

EXPERIMENTAL PLAN

A Detection of viral genome in extracted DNA

(i) DNA Preparation

5 micron frozen sections are cut from the biopsy specimens of OLP (n=10) healthy oral mucosa (n=10), leukoplakia (n=10) and chronic atrophic candidosis (n=10), taking precautions against cross-contamination, the relevant portion of the section micro-dissected and transferred to a sterile Eppendorf tube and the DNA extracted with a modified proteinase and phenol/chloroform technique, as detailed previously (Schifter et al, 1998).

(ii) Polymerase chain reaction (PCR)

HPV

The PCR for HPV has been established in the oral pathology laboratories, Westmead Hospital Centre for Oral Health and the conditions for HPV consensus and HPV 16 and 18 primers optimised. One microlitre (10 ng) of DNA is mixed with 1 μ M of each of the primers in a reaction mixture with final concentrations of 1 unit Taq polymerase, 200 μ m dNTPs, in 50 μ l of Taq buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 2 mM MgCl₂ and 200 mg/ml bovine serum albumin (BSA) using the following thermal cycles: Cycles 1-15, denaturation at 94°C for 30s, annealing 54°C for 30s, cycles 16-45, step 1 94°C for 30s, 54°C for 30s, polymerisation 72°C for 45s, cycle 46, 5°C for 10 min, stopped at 4°C. Ten microlitres are fractionated by electrophoresis in a 2% agarose gel containing ethidium bromide and the gels visualised under UV light.

Primers

Consensus primers GP5+ (5' TTT GTT ACT GTG GTA GAT AC)
 GP6+ (5' GAA AAA TAA ACT GTA AAT CA)

HPV16 E6 (320-339) ATT AGT GAG TAT AGA CAT TA

HPV16 E6 (410-429) GGC TTT TGA CAG TTA ATA CA

HPV18 E6 (102-125) ACT ATG GCG CGC TTT GAG GAT CCA

HPV18 E6 (417-436) GGT TTC TGG CAC CGC AGG CA

Controls

Positive	DNA extracted as above from Hela cells with integrated HPV-18 and Caski cells with integrated HPV-16
Negative	(i) Omission of primers, (ii) Taq polymerase, (iii) DNA from blood leucocytes from healthy volunteers

HSV 1 & 2

Primers (5' DNA polymerase) GCT CGA CTG CGA AAA AAC GTT C (primer) and CGG GGC GCT CGG CTA AC (primer)
GTA CAT CGG CGT CAT CTG CGG GGG CAA G-FLUOR (probe) and LC-Red 640-T CGC CAT CAA GGG CGT GGA TCT GGT GC-Phos (probe)

Thymidine Kinase GAC MAG CGC CCA GAT AAC AA (primer), MCA GCA TRG CCA GGT CAA GC (primer), AGG CGG TCG ATG TGT CTG TC (HSV-1 capture probe), and AGG CGG TCG GCG TGT TCG GC (HSV-2 capture probe)

CMV - primers 5' CGGTCACCAAGGAACGATGC
 CGCCAGACTGTGTGCTGAGT

B. *In situ PCR for viral DNA*

The tissue is fixed in 4% paraformaldehyde at room temperature, washed in PBS x 2 and stored at -70°C . Alternate 5 micron frozen sections are permeabilised with 0.3% Triton X /PBS for 15 min, washed and then equilibrated with 10 x PCR buffer (50 mM KCl, 10 mM Tris HCl, 15 mM MgCl_2 and pre-warmed to 75°C . A hot start PCR is performed using a 100 μL PCR solution, containing 50 mM KCl, 10 mM Tris HCl, 2.5 mM MgCl_2 , 0.2 mM dNTPs + 0.8 μM primers preheated to 75°C . Taq polymerase is added to pre-warmed PCR solution and applied to the sections. The PCR cycle is for an initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 1 minute, annealing at 64°C for 1 min 30s and extraction at 74°C for 1 min 30s.

Using the same primers, single stranded nucleic acid probes will be generated to detect the *in situ* PCR product starting with templates of the HPV sequences, using an asymmetric PCR and labeled with digoxigenin deoxyuridine triphosphate as previously established in our laboratory (Paine et al, 1995). The PCR product will be detected with anti-digoxigenin alkaline phosphatase-labeled antibody and visualised with BCIP and NBT substrates.

VZV (human herpes 3), FBV (human herpes 4), CMV (human herpes 5), Coxsackie A4, A10, A16

Realistically, the optimisation of the PCR for extracted DNA and the *in situ* PCR and its application to the OLP and control tissues for the HPV, HSV 1 and 2 will fully occupy the applicants in the first year of the project. They will then submit a

progress report and apply for further support for the study to detect VZV, EBV, CMV and Cocksackie viral genes in OLP.

Expected outcomes

Our hypothesis states that cytotoxic T lymphocytes may respond to viral antigens expressed by lesional keratinocytes in oral lichen planus. Hence, we may identify HSV, VZV, EBV, CMV, HPV or coxsackie virus genes in OLP lesions using type-specific primers and PCR. Later studies will use *in situ* hybridisation to locate viral genes within OLP lesions. These studies will enhance our understanding of the pathogenesis of OLP and may lead to novel therapies which are more specific and more effective than those used in current clinical practice.

Research time-table for the proposed research

We have already optimised the protocol for DNA and RNA extraction from human tissue samples and the PCR method. We therefore anticipate successful completion of this research project within the 12 month grant period.

Accommodation available for the proposed research

This project will be undertaken at Department of Dentistry, The University of Queensland and Faculty of Dentistry, University of Sydney (laboratory procedures). We routinely see this number of OLP, leukoplakia and chronic atrophic candidosis patients in the Oral Pathology and Oral Medicine Clinics at the respective institutions. The applicants are familiar with clinical procedures including biopsy of such patients. The applicants have complete access to the laboratory and equipment, thus ensuring successful completion of this research project within the 12 month grant period.

Ethical implications of the proposed research

The proposed research will use oral mucosa from patients and healthy control subjects. Biopsies of lesional tissue will be obtained from patients with oral lichen planus (n = 10), leukoplakia (n = 10) and chronic atrophic candidosis (n = 10). Healthy oral mucosa will be obtained from routine crown-lengthening procedures and trimming of surgical margins. Biopsies will be obtained under local anaesthesia as part of the routine work-up of these patients. In no instance will tissue be collected for the sole purpose of this research project. Patients will be recruited from the Oral Pathology and Medicine Clinic at The University of Queensland Dental School. The investigators have extensive experience in oral biopsy collection and procedures have passed ethical review several times previously. The project will follow guidelines set out in the NHMRC statement on human experimentation and the Helsinki Declaration. All subjects will be given a detailed explanation of the project and, if agreeable, will indicate their willingness to participate by reading and signing an informed consent form. All subjects will be over 18 years of age and there will be equal numbers of males and females. Participation in this project will not prejudice patient treatment and will allow voluntary withdrawal at any time without affecting routine care. In all cases, informed consent will be obtained and samples will be coded to maintain anonymity.

16 REFERENCES RELEVANT TO ITEM 15 ABOVE

- Axell T, Rundqvist L. Oral lichen planus - a demographic study. *Community Dent Oral Epidemiol* 1987; 15: 52-6.
- Berke G. The CTL's kiss of death. *Cell* 1995; 81: 9-12.
- Dekker NP, Lozada-Nur F, Lagenaur LA, MacPhail LA, Bloom CY, Regezi JA. Apoptosis-associated markers in oral lichen planus. *J Oral Pathol Med* 1997; 26: 170-75.
- Edwards KM, Davis JE, Browne KA, Sutton VR, Trapani JA. Anti-viral strategies of cytotoxic T lymphocytes are manifested through a variety of granule-bound pathways of apoptosis induction. *Immunol Cell Biol* 1999; 77: 76-89.
- Hashimoto K. Apoptosis in lichen planus and several other dermatoses. *Acta Dermatol Venereol* 1976; 56: 187-210.
- Jontell M, Watts S, Wallstrom M, Levin L, Sloberg K. Human papilloma virus in erosive oral lichen planus. *J Oral Pathol Med* 1990; 19: 273-7.
- Jungell P, Konttinen YT, Nortamo P, Malmstrom M. Immunoelectron microscopic study of distribution of T cell subsets in oral lichen planus. *Scand J Dent Res* 1989; 97: 361-7.
- Kilpi AM. Activation marker analysis of mononuclear cell infiltrates of oral lichen planus in situ. *Scand J Dent Res* 1987; 95: 174-80.
- Martz E, Howell DM. CTL: virus control cells first and cytolytic cells second? DNA fragmentation, apoptosis and the prelytic halt hypothesis. *Immunol Today* 1989; 10: 79-86.
- Mullbacher-A; Waring-P; Hla-RT; Tran-T; Chin-S; Stehle-T; Museteanu-C; Simon-MM. Granzymes are the essential downstream effector molecules for the control of primary virus infections by cytolytic leukocytes. *Proc Natl Acad Sci USA* 1999; 96: 13950-5.
- Nishioka WK, Welsh RM. Susceptibility to cytotoxic T lymphocyte-induced apoptosis is a function of the proliferative status of the target. *J Exp Med* 1994; 179: 769-74.
- Paine ML, Gibbins JR, Choi JK, McDonald DA, Walker DM, Kefford RF. Intranuclear post-transcriptional down-regulation responsible for loss of a keratin differentiation marker in tumor progression. *Anticancer Research* 1995; 15: 2145-54.
- Schifter M, Jones AM, Walker DM. p53 and PCNA immunohistochemistry and p53 gene mutational analysis in oral lichen planus. *J Oral Pathol Med* 1998; 27: 318-324.
- Shibata S, Kyuwa S, Lee SK, Toyoda Y, Goto N. Apoptosis induced in mouse hepatitis virus-infected cells by a virus-specific CD8+ cytotoxic T-lymphocyte clone. *J Virol* 1994; 68: 7540-5.
- Shimizu M, Higaki M, Kawashima M. The role of granzyme B-expressing CD8-positive T cells in apoptosis of keratinocytes in lichen planus. *Arch Dermatol Res* 1997; 289: 527-32.
- Simark-Mattsson C, Bergenholtz G, Jontell M, Eklund C, Seymour GJ, Sugerman PB, Savage NW, Dahlgren UI. Distribution of interleukin-2, -4, -10, tumour necrosis factor- α and transforming growth factor- β mRNAs in oral lichen planus. *Archives of Oral Biology* 1999; 44: 499-507.
- Sugerman PB, Savage NW, Walsh LJ, Seymour GJ. Disease mechanisms in oral lichen planus. A possible role for autoimmunity. *Australas J Dermatol* 1993; 34: 63-9.
- Sugerman PB, Savage NW, Seymour GJ, Walsh LJ. Is there a role for tumour necrosis factor alpha (TNF- α) in oral lichen planus? *J Oral Pathol Med* 1996; 25: 219-24.
- Sugerman PB, Shillitoe EJ. The high risk human papillomaviruses and oral cancer: evidence for and against a causal relationship. *Oral Diseases* 1997; 3: 130-147.

Sugerman PB, Satterwhite K, Bigby M. Auto-cytotoxic T cell clones in lichen planus. *Br J Dermatol* 2000; **142**: 449-56.

van der Meij EH, Schepman KP, Smeele LE, van der Wal JE, Bezemer PD, van der Waal I. A review of the recent literature regarding malignant transformation of oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1999; **88**: 307-10.

Weedon D: Apoptosis in lichen planus. *Clin Exp Dermatol* 1980; **5**: 425-30.

Zhou XJ, Savage NW, Sugerman PB et al. TCR V β gene expression in lesional T lymphocyte cell lines in oral lichen planus. *Oral Diseases* 1996; **2**: 295-8.

