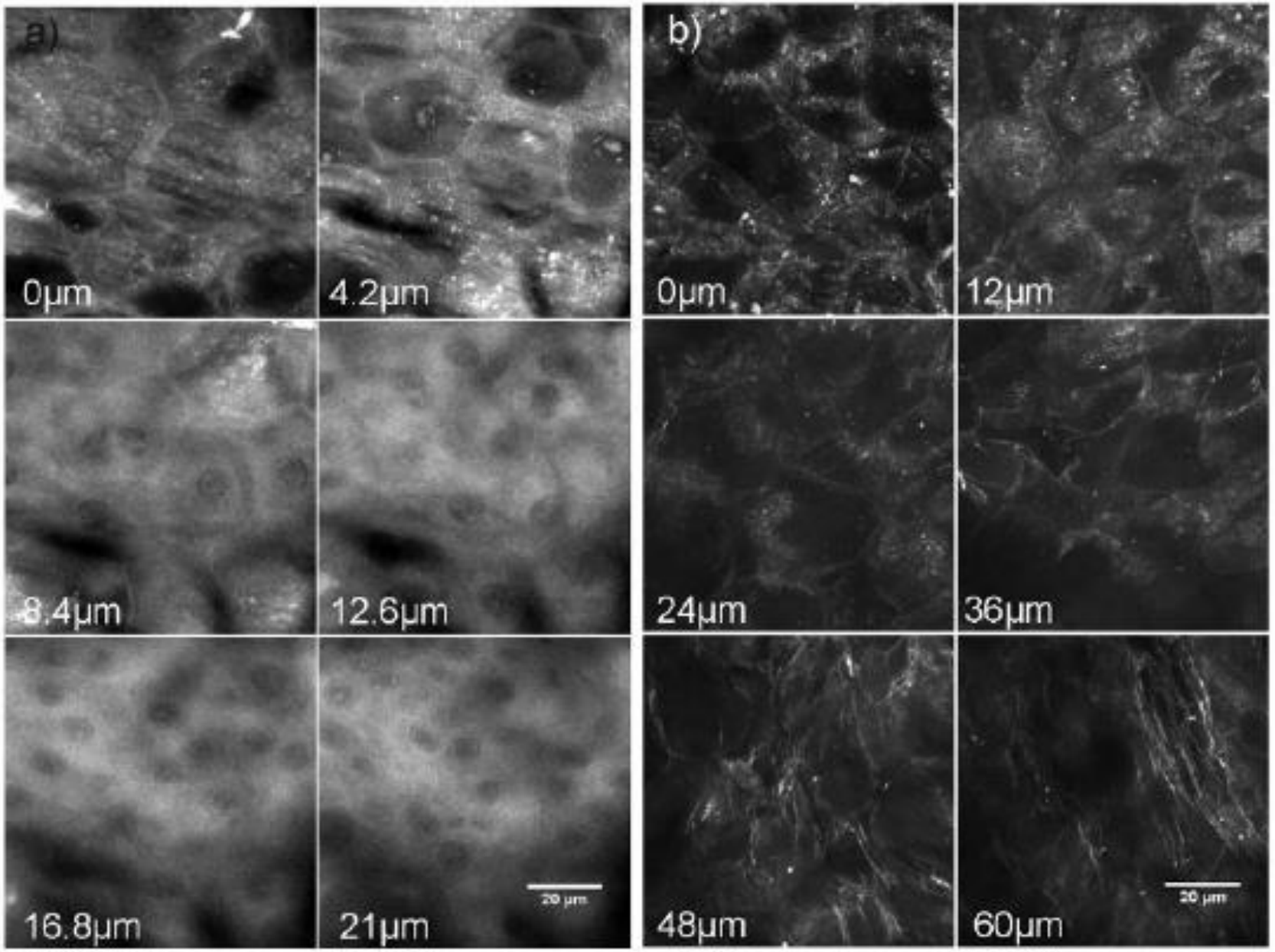


Figure 1. Summary of lipid analysis. Panel (a) shows the amount of lipid per 100 µg dry tissue for the stratum corneum (SC) and the cholesteatoma. There is no statistical difference between the control SC and the cholesteatoma. Panels (b) and (c) show the results of the quantitative high-performance thin-layer chromatography (HPTLC) measurements for cholesterol (Chol), cholesterol sulphate (CS), and cholesterol ester (CE). Panel (b) shows the quantities of cholesterols relative to the total dried tissue weight. Using an unpaired *t* test, there is a statistical difference between the amounts of cholesterol and cholesterol ester in the cholesteatoma and the control SC, $P = .0013$ and $P < .0001$, respectively. Panel (c) shows the quantities relative to the total lipid weight. There is a statistical difference between the amounts of cholesterol and cholesterol ester in the cholesteatoma and the control SC, $P = .012$ and $P < .0001$, respectively. There is no significant difference between the quantities of cholesterol sulphate. The data presented are the mean ± SD.