17 LIST OF PUBLISHED WORK AND REPORTS BY APPLICANT(S) (IN THE LAST FIVE YEARS ONLY)

(PLEASE APPEND THE LIST IF THE SPACE PROVIDED HERE IS INSUFFICIENT) ABSTRACTS AND PROCEEDINGS SHOULD NOT BE LISTED

Dr Philip B SUGERMAN publications 1995 - present

Editorial

Guest Editor for Clinics in Dermatology, Special Issue on Oral Pathology

Co-Editors: Drs Kathryn Bowers (Dermatologist) and John Sexton (Oral and Maxillofacial Surgeon), Boston, MA.

Chapters:

- Oral pathology and medicine in perspective - Dr Kathryn Bowers, Dr John Sexton and Dr Philip Sugerman;
- Oral histology and embryology - Dr Tracey Winning, A/Prof Grant Townsend;
- Autoimmune blistering diseases - Dr Kathryn Bowers;
- Oral drug reactions - Prof Stephen Porter, Prof Crispian Scully;
- Oral lichen planus - Dr Philip Sugerman, A/Prof Neil Savage, Dr Xijing Zhou, Prof Laurie Walsh, Dr Michael Bigby;
- Oral AIDS - Dr Sook-Bin Woo;
- Oral candidosis - Dr Camile Farah, A/Prof Robert Ashman, Prof Stephen Challacombe;
- Oral premalignancies and squamous cell carcinoma - Prof S Silverman, Dr Philip Sugerman;
- Recurrent aphthous stomatitis - A/Prof Neil Savage, Dr Bobby Joseph;
- Pigmented oral lesions - Dr Dore Eisen;
- Sjogren's syndrome - Dr Robert Fox;
- Oral surgical pathology - Dr John Sexton;
- Tongue pathology - Dr Bobby Joseph, Prof Neil Savage;
- Oral viral infections - Prof Denis Lynch.

Publication date: June, 2000.

Book Chapters

Chapters in Oral Pathology and Medicine Special Issue of "Clinics in Dermatology".

Publication date: June, 2000.

- Oral pathology and medicine in perspective - Drs Kathryn Bowers, John Sexton and Philip Sugerman;
- Oral lichen planus - Dr Philip Sugerman, Prof Neil Savage and Dr Michael Bigby;
- Oral premalignancies and squamous cell carcinoma - Prof S Silverman, Dr Philip Sugerman;

Original Research Papers

Sugerman PB, Savage NW, Xu LJ, Walsh LJ, Seymour GJ. Heat shock protein expression in oral lichen planus. *J Oral Pathol Med* 1995; **24**: 1-8.

Sugerman PB, Savage NW, Xu LJ, Walsh LJ, Seymour GJ. Heat shock protein expression in oral epithelial dysplasia and squamous cell carcinoma. *Eur J Cancer Part B: Oral Oncology* 1995; **31B**: 63-67.

Sugerman PB, Savage NW, Williams SL, Joynson OB, Daley TJ, Cowpe JG. A quantitative cytological study of lesional and non-lesional mucosa in oral lichen planus. *Arch Oral Biol* 1996; **41**: 117-120.

Zhou XJ, Savage NW, Sugerman PB, Walsh LJ, Aldred MJ, Seymour GJ. TCR V β gene expression in lesional T lymphocyte cell lines in oral lichen planus. *Oral Diseases* 1996; **2**: 295-298.

Simark-Mattsson C, Bergenholtz G, Jontell M, Eklund C, Seymour GJ, Sugerman PB, Savage NW, Dahlgren UI. Distribution of interleukin-2, -4, -10, tumour necrosis factor- α and transforming growth factor- β mRNAs in oral lichen planus. *Archives of Oral Biology* 1999; **44**: 499-507.

Sugerman PB, Bigby M. Preliminary functional analysis of human epidermal T cells. *Arch Derm Res* 2000; **292**: 9-15.

Sugerman PB, Satterwhite K, Bigby M. Auto-cytotoxic T cell clones in lichen planus. *Br J Dermatol* 2000; **142**: 449-456.

Review Papers

Sugerman PB, Joseph BK, Savage NW. The role of oncogenes, tumour suppressor genes and growth factors in oral squamous cell carcinoma: a case of apoptosis versus proliferation. *Oral Diseases* 1995; **1**: 172-188.

Sugerman PB, Savage NW. Exfoliative cytology in clinical oral pathology. *Aust Dent J* 1996; **41**: 71-74.

Sugerman PB, Savage NW, Seymour GJ, Walsh LJ. Is there a role for tumour necrosis factor alpha (TNF- α) in oral lichen planus? *J Oral Pathol Med* 1996; **25**: 219-224.

Sugerman PB, Shillitoe EJ. The high risk human papillomaviruses and oral cancer: evidence for and against a causal relationship. *Oral Diseases* 1997; **3**: 130-147.

Sugerman PB, Savage NW. Current concepts in oral cancer. *Aust Dent J* 1999; **44**: 147-156.

Joseph BK, Harbrow DJ, Sugerman PB, Smid JR, Savage NW, Young WG. Ameloblast apoptosis and insulin-like growth factor-1 receptor expression in the developing rat incisor model. *Apoptosis* 1999; **4**: 441-447.

Sugerman PB, Porter SR, Scully C, Luker J, Oakhill. A Orofacial manifestations in the Wiskott-Aldrich syndrome. *J South East Soc Paediatr Dent* (in press).

Case Reports

Sugerman PB, Savage NW, Young WG. Mucocele of the anterior lingual salivary glands (glands of Blandin and Nuhn): report of five cases. *Oral Surg Oral Path Oral Med* (in press; accepted November 2, 1999).

Dr Neil W SAVAGE publications 1995 - present

1. Savage, N.W., Walsh, L.J. 1995. Dermatopathology of the oral mucosa. In Dermatopathology, G.F. Murphy (ed). Philadelphia: W.B. Saunders, pp.421-444.
2. Sugerman, P.B., Savage, N.W., Xu, L.J., Walsh, L.J., Seymour, G.J. 1995. Heat shock protein expression in oral lichen planus. *J Oral Pathol Med.* 24:1-8.
3. Seymour, G.J., Savage, N.W., Walsh, L.J. 1995. Basic immunology. An Introduction for the Allied Health Sciences. McGraw Hill.
4. Walsh, L.J., Lange, P., Savage, N.W. 1995. Factors influencing the wearing of protective gloves in general dental practice. *Quint Int.* 26:203-9.
5. Athanasas-Platsis, S., Savage, N.W., Winning, T.A., Walsh, L.J. 1995. Induction of the CD1a Langerhans cell marker on human monocytes. *Arch Oral Biol.* 40:157-160.
6. Sugerman, P.B., Savage, N.W., Xu, L., Walsh, L.J., Seymour, G.J. 1995. Heat shock protein expression in oral epithelial dysplasia and squamous cell carcinoma. *Eur J Cancer.* 318:63-7.
7. Walsh, L.J., Davis, M.F., Xu, L.J., Savage, N.W. 1995. Relationship between mast cell degranulation and inflammation in the oral cavity. *J Oral Pathol Med.* 24:266-72.
8. Savage, N.W., Walsh, L.J. 1995. The use of autoclaves in the dental surgery. *Aust Dent J.* 40:197-200.
9. Ivanovski, S., Savage, N.W., Brockhurst, P.J., Bird, P.S. 1995. Disinfection of dental stone casts. Antimicrobial effects and physical property alterations. *Dent Materials.* 11:19-23.
10. Pietrzak, E.R., Savage, N.W., Xu, L.J., Walsh, L.J. 1995. Differential reactivity of anti-E-selectin monoclonal antibodies in human gingival tissue. *J Periodontal Res.* 30:375-81.
11. Sugerman, P.B., Joseph, B.K., Savage, N.W. 1995. The role of oncogenes, tumour suppressor genes and growth factors in oral squamous cell carcinoma: a case of apoptosis versus proliferation. *Oral Diseases* 1:172-8.
12. Walsh, L.J., Coyne, R.M., Xu, L.J., Savage, N.W. 1996. The role of adhesion molecules in oral cancer. *Aust Dent J.* 41:80-2.
13. Sugerman, P.B., Savage, N.W., Williams, S.L., Joynson, O.B., Daley, T.J., Cowpe, J.G. 1996. A quantitative cytological study of lesional and non-lesional mucosa in oral lichen planus. *Arch Oral Biol.* 41:117-20.
14. Sugerman, P.B., Savage, N.W. 1996. Exfoliative cytology in clinical oral pathology. *Aust Dent J.* 41:71-4.
15. Pietrzak, E.R., Savage, N.W., Walsh, L.J. 1996 Human gingival keratinocytes express E-selectin (CD62E). *Oral Dis.* 2:11-17.
16. Davis, M.F., Savage, N.W., Walsh, L.J. 1996. Lymphocyte homing molecules in periapical lesions. *Aust Endodon Newsletter* 22:29-30.
17. Gentner, M.R., Savage, N.W., Walsh, L.J. 1996. Modulation of dental pulp endothelial molecule expression in vitro. *Aust Endodon Newsletter* 22:32-4.
18. Lange, P., Savage, N.W., Walsh, L.J. 1996. Utilization of personal protective equipment in general dental practice. *Aust Dent J.* 41:164-8.
19. Walsh, L.J., Athanasas-Platsis, S., Savage, N.W. 1996. Reconstitution of cutaneous-neural immunological networks following bone marrow transplantation. *Transplantation* 61:413-7.
20. Joseph, B.K., Savage, N.W., Daley, T.D., Young, W.G. 1996. In situ hybridization evidence for a Paracrine/Autocrine role for insulin-like growth factor-1 in tooth development. *Growth Factors* 13:11-17.
21. Pietrzak, E.R., Savage, N.W., Aldred, M.J., Walsh, L.J. 1996. Expression of the E-selectin gene in human gingival epithelial tissue. *J Oral Pathol Med.* 25:320-4.
22. Sugerman, P.B., Savage, N.W., Seymour, G.J., Walsh, L.J. 1996. Is there a role for tumor necrosis factor alpha (TNF- α) in oral lichen planus? *J Oral Pathol Med.* 25:219-24.
23. Savage, N.W., Hu Yan. 1996. Recurrent aphthous ulceration: clinical assessment and management. *Periodontol.* 17:122-4.
24. Winning, T.A., Gemmell, E., Polak, B., Savage, N.W., Seymour, G.J., Walsh, L.J. 1996. The expression of CD1a on monocytes cultured with supernatants from periodontally diseased gingival epithelial cells. *Oral Dis.* 2:247-52.

- 25.Savage, N.W., Joseph, B.K., Monsour, P.A., Young, W.G. 1996. The glandular odontogenic cyst - report of a case. *Pathol.* 28:370-2.
- 26.Savage, N.W. 1996. Burning mouth syndrome: patient management. *Aust Dent J.* 41:363-6.
- 27.Zhou, X.J., Savage, N.W., Sugerman, P.B., Walsh, L.J., Aldred, M.J., Seymour, G.J. 1996. TCR Vb gene expression in lesional T lymphocyte cell lines in oral lichen planus. *Oral Dis.* 2:295-8.
- 28.McDonald, R.I., Walsh, L.J., Savage, N.W. 1997. Analysis of workplace injuries in a dental school environment. *Aust Dent J.* 42:109-13.
- 29.Clayden, A.M., Bleys, C.M., Jones, S.F., Savage, N.W., Aldred, M.J. 1997. Orofacial granulomatosis: a diagnostic problem for the unwary and management dilemma. *Aust Dent J.* 42:228-32.
- 30.Zhao, Z-Z., Savage, N.W., Pujic, Z., Walsh, L.J. 1997. Immunohistochemical localization of mast cells and mast cell-nerve interactions in oral lichen planus. *Oral Dis.* 3:71-6.
- 31.Savage, N.W. Oral lichenoid drug eruptions. Guest Editorial. 1997. *Oral Dis.* 3:55-7.
- 32.Savage, N.W., Walsh, L.J. 1997. Integrating infection control into the dental curriculum. *Aust Dent J.* 42:395-8.
- 33.Joseph, B.K., Savage, N.W., Harbrow, D.J., Young, W.G. 1997. Non-expression of insulin-like growth factor-1 receptor is associated with apoptosis: an ultrastructural study on rat ameloblasts. *Apoptosis* 2:471-7.
- 34.Elvery, M., Savage, N.W., Wood, W. 1998. A radiographic study of the Broadbeach Aboriginal dentition. *Amer J Physical Anthropol.* 107:211-9.
- 35.Savage, N.W., Gentner, M., Symons, A.L. 1998. Pre-eruptive intracoronal radiolucencies: review and case report. *J Dent Child.* 65:36-40.
- 36.Pujic, Z., Savage, N.W., Bartold, P.M., Walsh, L.J. 1998. Simultaneous detection of multiple antigens with triple immunolabelling. *App Immunohistochem.* 6:150-3.
- 37.Cockings, J.M., Savage, N.W. 1998. Minocycline induced oral pigmentation. *Aust Dent J.* 43:14-6.
- 38.Pietrzak, E.R., Polak, B., Walsh, L.J., Savage, N.W., Seymour, G.J. 1998. Characterization of serum antibodies to *Porphyromonas gingivalis* in individuals with and without periodontitis. *Oral Microbiol Immunol.* 13:65-72.
- 39.Zhao, Z-Z., Savage, N.W., Walsh, L.J. 1998. Associations between mast cells and laminin in oral lichen planus. *J Oral Pathol Med.* 27:163-7.
- 40.Cockings, J.M., Walsh, L.J., Savage, N.W. 1998. Genetic factors in cyclosporin A induced gingival overgrowth. *Periodontol.* 19:92-7.
- 41.Hu Yan, Savage, N.W. 1998. The changes of suppressor function of T cells in patients with RAU. *J Chinese Stomatol.* 44:103-5.
- 42.Hu Yan, Savage, N.W. 1998. The hypotheses for the aetiology of RAU (review). *J of Dent Prevent of Guangdong* 6:60-2.
- 43.Joseph, B.K., Harbrow, D.J., Sugerman, P.B., Smid, J.R., Savage, N.W. , Young, W.G. 1999. Ameloblast apoptosis and IGF-1 receptor expression in the continuously erupting rat incisor model. *Apoptosis.* 4:441-7.
- 44.Simark-Mattson, C., Bergenholtz, G., Jontell, M., Eklund, C., Seymour, G.J., Sugerman, P.B., Savage, N.W., Dahlgren, U.I. 1999. Distribution of interleukin-2,-4,-10, tumour necrosis factor- α and transforming growth factor- β mRNA's in oral lichen planus. *Arch Oral Biol.* 44:499-507.
- 45.Savage, N.W. 1999. White patches on the oral mucosa. *Hygiene Today.* 9:4-6.
- 46.Sugerman, P.G., Savage, N.W. 1999. Current concepts in oral cancer. *Aust Dent J.* 44:147-56.
- 47.Joseph, B.K., Savage, N.W., Macleod, A.W.G. 1999. Cat Scratch Disease: A case report.

Dr D Murray WALKER publications 1995 - present

WALKER DM. "The Periodontal Ligament in Health & Disease" Chapter: Neoplastic involvement of the periodontal ligament. Eds, Newman HN, Berkovitz BKB, Moxham B J. Mosby Year Book Ltd, U K, 1995

HERBERT A-M, BAGG J, WALKER DM, DAVIES KJ & WESTMORELAND D. The seroepidemiology of herpes virus infections among dental personnel. *Journal of Dentistry*, 1995; 23 (6): 339-342.

PAINE ML, GIBBINS JR, CHOI JK, McDONALD DA, WALKER DM & KEFFORD RF. Intranuclear post-transcriptional down-regulation responsible for loss of a keratin differentiation marker in tumor progression. *Anticancer Research*, 1995, 15: 2145-54.

COX SC & WALKER DM. Oral submucous fibrosis: a review. *Australian Dental Journal*, 1996, 41: 294-299.

COX SC & WALKER DM. Epithelial Growth Fraction and Expression of p53 Tumour Suppressor Gene in Oral Submucous Fibrosis. *Australian Dent Journal*, 1996; 41: 91-96

COX SC & WALKER DM. Establishing a normal range for mouth opening in Nepal: its use in screening for oral submucous fibrosis. *British Journal of Oral & Maxillofacial Surgery*, 1997, 35: 40-42

YOUNES SI, J, WALKER, DM, ELLIS, P & DWYER, DE. Fatal Staphylococcus aureus Infective Endocarditis: The Dental Implications. *Oral Surg*, 1997; 85: (2) 168-72.

WALKER DM. Principles and Practices of Geriatric Medicine, Third Edition. Chap 27. Oral Diseases. Publ. John Wiley & Sons Ltd. Chichester, UK, Ed. MSJ Pathy. 1998 (two volumes) 313-332.

McDONALD LA, WALKER DM & GIBBINS JR. Cervical lymph node involvement in head and neck cancer detectable as expression of a spliced transcript of type II keratin K5. *European Journal of Cancer. B. Oral Oncology*, 1998; 34: 276-283.

SCHIFTER M, JONES AM & WALKER, DM. P53 and PCNA immunohistochemistry and p53 gene mutational analysis in oral lichen planus. *Journal of Oral Pathology & Oral Medicine*, 1998; 27: 318-324.

CURTIS NJ, LAUTO A, TRICKETT R, OWEN E & WALKER DM. Preliminary study of microsurgical repairs of the inferior alveolar nerve in rats, using primary suturing and laser weld techniques. *International Journal of Oral & Maxillofacial Surgery*, 1998; 27: 476-81.

CURTIS N, OWEN D, TRICKETT, E, LAUTO R, WALKER DM & LANZETT A. Intra-osseous repair of inferior alveolar nerve in rats: an experimental model. *J Reconstructive Microsurgery, Laser Surgery SPIE*, 1998; 2973: 197-207.

BOULOUX GF, WALKER DM & McKELLAR G. Massive Osteolysis of the Mandible: Report of a Case with Multifocal Bone Loss. Oral Surgery, Oral Medicine, Oral Pathology, 1999; 87 (3): 357-361

WALKER DM. Oral Cancer in New South Wales: NSW Public Health Bulletin, February 1999; 10 (8): 98-101.

In press

LOW EOW, GIBBINS JR & WALKER DM. Analysis of the amplification refractory mutation allele specific polymerase chain reaction system for sensitive and specific detection of p53 mutations in DNA (2594). Journal of Pathology.

Provisionally accepted

LOW EOW, GIBBINS JR & WALKER DM. in situ detection of specific p53 mutations in cultured cells using the amplification refractory mutation system polymerase chain reaction. Diagnostic Molecular Pathology.

18 OTHER RESEARCH PROGRAMMES BEING UNDERTAKEN OR SUPERVISED BY THE APPLICANT(S)

Dr Philip B SUGERMAN

Student: Dr Zhen Zhen Zhao (enrolled in PhD).

Location: Oral Biology and Pathology, Department of Dentistry, The University of Queensland.

Co-supervisors: Dr PB Sugerman, A/Prof Neil Savage, A/Prof Laurie Walsh.

Duration: current

Project: Mast cell degranulation in oral lichen planus.

Student: Dr Jane Manakil (enrolled in PhD).

Location: Periodontology Research Laboratories, Department of Dentistry, The University of Queensland.

Co-supervisors: Dr PB Sugerman, Professor Gregory Seymour, Professor Mark Bartold.

Duration: current

Project: Proteoglycan production by human peripheral blood and periodontal T cells.

Dr Neil W SAVAGE

Student: Dr Zhen Zhen Zhao (enrolled in PhD).

Location: Oral Biology and Pathology, Department of Dentistry, The University of Queensland.

Co-supervisors: Dr PB Sugerman, A/Prof Neil Savage, A/Prof Laurie Walsh.

Duration: current

Project: Mast cell degranulation in oral lichen planus.

Student: Dr Ali Nawshad (enrolled in PhD).

Location: Oral Biology and Pathology, Department of Dentistry, The University of Queensland.

Co-supervisors: A/Prof Neil Savage, A/Prof Laurie Walsh.

Duration: current

Project: Genetics of odontogenic tumours.

Dr D Murray WALKER

Student: Dr Louise MacDonald

Location: Oral Pathology & Oral Medicine, Faculty of Dentistry, University of Sydney.

Co-supervisor: Dr JR Gibbins

Duration: current

Project: Detection of lymph node metastases in oral cancer by reverse transcriptase – polymerase chain reaction for keratin 5 exon 5 mRNA

19 A CERTIFICATE OF ETHICAL CLEARANCE

- (1) IS APPENDED ☒
- (2) WILL FOLLOW THIS APPLICATION ☐
- (3) IS UNNECESSARY ☐ (IF SO, PLEASE SPECIFY WHY)

20 SIGNATURE(S) OF APPLICANT(S)

THE APPLICANT(S) BY THE EXECUTION OF THIS APPLICATION FORM ACKNOWLEDGES AND ACCEPTS THE ABSOLUTE DISCRETION OF THE DIRECTORS OF THE AUSTRALIAN DENTAL RESEARCH FOUNDATION INC. TO DECIDE IN ANY YEAR WHICH PROJECTS WILL RECEIVE GRANTS FROM THE FOUNDATION, THE SIZE OF THOSE GRANTS AND TO USE WHATEVER MEANS, METHODS AND CRITERIA THEY CONSIDER APPROPRIATE TO MAKE SUCH DECISIONS, AND AGREES THAT HE OR SHE WILL NOT, AND HAS NO RIGHT TO, CHALLENGE SUCH DECISIONS OF THE DIRECTORS OF THE FOUNDATION.

SIGNATURE(S) _____

**21 CERTIFICATE OF HEAD OF DEPARTMENT WHERE APPLICANT IS TO
WORK IN AN INSTITUTION OR UNIVERSITY DEPARTMENT (NOT
REQUIRED FOR RESEARCH UNDERTAKEN IN A PRIVATE PRACTICE)**

I CERTIFY THAT THE PROJECT IS APPROPRIATE TO THE GENERAL FACILITIES IN MY DEPARTMENT/INSTITUTION
AND I AM PREPARED TO HAVE THE PROJECT CARRIED OUT IN THAT DEPARTMENT/INSTITUTION. I HAVE NOTED
THE CONTENTS OF ITEM 19 REGARDING ETHICS APPROVAL.

SIGNATURE _____

NAME _____

DATE _____

APPENDIX II

WESTERN SYDNEY AREA HEALTH SERVICE
WESTMEAD NSW 2145

PATIENT INFORMATION

Title of Project:

Viral Genes in Oral Lichen Planus

Names of Investigators:

Professor D M Walker

Dr Mark Schifter

Dr Anastasia Georgiou

What is the purpose of this Study?

From your history and examination of your mouth, we believe that you have Lichen Planus and advise you have a biopsy to confirm the diagnosis in order that you are given the appropriate treatment. Please read the leaflet supplied which explains what is presently known about oral lichen planus

The tissue will be investigated for viral genes to obtain greater understanding of the cause of oral lichen planus which may lead to new treatments than those used at present.