Normal skin

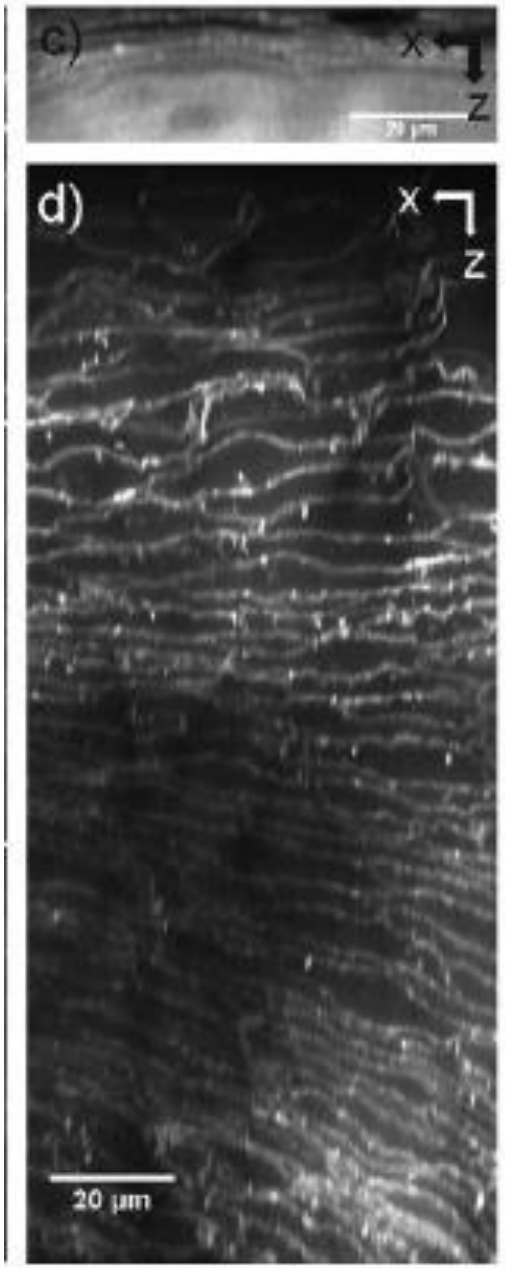
Cholesteatoma



CARS microscopy

c) Retroauricular skin
Stratum corneum 4 layers thick

d) Cholesteatoma
Many times thicker stratum corneum



Conclusion

- The main structure in cholesteatoma resembles very thick stratum corneum
- The cholesterol content is higher in cholesteatoma than in normal skin, both relative to the total dried tissue weight and to the total lipid weight

Perspective

- The name cholesteatoma is suitable
- Supportive of the theory ”skin in the wrong place”

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Abstract

Objectives: To quantify the barrier sterols and image the lipid structures in the matrix of acquired cholesteatoma and compare the distribution with that found in stratum corneum from normal skin, with the goal to resolve their potential influence on cholesteatoma growth.

Methods: High-performance thin-layer chromatography (HPTLC) was used to achieve a quantitative biochemical determination of the sterols. The intercellular lipids were visualized by Coherent Anti-Stokes Raman scattering (CARS) microscopy, which enables label-free imaging of the lipids in intact tissue samples.

Results: The results show that the total lipid content of the cholesteatoma matrix is similar to that of stratum corneum from skin and that the cholesteatoma matrix unquestionably contains cholesterol. The cholesterol content in the cholesteatoma matrix is increased by over 30% (w/w dry weight) compared to the control. The cholesterol sulfate content is below 1% of the total lipids in both the cholesteatoma and the control. Cholesterol ester was reduced by over 30% when compared to the control.

Conclusions: The content of cholesterol in the cholesteatoma matrix is significantly different from that in stratum corneum from skin, and we confirm that the main structure of the cholesteatoma resembles very thick stratum corneum.