WESTERN SYDNEY AREA HEALTH SERVICE

WESTMEAD NSW 2145

Who will be asked to enter the Study?

Patients who are having an incisional tissue biopsy of the lining of the mouth (oral mucosa) for the diagnosis and management of oral lichen planus.

What will happen on the Study?

A small piece of tissue will be taken from the mucosal lining of your mouth, having made the area quite numb with a local anaesthetic. The tissue will be examined by a pathologist – this biopsy is a routine procedure for confirming that you have lichen planus of the mouth. Two or three stitches will be placed to help healing, these will be removed at the next visit. The biopsy site will be slightly sore and swollen for approximately 3 days, we will give you a chlorhexidine antiseptic mouthwash to aid healing.

Part of the biopsy will be studied to see whether oral lichen planus could be caused by common viruses such as the human papillomavirus (which also causes warts).

If you do not wish for your biopsy to be studied for virus DNA, please say so and the biopsy will then be used only for diagnosis by the pathologist. Your treatment will not be altered in any way.

WESTERN SYDNEY AREA HEALTH SERVICE

WESTMEAD NSW 2145

Are there any risks?

There are no risks involved with the research, but there will be some discomfort of your lining of the mouth following the routine diagnostic biopsy which you have been advised to have. This discomfort is usually not severe and usually lasts for approximately three days.

Do you have a choice?

We advise you to have a biopsy to confirm that you have oral lichen planus as the treatment of lichen planus differs from that of other mouth conditions.

If you decide not to have a biopsy we will advise the standard treatment of lichen planus and review your progress from time to time as usual. Alternatively you can opt to have the biopsy, but not allow any of the tissue to be used for research.

If you have any problems whilst on this study, please contact Dr Anastasia Georgiou

Working hours: 02 9845 7834

After hours: 02 9845 5555 (and ask for the doctor to be paged)

Patient's Name: _____ **Date:** _____

Signature: _____

Witness: _____

APPENDIX III

WESTERN SYDNEY AREA HEALTH SERVICE
WESTMEAD NSW 2145

CONSENT TO PARTICIPATE IN RESEARCH

Title of Project: **Viral Genes in Oral Lichen Planus**

Names of Investigators: **Professor D M Walker, Dr Mark Schifter**
Dr Anastasia Georgiou

1. I understand that the investigator will conduct this study in a manner conforming with ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.
2. The general purposes, methods and demands, and the possible risks, inconveniences which may occur to me during the study have been read and explained to me by Dr *Anastasia Georgiou* and I, being over the age of 16 years, acknowledge that I understand the methods and demands relating to the treatment and the possible risks, inconveniences and discomforts which may occur.
3. I acknowledge that I have been given time to consider the information and to seek other advice.
4. Refusal to take part in this study will not affect the usual treatment of my condition.
5. I am volunteering to take part in this study and I may withdraw at any time.
6. This research has been approved by the Western Sydney Area Health Service Human Research Ethics Committee.
7. I acknowledge that I have received a copy of this form and the participant information sheet, which I have signed.
8. Sponsoring pharmaceutical companies and any regulatory authorities may have access to my medical records to monitor the research in which I am agreeing to participate. However, my identity will not be disclosed to them or anyone else.

Name of participant: _____

Address: _____

Signature of participant: _____
(refer below for definition)

Signature of Investigator: _____

WESTERN SYDNEY AREA HEALTH SERVICE

WESTMEAD NSW 2145

Definition of Participant:

This may only be signed by:

1. Participants over 16 years of age: or
2. Participants between the age of 14 and 16 years together with the signature of their patient or guardian.
3. Parent or guardian of participants under 14 years.
4. Where patient or participant has a medical or legal disability then signature must be that of:
 - a) The legal guardian; or
 - b) Spouse or de factor spouse; or
 - c) Caregiver; or
 - d) The Guardianship Board (Telephone 02 9555 8500); or
 - e) Family member or friend but not a professional caregiver (e.g. medical superintendent, director of nursing, nursing home director)

INDEPENDNT WITNESS / INTERPRETER:

Referring to participation in research: **Viral Genes in Oral Lichen Planus**

Independent Witness:

Name of independent witness: * _____

Address: _____

Signature of independent witness: _____

Relationship to participant of independent witness: _____

*Independent witness is not the investigator nor his/her delegate

Interpreter: following is necessary if an interpreter is used: I _____ of

_____ Interpreter, not being a party to the research,

certify that I was present when Dr Anastasia Georgiou informed the participant of the

nature and content of this form and that I have read the contents of this form in English /

_____ language to _____ (participant and /or responsible person)

who acknowledge to me that he / she understood the method of treatment and possible risk

and freely and voluntarily signed this form of consent.

Signed by the above-named interpreter: _____

Date: _____

WESTERN SYDNEY AREA HEALTH SERVICE

WESTMEAD NSW 2145

INFORMATION FOR NORMAL CONTROL SUBJECTS

Title of Project:

Viral Genes in Oral Lichen Planus

Names of Investigators

**Professor D M Walker
Dr Mark Schifter
Dr Anastasia Georgiou**

What is the purpose of this Study?

Oral Lichen Planus is a relatively common condition in the mouth, the cause of which remains unknown. The lesions may be painful and causes a slightly increased risk of oral cancer. This project will investigate Oral Lichen Planus tissue and normal tissue for viral genes known to affect the oral tissues, in order to obtain greater understanding of the cause of Oral Lichen Planus, which may lead to new treatments than those used at present.

Who will be asked to enter the Study?

Patients who are thought to have Oral Lichen Planus, and who are having an incisional biopsy of the lining of the mouth (oral mucosa) for the diagnosis and management of Oral Lichen Planus.

Patients who require surgical extraction of teeth. You do not have lichen planus, and are merely asked to provide normal tissue to help this research, which would be removed as usual during extraction of your tooth, or teeth.

What will happen on the Study?

Normal oral tissue is needed to compare investigations of Oral Lichen Planus tissue. Once the tooth that is required to be removed is extracted, the tissue that is still attached to the tooth is trimmed. This tissue is then used as normal control tissue in the Study.

WESTERN SYDNEY AREA HEALTH SERVICE

WESTMEAD NSW 2145

INFORMATION FOR NORMAL CONTROL SUBJECTS

Are there any risks?

There are no risks involved in the research, there will be some discomfort of your mouth following extraction of teeth.

Do you have a choice?

You can opt to have the tooth extracted, but not allow the tissue to be used for research.

If you have any problems while on the study, please contact Dr Anastasia Georgiou

Working hours: 02 9845 7834

After hours: 02 9845 5555 – page 12533

Patient's Name: _____ Date: _____
Signature: _____

If you have any concerns about the conduct of the study, you may contact the Westmead Hospital Patient Representative, Ms Jilian Gwynne Lewis, 02 9845 7014.

REFERENCES

-
- ¹ Wilson E. On liechen planus. *J Cutan Med Dis Skin* 1869; **3**: 117-32.
- ² Andreassen JO. Oral lichen planus 1. A clinical evaluation of 115 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1986; **25**: 31-42.
- ³ Scully C, el-Kom M. Lichen planus: review and update on pathogenesis. *J Oral Pathol Med* 1985; **14**: 431-58.
- ⁴ Bermejo A, Bermejo MD, Roman P, Botella R, Bagan JV. Lichen planus with simultaneous involvement of the oral cavity and genitalia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1990; **69**: 209-16.
- ⁵ Axell T. A prevalence study of oral mucosal lesions in an adult Swedish population. *Odontol Rev* 1976; Suppl 36, **27**: 1-103.
- ⁶ Axéll T, Rundquist L. Oral lichen planus: a demographic study. *Community Dent Oral Epidemiol* 1987; **15**: 52-6.
- ⁷ Bouquot JE, Gorlin RJ. Leukoplakia, lichen planus, and other oral keratoses in 23,616 white Americans over the age of 35 years. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1986; **61**: 373-81.
- ⁸ Pindborg JJ, Mehta FS, Daftary DK, Gupta PC, Bhonsle RB. Prevalence of oral lichen planus among 7639 Indian villagers in Kerala, South India. *Acta Dermatovenereol (Stockh)* 1972; **52**: 216-20.
- ⁹ Shklar G. Lichen planus as an oral ulcerative disease. *Oral Surg Oral Med Oral Pathol* 1972; **33**: 376-88.
- ¹⁰ Kovesi G, Banoczy J. Follow up studies in oral lichen planus. *Int J Oral Surg* 1979; **2**: 13-9.

-
- ¹¹ Silverman S Jr, Griffith M. Studies on oral lichen planus II. Follow-up on 200 patients, clinical characteristics, and associated malignancy. *Oral Surg Oral Med Oral Pathol* 1974; **37**: 705-10.
- ¹² Jungell P. Oral lichen planus. A review. *Int J Oral Maxillofac Surg*. 1991; **20**: 129-35.
- ¹³ Altman J, Perry HO. The variations and course of lichen planus. *Arch Dermatol* 1961; **84**: 179-91.
- ¹⁴ Cooke BED. The oral manifestations of lichen planus: 50 cases. *Br Dent J* 1954; **96**: 1-9.
- ¹⁵ Shklar G, McCarthy PL. The oral lesions of oral lichen planus. Observations on 100 cases. *Oral Surg Oral Med Oral Pathol* 1961; **14**: 164-81.
- ¹⁶ WHO Collaborating centre for oral precancerous lesions. Definition of leukoplakia and related lesions. An aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol* 1978; **46**: 518-39.
- ¹⁷ Mollaoglu N. Oral lichen planus: a review. *Br J Oral & Maxillofac Surg* 2000; **38**: 370-7.
- ¹⁸ Bricker SL. Oral lichen planus. A review. *Semin Dermatol* 1994; **13**: 87-90.
- ¹⁹ Marder MZ, Deesen KC. Transformation of oral lichen planus to squamous cell carcinoma: a literature review and report of case. *J Am Dent Assoc* 1982; **105**: 55-60.
- ²⁰ Thorn JJ, Holmstrup P, Rindum J, Pindborg JJ. Course of various clinical forms of oral lichen planus. A prospective follow-up study of 611 patients. *J Oral Pathol Med* 1988; **17**: 213-8.
- ²¹ Zegarelli DJ. The treatment of oral lichen planus. *Ann Dent* 1993; **52**: 3-8.

-
- ²² Scully C, Beyli M, Ferreiro MC, Ficarra G, Gill Y, Griffiths M, Holmstrup P, Mutlu S, Porter S, Wray D. Update on oral lichen planus: Etiopathogenesis and management. *Crit Rev Oral Biol Med* 1998; **9**: 86-122.
- ²³ Eisen D. The vulvovagina-gingival syndrome of lichen planus. *Arch Dermatol* 1994; **130**: 1370-82.
- ²⁴ Scott MJ, Scott MJ. Ungual lichen planus. *Arch Dermatol* 1979; **115**: 1197-99.
- ²⁵ Mahood JM. Familial lichen planus. *Arch Dermatol* 1983; **119**: 292-4.
- ²⁶ Oomen C, Temmerman L, Kint A. Lichen planus pemphigoides. *Clin Exp Dermatol* 1986; **11**: 92-6.
- ²⁷ Allen CM, Camisa C, Grimwood R. Lichen planus pemphigoides: report of a case with oral lesions. *Oral Surg Oral Med Oral Pathol* 1987; **63**: 184-8.
- ²⁸ Fellner MJ. Lichen planus. *Int J Dermatol* 1980; **19**: 71-5.
- ²⁹ Dubreuil W. Histologie du lichen plan des muqueuses. *Ann Dermatol* 1906; **7**: 123-9.
- ³⁰ Lever WF, Schaumburg-Lever G. Histopathology of the Skin, 7th edn. Philadelphia: JB Lippincott, 1990: 169-70
- ³¹ Shafer WG, Hine MK, Levy BM. A textbook of oral pathology. 4th ed. Philadelphia: W.B. Saunders, 1983: 808-14.
- ³² Ragaz A, Ackerman AB. Evolution, maturation, and regression of lesions of lichen planus, new observations and correlations of clinical and histological findings. *Am J Dermatopathol* 1981; **3**: 5-15.

³³ El-Labban NG, Kramer IR. Civatte bodies and the actively dividing epithelial cells in oral lichen planus. *Br J Dermatol* 1974; **90**: 13-23.

³⁴ Firth NA, Reade PC. Comparison of eosinophil densities in oral mucosal lichen planus and lichenoid drug reactions. *J Oral Pathol Med* 1990; **19**: 86-8.

³⁵ Winer LH, Leeb AJ. Lichenoid eruptions: a histopathological study. *Arch Dermatol Syphil* 1954; **70**: 274-81.

³⁶ Watanabe C, Hayashi T, Kawadi A. Immunofluorescence study of drug induced lichen planus-like lesions. *J Dermatol* 1981; **8**: 473-7.

³⁷ Odell EW, Morgan PR. In "Biopsy pathology of the oral tissues" First Edition. Chapman & Hall Medical London 1998 pp 44-6.

³⁸ Krutchkoff DJ, Eisenberg E. Lichenoid dysplasia: a distinct histopathologic entity. *Oral Surg Oral Med Oral Pathol* 1985; **60**: 308-15.

³⁹ Firth NA, Rich AM, Radden BG, Reade PC. Assessment of the value of immunofluorescence microscopy in the diagnosis of oral mucosal lichen planus. *J Oral Pathol Med* 1990; **19**: 295-7.

⁴⁰ Daniels TE, Quadra-White C. Direct immunofluorescence in oral mucosal disease: A diagnostic analysis of 130 cases. *Oral Surg Oral Med Oral Pathol* 1981; **51**: 38-46.

⁴¹ Rogers RS, Jordon RE. Immunopathology of oral mucosal inflammatory diseases. *Clin Exp Dermatol* 1977; **2**: 97-107.

-
- ⁴² Hashimoto K, DiBella RJ, Shklar G, Lever WF. Electron microscopic studies of oral lichen planus. *G Ital Derm* 1966; **107**: 765-88.
- ⁴³ Jungell P, Malmström M, Wartiovaara J, Konttinen Y, San J. Ultrastructure of oral leukoplakia and lichen planus. I. Basal region and inflammatory cells. *J Oral Pathol* 1987; **16**: 170-8.
- ⁴⁴ Jungell P. Immunoelectron microscopic study of the basement membrane in oral lichen planus. *J Cutan Pathol* 1990; **17**: 72-7.
- ⁴⁵ Shklar G, Flynn E, Szabo G. Basement membrane alterations in oral lichen planus. *J Invest Dermatol* 1978; **70**: 45-50.
- ⁴⁶ Lowe NJ, Cudworth AG, Woodrow JC. HLA antigens in lichen planus. *Br J Dermatol* 1976; **96**: 169-71.
- ⁴⁷ Powell, FC, Rogers RS, Dickson ER, Moore SB. An association between HLA-DR1 and lichen planus. *Br J Dermatol* 1986; **114**: 473-78.
- ⁴⁸ Watanabe T, Ohishi M, Tanaka K, Sato H. Analysis of HLA antigens in Japanese with oral lichen planus. *J Oral Pathol* 1986; **15**: 529-33
- ⁴⁹ Lin SC, Sun A. HLA-DR and DQ antigens in Chinese patients with oral lichen planus. *J Oral Pathol Med* 1990; **19**: 298-300
- ⁵⁰ Holmstrup P. Reactions of the oral mucosa related to silver amalgam. *J Oral Pathol Med* 1991; **20**: 1-7.
- ⁵¹ Jacob FM, Helmbold TR. Bacteriologic studies on lichen planus: preliminary report. *Arch Derm Syphilol* 1933; **27**: 472.

-
- ⁵² Lehnhoff C. Spirochaetes in aetiologically obscure diseases. *Acta Derm Venereol (Stockh)* 1948; **28**: 295.
- ⁵³ Ficarra G, Flaitz CM, Gaglioti D, Piluso S, Milo D, Adler-Storthz K, Eversole LR. White lichenoid lesions of the buccal mucosa in patients with HIV infection. *Oral Surg Oral Med Oral Pathol* 1993; **76**: 460-6.
- ⁵⁴ Maitland NJ, Cox MV, Lynas C, Prime SS, Meanwell CA, Scully C. Detection of human papillomavirus DNA in biopsies of human oral tissue. *Br J Cancer* 1987; **56**: 245-9.
- ⁵⁵ Bagan JV, Aguirre JM, del Olmo JA, Milian A, Penarrocha M, Rodrigo JM *et al*. Oral lichen planus and chronic liver disease: a clinical and morphometric study of the oral lesions in relation to transaminase elevation. *Oral Surg Oral Med Oral Pathol* 1994; **78**: 337-42.
- ⁵⁶ Eisen D. The therapy of oral lichen planus. *Crit Rev Oral Biol Med* 1993; **4**: 141-58.
- ⁵⁷ Allen CM, Blozis GG. Oral mucosal reactions to cinnamon-flavored chewing gum. *J Am Dent Assoc* 1988; **116**: 664-7.
- ⁵⁸ Bagan JV, Donat JS, Penarrocha M, Milian MA, Sanchis JM. Oral lichen planus and diabetes mellitus. A clinico-pathological study. *Bull Group Int Rech Sci Stomatol et Odontol* 1993; **36**: 3-6.
- ⁵⁹ Christensen E, Holmstrup P, Wilberg-Jørgensen F, Neumann-Jensen B, Pindborg JJ. Arterial blood pressure in patients with oral lichen planus. *J Oral Pathol* 1977; **6**: 143-51.
- ⁶⁰ Choudhary SD, Gupta S, Jain VK. Lichen planus hypertrophicus with carcinoma breast in a male patient (a case report). *Indian J Dermatol* 1988; **33**: 40-1.
- ⁶¹ Helm TM, Camisa C, Liu Ay, Valenzuela R, Bergfeld WF. Lichen planus associated with neoplasia: a cell-mediated immune response to tumor antigens? *J Am Acad Dermatol* 1994; **30**: 219-24.

-
- ⁶² Okaki H, Hashiro K, Tsuchida T, Seki Y, Tamaki K. Lichen planus pemphigoides: case report and results of immunofluorescence and immunoelectron microscopic study. *J Am Acad Dermatol* 1990; **22**: 626-31.
- ⁶³ Ashinoff R, Cohen R, Lipkin G. Castleman's tumor and erosive lichen planus: coincidence or association? Report of a case. *J Am Acad Dermatol* 1989; **21**: 1076-80.
- ⁶⁴ Jansen T, Plewig G, Anhalt GJ. Paraneoplastic pemphigus with clinical features of erosive lichen planus associated with Castleman's tumor. *Dermatol* 1995; **190**: 245-50.
- ⁶⁵ Magnusson B. Lichen ruber bullosus and tumors in internal organs. *Dermatologica* 1967; **134**: 166-72.
- ⁶⁶ Gruppo Italiano Studie Epidemiologici in Dermatologia (GISED). Epidemiological evidence of the association between lichen planus and two immune-related diseases: Alopecia areata and ulcerative colitis. *Arch Dermatol* 1991; **127**: 688-91.
- ⁶⁷ Graells J, Notario J, Badia F. Lichen planus in monozygotic twins (letter). *Clin Exp Dermatol* 1998; **23**: 290-9.
- ⁶⁸ Gibstine CF, Esterly NB. Lichen planus in monozygotic twins (letter) .*Arch Dermatol* 1984; **120**: 580.
- ⁶⁹ Lin SC, Sun A. HLA-DR and DQ antigens in Chinese patients with oral lichen planus. *J Oral Pathol Med* 1990; **19**: 298-300.
- ⁷⁰ Hietanen J, Pihlman K, Forström L, Linder E, Reunala T. No evidence of hypersensitivity to dental restorative metals in oral lichen planus. *Scand J Dent Res* 1987; **95**: 320-7.
- ⁷¹ Shovelton DS. Silver amalgam and mercury allergy. *Oral Surg Oral Med Oral Pathol* 1968; **25**: 29-30.

⁷² Finne K, Coransson K, Winkler L. Oral lichen planus and contact allergy to mercury. *Int J Oral Surg* 1982; **11**: 236-9.

⁷³ Eversole LR, Ringer M. The role of restorative metals in the pathogenesis of oral lichen planus. *Oral Surg, Oral Med, Oral Pathol* 1984; **57**: 383-7.

⁷⁴ Smart ER, MacLeod RI, Lawrence CM. Resolution of lichen planus following removal of amalgam restorations in patients with proven allergy to mercury salts: a pilot study. *Br Dent J* 1995; **178**: 108-12.

⁷⁵ Skoglund A, Egelrud T. Hypersensitivity reactions to dental materials in patients with lichenoid oral mucosal lesions and in patients with burning mouth syndrome. *Scand J Dent Res* 1991; **99**: 320-8.

⁷⁶ Pang BK, Freeman S. Oral lichenoid lesions caused by allergy to mercury in amalgam fillings. *Contact Dermatitis* 1995; **33**: 423-7.

⁷⁷ Skoglund A. Value of epicutaneous patch testing in patients with oral mucosal lesions of lichenoid character. *Scand J Dent Res* 1994; **102**: 216-22.

⁷⁸ Conklin RJ, Blasberg B. Oral lichen planus. *Dermatol Clin* 1987; **5**: 663-73.

⁷⁹ Laeijendecker R, Van Joost TH. Oral manifestations of gold allergy. *J Am Acad Dermatol* 1994; **30**: 205-9.

⁸⁰ Torresani C, Nannini R, Bondi A, Guadagni M, Manara GC. Erosive lichen planus due to sensitization to cobalt chloride. *Clin Exp Dermatol* 1994; **167**: 535-6.

⁸¹ Lind P. Oral lichenoid reactions related to composite restorations. *Acta Odontol Scand* 1988; **46**: 63-5.

⁸² Schmitt CL, Alpini O, Chambers G. Clinical investigation of a new cutaneous entity. *Arch Dermatol Syph* 1945; **52**: 226-8.

-
- ⁸³ Savage J. Lichenoid dermatitis due to chloroquine. *J Dermatol* 1958; **70**: 181.
- ⁸⁴ Penneys NS, Ackerman AB, Gottlieb NL. Gold dermatitis. *Arch Dermatol* 1974; **109**: 372-6.
- ⁸⁵ Downham TF. Spironolactone-induced lichen planus (letter). *J Am Med Assoc* 1978; **240**: 1138.
- ⁸⁶ Kirby JD, Black M, McGibbon D. Levamisole induced lichenoid eruptions. *J R Soc Med* 1980; **73**: 208-11.
- ⁸⁷ Fitzpatrick TB. Lichen planus-like drug eruption. *Arch Dermatol* 1963; **88**: 352.
- ⁸⁸ Potts AJC, Hamburger J, Scully C. The medication of patients with oral lichen planus and the association of nonsteroidal anti-inflammatory drugs with erosive lesions. *Oral Surg Oral Med Oral Pathol* 1987; **64**: 541-3.
- ⁸⁹ Firth NA, Reade PC. Angiotensin-converting enzyme inhibitors implicated in oral mucosal lichenoid reactions. *Oral Surg Oral Med Oral Pathol* 1989; **67**: 41-4.
- ⁹⁰ Fry L, Withers M. Lichen planus: failure to cultivate viruses or mycoplasma. *Br J Dermatol* 1969; **80**: 384.
- ⁹¹ Holmstrup P, Schiotz AW, Hyug D, Estergaard W. Effect of dental plaque control on gingival lichen planus. *Oral Surg Oral Med Oral Pathol* 1990; **69**: 585-90.
- ⁹² Voltz MJ. Flow cytometric analysis of peripheral blood lymphocyte subsets in oral lichen planus (thesis). Queensland, Australia: University of Queensland.
- ⁹³ Olsen RG, Du Plessis DP, Barron C, Schulz EJ, Villet W. Lichen planus dermopathy: demonstration of a lichen planus specific epidermal antigen in affected patients. *J Clin Lab Immunol* 1983; **10**: 9-15.

-
- ⁹⁴ Camisa C, Allen CM, Bowen B, Olsen RG. Indirect immunofluorescence of oral lichen planus. *J Oral Pathol* 1986; **15**: 218-20.
- ⁹⁵ Rebora A. Lichen planus and the liver. *Lancet* 1981; **ii**: 805-6.
- ⁹⁶ Rebora A, Rongioletti F. Lichen planus and chronic active hepatitis. *J Am Acad Dermatol* 1984; **10**: 841.
- ⁹⁷ Rebora A, Rongioletti F, Canepa A. Chronic active hepatitis and lichen planus. *Acta Derm Venereol* 1982; **62**: 351-2.
- ⁹⁸ Gruppo Italiano Studi Epidemiologici in Dermatologia (GISED). Lichen planus and liver diseases: a multicentre case-control study. *Br Med J* 1990; **300**: 227-30.
- ⁹⁹ Powell F, Rogers R, Dickson E. Lichen planus, primary biliary cirrhosis and penicillamine. *Br J Dermatol* 1983; **9**: 540-5.
- ¹⁰⁰ Bagan JV, Milian-Masanet MA, Penarrocha-Diago M, Jimenez Y. A clinical study of 205 patients with oral lichen planus. *J Oral Maxillofac Surg* 1992; **50**: 116-8.
- ¹⁰¹ Cottoni F, Solinas A, Piga MR, Tocco A, Lissia M, Cerimele D. Lichen planus, chronic liver diseases, and immunologic involvement. *Arch Dermatol* 1988; Res **280** (Suppl): 55-60.
- ¹⁰² Nagao Y, Sata M, Tanikawa K, Itoh K, Kameyama T. Lichen planus and hepatitis C virus in the Northern Kyushu region of Japan. *Eur J Clin Invest* 1995; **25**: 910-4.
- ¹⁰³ Scully C, Potts J, Hamburger J, Wiesenfeld S, McKee JJ, el-Kom M. Lichen planus and liver disease: how strong is the association? *J Oral Pathol* 1984; **14**: 224-6.

-
- ¹⁰⁴ Tan RSH. Ulcerative colitis, myaesthesia gravis, atypical lichen planus, alopecia areata, vitiligo. *Proc R Soc Med* 1974; **67**: 195-6.
- ¹⁰⁵ Grupper C, Bourgeois-Spinasse J, Buisson J. Duhring-Broq disease followed by or associated with lichen planus. *Bull Soc Fr Syph* 1972; **79**: 231-2.
- ¹⁰⁶ Cottonnii F, Tedde G, Solinas A, Delpano A. Lichen planus associated with anti-liver-kidney microsome-positive chronic active hepatitis and hyperthyroidism. *Arch Dermatol* 1991; **127**: 1730-1.
- ¹⁰⁷ Davies MG, Corkiewicz A, Knight A, Marks R. Is there a relationship between lupus erythematosus and lichen planus? *Br J Dermatol* 1977; **96**: 145-54.
- ¹⁰⁸ Van der Horst JC, Cirkel PKS, Nieboer C. Mixed lichen planus-lupus erythematosus disease. A distinct entity? Clinical, histopathological, immunopathological studies in six patients. *Clin Exp Dermatol* 1983; **8**: 631-40.
- ¹⁰⁹ Connelly MG, Winklemann RK. Coexistence of lichen sclerosus, morphea and lichen planus. Report of four cases and review of the literature. *J Am Acad Dermatol* 1985; **12**: 844-51.
- ¹¹⁰ Neumann-Jensen B, Worsaae N, Dabelsteen E, Ullman S. Pemphigus vulgaris and pemphigus foliaceus coexisting with oral lichen planus. *Br J Dermatol* 1980; **102**: 585-90.
- ¹¹¹ Lee CW. Pemphigus vulgaris coexisting with generalised lichen planus. *J Dermatol* 1987; **14**: 388-91.
- ¹¹² Rustin MH. A case of atrophic lichen planus in association with Addisonian pernicious anaemia. *Clin Exp Dermatol* 1986; **11**: 188-90.

-
- ¹¹³ Blasberg B, Dorey JL, Stein HB, Chalmers A, Conklin RJ. Lichenoid lesions of the oral mucosa in rheumatoid arthritis patients treated with penicillamine. *J Rheum* 1984; **11**: 348-51.
- ¹¹⁴ Kaplan S, McDonald E, Marino C. Lichen planus in patient with rheumatoid arthritis treated with sulfasalazine. *J Rheum* 1995; **22**: 191-2.
- ¹¹⁵ Bermejo Fennoll A, Lopez Jornet MP. Oral lichen planus and Sjögren's syndrome. 2 cases of association. (Spanish) *Advances en Odontoestomatol* 1991; **7**: 29-38
- ¹¹⁶ Gart GS. Ulcerative and oral lichen planus associated with sicca syndrome and primary biliary cirrhosis. *Cutis* 1994; **53**: 249-50.
- ¹¹⁷ Brenner W, Diem E, Gaschnait F. Coincidence of vitiligo, alopecia areata, onychodystrophy, localized scleroderma and lichen planus. *Dermatologica* 1979; **159**: 356-60.
- ¹¹⁸ Parodi A, Gallo R, Rebora A. Lichen planus presenting with erythema-multiforme-like bullous lesions in a patient with systemic scleroderma. *Dermatol* 1995; **190**: 156-9.
- ¹¹⁹ Jubert C, Pawlotsky JM, Pouget F, Andre C, DeForges L, Bretagne S, Mavie JP, Duval J, Revuz J, Dhumeaux D, Bagot M. Lichen planus and hepatitis C virus-related chronic active hepatitis. *Arch Dermatol* 1994; **130**: 73-6.
- ¹²⁰ Mokini M, Rybojad M, Puppin D Jr, Catala S, Venezia F, Dijan R, Morel P. Lichen planus and hepatitis C virus (case report). *J Am Acad Dermatol* 1991; **24**: 792.
- ¹²¹ Nagao Y, Sata M, Noguchi S, Seno'o T, Kinoshita M, Kameyama T, Ueno T. Detection of hepatitis C virus RNA in oral lichen planus and oral cancer tissues. *J Oral Pathol & Medicine* 2000; **29**: 59-66.

¹²² Hampf BG, Malmström MJ, Aalberg VA, Hannula Ja, Vikkula J. Psychiatric disturbances in patients with oral lichen planus. *Oral Surg, Oral Med, Oral Pathol* 1987; **63**:429-32.

¹²³ Lundström I. Incidence of diabetes mellitus in patients with oral lichen planus. *Int J Oral Surg* 1983; **12**: 147-52.

¹²⁴ Borghelli RF, Pettiniari II, Chuchurru JA, Stirparo MA. Oral lichen planus in patients with diabetes: an epidemiologic study. *Oral Surg Oral Med Oral Pathol* 1993; **75**: 498-500.

¹²⁵ Lozada-Nur F, Luangjarmekorn L, Silverman S Jr, Karam J. Assessment of plasma glucose in 99 patients with oral lichen planus. *J Oral Med* 1985; **40**: 60-1.

¹²⁶ Albrecht M, Bánóczy J, Dinya E, Tamas G Jr. Occurrence of oral leukoplakia and lichen planus in diabetes mellitus. *J Oral Pathol Med* 1992; **21**: 364-6.

¹²⁷ Van Dis ML, Parks ET. Prevalence of oral lichen planus in patients with diabetes mellitus. *Oral Surg Oral Med Oral Pathol* 1995; **79**: 696-700.

¹²⁸ Grinspan D, Diaz J, Villapol LO, Schneiderman J, Berdichesky R, Palese D, *et al.* *Bull Soc Franc Dermatol Syph* 1966; **73**: 898-9.

¹²⁹ Dockrell H, Greenspan JS. Histochemical identification of T-cells in oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1979; **48**: 42-6.

¹³⁰ Smolle J. Mononuclear cell patterns in the skin. An immunohistological and morphometric analysis. *Am J Dermatopathol* 1988; **10**: 34-46.

-
- ¹³¹ Gomes MA, Schmitt D, Souteyrand P, Ohrt C, Brochier J, Thivolet J. Lichen planus and chronic graft-versus-host reaction. In situ identification of immunocompetent cell phenotypes. *J Cutan Pathol* 1982; **9**: 249-57.
- ¹³² Walsh LJ, Savage NW, Ishii T, Seymour GJ. Immunopathogenesis of oral lichen planus. *J Oral Pathol Med* 1990; **19**: 389-96.
- ¹³³ Tammi R, Hyrylainen A, Aki JE. Histologic characteristics of lichen planus transplanted onto nude mice and cultured in vitro. *Arch Dermatol Res* 1988; **280**: 23-8.
- ¹³⁴ Shiohara T, Moriya N, Tsuchiya K, Nagashima M, Narimatsu H. Lichenoid tissue reaction induced by local transfer of Ia-reactive T-cell clones. *J Invest Dermatol* 1986; **87**: 33-8.
- ¹³⁵ Becker J, Löning JH, Reichart P, Hartmann N. Oral lichen planus. Characterization of immunocompetent cells with hybridoma antibodies. *J Oral Pathol* 1983; **12**: 117-23.
- ¹³⁶ Matthews JB, Scully CM, Potts AJ. Oral lichen planus: an immunoperoxidase study using monoclonal antibodies to lymphocyte subsets. *Br J Dermatol* 1984; **3**: 587-94.
- ¹³⁷ Rich AM, Reade PC. A quantitative assessment of Langerhans cells in oral mucosal lichen planus and leukoplakia. *Br J Dermatol* 1989; **120**: 223-8.
- ¹³⁸ Murphy GF. Cell membrane glycoproteins and Langerhans cells. *Human Pathol* 1985; **16**: 103-12.
- ¹³⁹ Gonwa TA, Picker LJ, Raff HV, Goyer SM, Silver J, Stobo JD. Antigen-presenting capabilities of human monocytes correlate with their expression of HLA-DS and Ia determinants distinct from HLA-DR. *J Immunol* 1983; **130**: 706-11.

-
- ¹⁴⁰ Shimada S, Caughman SW, Sharrow SO, Stephany D, Katz SI. Enhanced antigen-presenting capacity of cultured Langerhans Cells is associated with markedly increased expression of Ia antigen. *J Immunol* 1987; **139**: 2551-6.
- ¹⁴¹ Rheins LA, Nordlund JJ. Modulation of the population density of identifiable epidermal Langerhans cells associated with enhancement or suppression of cutaneous immune reactivity. *J Immunol* 1987; **136**: 867-76.
- ¹⁴² Chen ES-Y, Thornhill M, Zakrzewska J. Interventions for treating oral lichen planus. *Cochrane Database of Systemic Reviews* 2001; **1**:1-19.
- ¹⁴³ Lehner T, Lyne C. Adrenal function during topical oral corticosteroid treatment. *Br Med J* 1969; **4**: 138-41.
- ¹⁴⁴ Plemons JM, Rees TD, Zachariah NY. Absorption of a topical steroid and evaluation of adrenal suppression in patients with erosive lichen planus. *Oral Surg Oral Med Oral Path* 1990; **69**: 688-93.
- ¹⁴⁵ Beckman BI. Valisone aerosol spray contraindicated in mucous membranes (letter). *J Am Acad Dermatol* 1981; **4**: 233.
- ¹⁴⁶ Vincent SD, Fotos PG, Baker KA, Williams TP. Oral lichen planus: the clinical, historical and therapeutic features of 100 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1990; **70**: 165-71.
- ¹⁴⁷ Thongprasom K, luangjarmekorn L, Sererat T, Taweasap W. Relative efficacy of fluocinolone acetonide compared with triamcinolone acetonide in the treatment of oral lichen planus. *J Oral Pathol Med* 1992; **21**: 456-8.
- ¹⁴⁸ Tyldesley WR, Harding SM. Betamethasone valerate aerosol in the treatment of oral lichen planus. *Br J Dermatol* 1977; **96**: 659-62.

-
- ¹⁴⁹ Lozada-Nur F, Huang MZ. Open preliminary clinical trial of clobetasol proprionate ointment in adhesive paste for treatment of chronic oral vesiculo-erosive diseases. *Oral Surg Oral Med Oral Pathol* 1991; **71**: 283-7.
- ¹⁵⁰ Carbone M, Conrotto D, Carozzo M Broccoletti R, Gandolfo S, Scully C. Topical corticosteroids in association with miconazole and chlorhexidine in the long-term management of atrophic-erosive oral lichen planus: a placebo-controlled and comparative study between clobetasol and fluocinonide. *Oral Dis* 1999; **5**: 44-9.
- ¹⁵¹ Zegarelli DJ. Multimodality steroid therapy of erosive and ulcerative oral lichen planus. *J Oral Med* 1983; **38**: 127-30.
- ¹⁵² Setterfield JF, Black MM, Challacombe SJ. The management of oral lichen planus. *Clin Exp Dermatol* 2000; **25**: 176-82.
- ¹⁵³ Dusek J, Frick W. Lichen planus: oral manifestations and suggested treatments. *J Oral Maxillofac Surg* 1982; **40**: 240-4.
- ¹⁵⁴ Gunther S. The therapeutic value of retinoic acid (vitamin A acid) in lichen planus of the oral mucous membranes. *Dermatologica* 1973; **147**: 130-6.
- ¹⁵⁵ Handler HL. Isotretinoin for oral lichen planus (letter to the editor). *J Am Acad Dermatol* 1984; **10**: 674.
- ¹⁵⁶ Hersle K, Mobacken H, Sloberg K, Thilander H. Severe oral lichen planus: treatment with an aromatic retinoid (etretinate). *Br J Dermatol* 1982; **106**: 77-80.
- ¹⁵⁷ Sloberg K, Hersle K, Mobacken H, Thilander H. Severe oral lichen planus: remission and maintenance with vitamin A analogues. *J Oral Pathol* 1983; **12**: 473-7.

-
- ¹⁵⁸ Stern RS. Treatment of oral lichen planus with low-dose isotretinoin (letter to the editor). *J Am Acad Dermatol* 1984; **11**: 527-8.
- ¹⁵⁹ Woo TY. Systemic isotretinoin treatment of oral and cutaneous lichen planus. *Cutis* 1985; **35**: 385-93.
- ¹⁶⁰ Ferguson M, Simpson N, Hammersley N. The treatment of erosive lichen planus with a retinoid-etretinate. *Oral Surg Oral Med Oral Pathol* 1984; **58**: 283-7.
- ¹⁶¹ Regezi JA, Ellis CN, Stewart JC, Giustina TA, Arbor A. Histologic changes associated with the topical use of isotretinoin on oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1986; **61**: 479-84.
- ¹⁶² Zegarelli DJ. Treatment of oral lichen planus with topical vitamin A acid. *J Oral Med* 1984; **39**: 186-91.
- ¹⁶³ Jungell P, Malmstrom M. Cyclosporin A mouthwash in the treatment of oral lichen planus. *Int J Oral & Maxillofac Surg* 1996; **25**: 60-2.
- ¹⁶⁴ Sieg P, Domarus H, Von Zitzewitz V, Iven H, Farber L. Topical cyclosporine in oral lichen planus: a controlled, randomised, prospective trial. *Br J Dermatol* 1995; **132**: 790-4.
- ¹⁶⁵ Harpenau LA, Plemons JM, Rees TD. Effectiveness of a low dose of cyclosporine in the management of patients with oral erosive lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **80**: 161-7.
- ¹⁶⁶ Eisen D, Ellis CN, Duell Ea, Griffiths CEM, Voorhees JJ. Effect of topical cyclosporine rinse on oral lichen planus. *N Eng J Med* 1990; **323**: 290-4.
- ¹⁶⁷ Epstein JB, Truelove EL. Topical cyclosporine in a bioadhesive for treatment of oral lichenoid mucosal reactions: an open label clinical trial. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; **82**: 532-6.

-
- ¹⁶⁸ Voute AB, Schulten EA, Langendijk PN, Nieboer C, van der Waal I. Cyclosporin A in an adhesive base for treatment of recalcitrant oral lichen planus. An open trial. *Oral Surg Oral Med Oral Pathol* 1994; **78**: 437-41.
- ¹⁶⁹ Araujo OE, Flowers FP, King MM. Griseofulvin: a new look at an old drug. *Annals Pharmacotherapy* 1990; **24**: 851-4.
- ¹⁷⁰ Naylor GD. Treating erosive lichen planus with griseofulvin: a report of four cases. *Quintessence Int* 1990; **21**: 943-7.
- ¹⁷¹ McCreary CE, McCartan B. Clinical management of oral lichen planus. *Br J Oral & Maxillofac Surg* 1999; **37**: 338-43.
- ¹⁷² Sehgal VN, Bikhchandani R, Koranne RV, Nayar M, Saxena HM. Histopathological evaluation of griseofulvin therapy in lichen planus – a double-blind controlled study. *Dermatologica* 1980; **161**: 22-7.
- ¹⁷³ Aufdemorte TB, DeVillez RL, Gieseke DR. Griseofulvin in the treatment of three cases of oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1983; **55**: 459-62.
- ¹⁷⁴ Bagan JV, Silvestre FJ, Mestre S, Gisbert C, Bermejo, Agramunt J. Treatment of lichen planus with griseofulvin: report of seven cases. *Oral Surg Oral Med Oral Pathol* 1985; **60**: 608-10.
- ¹⁷⁵ Matthews RW, Scully C. Griseofulvin in the treatment of oral lichen planus: adverse drug reactions, but little beneficial effect. *Ann Dent* 1992; **51**: 10-1.
- ¹⁷⁶ Beck HI, Brandrup F. Treatment of erosive lichen planus with dapsone. *Act Dermato-Venereologica* 1986; **66**: 366-7.

¹⁷⁷Falk DK, Latour DL, King LE Jr. Dapsone in the treatment of erosive lichen planus. *J Am Ac Dermatol* 1985; **12**: 567-70.

¹⁷⁸Boyd AS. New and emerging therapies for lichenoid dermatoses. *Derm Clin* 2000; **18**: 21-9.

¹⁷⁹LuLu SY, Chen WJ, Eng HL. Dramatic response to Levamisole and low-dose prednisolone in 23 patients with oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **80**: 705-9.

¹⁸⁰Klein LR, Callen JP. Azathioprine: Effective steroid-sparing therapy for generalized lichen planus. *Southern Med J* 1992; **85**: 198-201.

¹⁸¹Rapini RP, Jordon RE, Wolverton SE. Chapter 5:Cytotoxic agents in "Systemic drugs for skin diseases" (Editors Wolverton SE, Wilkin JK) W.B Saunders 1991, 130-5.

¹⁸²Lear JT, English JS. Erosive and generalized lichen planus responsive to azathioprine. *Clin Exp Dermatol* 1996; **21**: 26-57.

¹⁸³Camisa C, Popovsky JL. Effective treatment of oral erosive lichen planus with thalidomide. *Arch Dermat.* 2000; **136**: 1442-3.

¹⁸⁴Dereure O, Basset-Seguin N, Guilhou JJ, Erosive lichen planus: dramatic response to thalidomide. *Arch Dermatol* 1996; **132**: 1392-3

¹⁸⁵Jubran RF, Dinndorf PA. Successful therapy of refractory graft versus host disease with tacrolimus and psoralen plus ultraviolet light. *Ther Drug Monit* 1998; **20**: 236-9.

¹⁸⁶Kaliakatsou F, Hodgson TA, Lewsey JD, Hegarty AM, Murphy AG, Porter SR. Management of recalcitrant ulcerative oral lichen planus with topical tacrolimus. *J Am Acad Dermatol* 2002; **46**: 35-41.

-
- ¹⁸⁷ Rozycki TW, Rogers RS 3rd, Pittelkow MR, McEvoy MT, el-Azhary RA, Bruce AJ, Fiore JP, Davis MD. Topical tacrolimus in the treatment of symptomatic oral lichen planus: a series of 13 patients. *J Am Acad Dermatol* 2002; **46**: 27-34.
- ¹⁸⁸ Vente C, Reich K, Rupprecht R, Neumann C. Erosive mucosal lichen planus: response to topical treatment with tacrolimus. *Brit J Dermatol* 1999; **140**: 338-42.
- ¹⁸⁹ Lundquist G, Forsgren H, Gajecki M, Emtestam L. Photochemotherapy of oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **79**: 554-8.
- ¹⁹⁰ Kuusilehto A, Lehtinen R, Happonen RP, Heikinheimo K, Lehtimäki K, Jansen CT. An open clinical trial of a new mouth-PUVA variant in the treatment of oral lichenoid lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol & Endod* 1997; **84**: 502-5.
- ¹⁹¹ Viander M, Jansen CT, Eskola J. Influence of whole-body PUVA treatments on peripheral blood: NK cell activity. *Photodermatol* 1984; **1**: 23-9.
- ¹⁹² Becherel PA, Bussell A, Chosidow O, Rabian C, Piette JC, Frances C. Extracorporeal photochemotherapy for chronic erosive lichen planus. *Lancet*. 1998; **351**: 805.
- ¹⁹³ Skinner RB Jr. Psoralens Chapter 9 In "Systemic drugs for skin diseases" (edited by Wolverton SE, Wilkin JK) WB Saunders Co 1991 pp 219-46.
- ¹⁹⁴ Miles DA, Howard MM. Diagnosis and management of oral lichen planus. *Derm Clin* 1996; **14**: 281-90.
- ¹⁹⁵ Baujeeb W, Kraivaphan P, Pobruska C. Efficacy of topical retinoic acid compared with topical fluocinolone acetonide in the treatment of oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997; **83**: 21-5.

-
- ¹⁹⁶ Giustina TA, Stewart JC, Ellis CN, Regezi JA, Annesley T, Woo TY, Voorhees JJ. Topical application of isotretinoin gel improves oral lichen planus. *Arch Dermatol* 1986; **122**: 534-6.
- ¹⁹⁷ Hersle K, Mobacken H, Slobere K, Thilander H. Severe oral lichen planus: treatment with an aromatic retinoid (etretinate). *Br J Dermatol* 1982; **106**: 77-80.
- ¹⁹⁸ Tal H, Rafkin B. Cryosurgical treatment of gingival lichen planus: report of a case. *J Am Dent Assoc* 1986; **113**: 629-31.
- ¹⁹⁹ Malurström M, Leikomaa H. Experiences with cryosurgery in the treatment of oral lesions. *Proc Finn Dent Soc* 1980; **76**: 117-23.
- ²⁰⁰ Leopard PJ, Poswillo DE. Practical cryosurgery for oral lesions. *Br Dent J* 1974; **136**: 185-96.
- ²⁰¹ Vedtofte P, Homstrup P, Hjørting-Hansen E, Pindborg JJ. Surgical treatment of premalignant lesions of the oral mucosa. *Int J Oral Maxillofac Surg* 1987; **16**: 656-64.
- ²⁰² Loh HS. A clinical investigation of the management of oral lichen planus with CO₂ laser surgery. *J Clin Laser Med & Surg* 1992; **10**: 445-9.
- ²⁰³ Salem G. Oral lichen planus among 4277 patients from Gizan, Saudi Arabia. *Comm Dent Oral Epidemiol* 1989; **17**: 322-4.
- ²⁰⁴ Barnard NA, Scully C, Eveson JW, Cunningham S, Porter SR. Oral cancer development in patients with oral lichen planus. *J Oral Pathol Med* 1992; **22**: 421-4.
- ²⁰⁵ Krutchkoff DJ, Cutler L, Laskowski S. Oral lichen planus: the evidence regarding potential malignant transformation. *J Oral Pathol* 1978; **7**: 1-7.

-
- ²⁰⁶ van der Meij EH, Schepman KP, Smeele LE, van der Wal JE, Bezemer PD, van der Waal I. A review of the recent literature regarding malignant transformation of oral lichen planus. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol Endod* 1999; **88**: 307-10.
- ²⁰⁷ Silverman S, Gorsky M, Lozada-Nur F. A prospective follow-up study of 570 patients with oral lichen planus: Persistence, remission, and malignant association. *Oral Surg Oral Med Oral Pathol* 1985; **60**: 30-4.
- ²⁰⁸ Silverman S Jr, Gorsky M, Lozada-Nur F, Giannotti K. A prospective study of findings and management in 214 patients with oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1991; **72**: 665-70.
- ²⁰⁹ Lozada-Nur F. Oral lichen planus and oral cancer: Is there enough epidemiologic evidence? *Oral Surg, Oral Med, Oral Pathol, Oral Radiol Endod* 2000; **3**: 265-6.
- ²¹⁰ Block G, Patterson B, Subar A. Fruit, vegetables and cancer prevention: a review of the epidemiologic evidence. *Nutr Cancer* 1992; **18**: 1-29.
- ²¹¹ McLauchlin JK, Gridley G, Block G, Winn DM, Preston-Martin S, Schoenberg JB et al. *J Natl Cancer Inst* 1988; **80**: 1237-43.
- ²¹² Sigurgeirsson B. Skin disease and malignancy. An epidemiological study. *Acta Dermato-Venereologica. Suppl* 1992; **178**: 1-110.
- ²¹³ Otsubo H, Yokoe H, Miya T, Atsuta F, Miura N, Tanzawa H, Sato K.. Gingival squamous cell carcinoma in a patient with chronic graft-versus-host disease. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997; **84**: 171-4.
- ²¹⁴ Kim J, Yook JI, Lee EH, Ryu MH, Yoon JH, Hong JC, Kim DJ, Kim HS. Evaluation of premalignant potential in oral lichen planus using interphase cytogenetics. *J Oral Pathol Med* 2001; **30**: 65-72.

-
- ²¹⁵ Zhang L, Michelsen C, Cheng X, Zeng T, Priddy R, Rosin MP. Molecular analysis of oral lichen planus. A premalignant lesion? *Am J Pathol* 1997; **151**: 323-7.
- ²¹⁶ Zhang L, Cheng X, Li Y, Poh C, Zeng T, Priddy R, Lovas J, Freedman P, Daley T, Rosin MP. High frequency of allelic loss in dysplastic lichenoid lesions. *Lab Invest* 2000; **80**: 233-7.
- ²¹⁷ Rajentheran R, McLean NR, Kelly CG, Reed MF, Nolan A. Malignant transformation of oral lichen planus. *Eur J Surg Oncol* 1999; **25**: 520-3.
- ²¹⁸ de Villiers EM. Taxonomic classification of Papillomaviruses. *Papillomavirus Rep* 2001; **12**: 57-63.
- ²¹⁹ Shope RE, Hurst EW. Infectious papillomatosis of rabbits. *J Exp Med* 1933; **58**: 607-23.
- ²²⁰ Garland SM. Human papillomavirus update with particular focus on cervical disease. *Pathology* 2002; **34**: 213-24.
- ²²¹ Chang F, Syrjänen S, Kellokoski J, Syrjänen K. Human papillomavirus (HPV) infections and their associations with oral disease. *J Oral Pathol Med* 1991; **20**: 305-17.
- ²²² Giri I, Danos O. Papillomavirus genomes: From sequence data to biological properties. *Trends Genet* 1986; **2**: 227-32.
- ²²³ Garlick JA, Taichman LB. Human papillomavirus infection of the oral mucosa. (Review) *Am J Dermatopathol* 1991; **13**: 143-9.
- ²²⁴ Heilman CA, Engel L, Lowy DR, Howley PM. Virus specific transcription in bovine papillomavirus-transformed mouse cells. *Virology* 1982; **119**: 22-34.

-
- ²²⁵ Doorbar J, Ely S, Sterlin J, et al. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature* 1991; **352**: 824-7.
- ²²⁶ Turek LP, Smith EM. The genetic program of genital human papillomaviruses in infection and cancer. *Obs & Gyn Clin of N America* 1996; **23**: 735-57
- ²²⁷ McKaig RG, Baric RS, Olshan AF. Human papillomavirus and head and neck cancer: Epidemiology and molecular biology. *Head & Neck* 1998; **20**: 250-65.
- ²²⁸ Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 1990; **248**: 76-9.
- ²²⁹ Terai M, Takagi M. Human papillomavirus in the oral cavity. *Oral Med Pathol* 2001; **6**: 1-12.
- ²³⁰ Adler-Storthz K, Newland JR, Tessin BA, et al. Identification of human papillomavirus types in oral verruca vulgaris. *J Oral Pathol* 1986; **15**: 230-3.
- ²³¹ de Villiers . Papillomavirus and HPV typing. *Clinics in Dermatology* 1997; **15**: 199-206.
- ²³² Sang BC, Barbosa MS. Single amino acid substitutions in "low-risk" human papillomavirus (HPV) type 6 E7 protein enhance features characteristic of the "high-risk" HPV E7 oncoproteins. *Proc Natl Acad Sci USA* 1992; **89**: 8063-7.
- ²³³ Morrison EA, Ho GYF, Vermund SH, Goldberg GL, Kadish AS, Kelley KF, Burk RD. Human papillomavirus infection and other risk factors for cervical neoplasia: A case control study. *Int J Cancer* 1991; **49**: 6-13.

-
- ²³⁴ Richart RM, Masson S, Syrjänen KJ, Vassilakos P, et al. Human papillomavirus: IAC Task Force Summary. *Acta Cytol* 1998; **42**: 50-8.
- ²³⁵ Richart RM, Wright TC. The pathology of cervical neoplasia. In "Intraepithelial Neoplasia of the Lower Genital Tract". Edited by Luesley D, Jordan J, Richart RM. New York, Churchill Livingstone, 1995.
- ²³⁶ Swygart C. Human papillomavirus: disease and laboratory diagnosis. *British J Biomed Science* 1997; **54**: 299-303.
- ²³⁷ Syrjänen KJ, Heinonen UM, Kauraniemi T. Cytological evidence of the association of condylomatous lesions with the dysplastic and neoplastic changes in uterine cervix. *Acta Cytol* 1981; **25**: 17-22.
- ²³⁸ Sugerman PB, Shillito EJ. The high risk human Papillomaviruses and oral cancer: evidence for and against a causal relationship. *Oral Diseases* 1997; **3**: 130-47.
- ²³⁹ Syrjänen KJ. Human papillomavirus (HPV) infection of the female genital tract and their associations with intraepithelial neoplasms and squamous cell carcinoma. *Pathol Annu* 1986; **21**: 53-87.
- ²⁴⁰ Brandsma J, Burk RD, Lancaster WD, Pfister H, Schiffman MH. Inter-laboratory variation as an explanation for varying prevalence estimates of human papillomavirus infection. *Int J Cancer* 1989; **43**: 260-2.
- ²⁴¹ Sryjanen SM. Basic concepts and practical applications of recombinant DNA techniques in detection of human papillomavirus infection. Review article. *APMIS* 1990; **98**: 95-110.
- ²⁴² Baay MF, Quint WG, Koudstaal J, Hollema H, Duk JM, Burger MP, Stolz E, Herbrink P. Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by PCR in paraffin-embedded cervical carcinomas. *J Clin Microbiol* 1996; **34**: 745-7.

²⁴³ de Roda Husman AM, Walboomers JMM, van den Brule AJC, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1985; **76**: 1057-62.

²⁴⁴ Miller CS, Zeuss MX, White DK. Detection of HPV DNA in oral carcinoma using polymerase chain reaction together with in situ hybridization. *Oral Surg Oral Med Oral Pathol* 1994; **77**: 480-6.

²⁴⁵ Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I. Human papillomavirus in normal oral mucosa: a comparison of methods for sample collection. *J Oral Pathol Med* 1992; **21**: 265-9.

²⁴⁶ Ostwald C, Muller P, Barten M, Rutsatz K, Sonnenburg M, Milde-Langosch K, Loning T. Human papillomavirus DNA in oral squamous cell carcinomas and normal mucosa. *J Oral Pathol Med* 1994; **23**: 220-5.

²⁴⁷ Miller CS, White DK. Human papillomavirus expression in oral mucosa, premalignant conditions and squamous cell carcinoma: A retrospective review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; **82**: 57-68.

²⁴⁸ Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *New Eng J Med* 1998; **7**: 423-8.

²⁴⁹ Jungell P, Konttinen YT, Nortamo P, Malmstrom M. Immunoelectron microscopic study of distribution of T cell subsets in oral lichen planus. *Scand J Dent Res* 1989; **10**: 79-86.

²⁵⁰ Sugerman PB, Satterwhite K, Bigby M. Autocytotoxic T-cell clones in lichen planus. *Br J Dermatol* 2000; **142**: 449-56.

-
- ²⁵¹ Martz E, Howell DM. CTL: virus control cells first and cytolytic cells second? DNA fragmentation, apoptosis and the prelytic halt hypothesis. *Immunol Today* 1989; **10**: 79-86.
- ²⁵² Shibata S, Kyuwa S, Lee SK, Toyoda Y, Goto N. Apoptosis induced in mouse hepatitis virus-infected cells by a virus specific CD8₊ cytotoxic T-lymphocyte clone. *J Virol* 1994; **68**: 7540-55.
- ²⁵³ Meissner JD. Nucleotide sequences and further characterization of human papillomavirus DNA present in the CaSki and HeLa cervical carcinoma cell lines. *J General Virol* 1999; **80**: 1725-33.
- ²⁵⁴ Callahan DE, Karim A, Zheng G, Ts'o PO, Lesko SA. Quantitation and mapping of integrated human metaphase chromosomes using a fluorescence microscope imaging system. *Cytometry* 1992; **13**: 453-61.
- ²⁵⁵ Mincheva A, Gissmann L, zur Hausen H. Chromosomal integration sites of human papillomavirus DNA in three cervical cancer cell lines mapped by in situ hybridization. *Med Microbiol & Immunol* 1987; **176**: 245-56.
- ²⁵⁶ Popescu NC, DiPaolo JA. Preferential sites for viral integration on mammalian genome. *Cancer Genetics & Cytogen* 1989; **42**: 157-71.
- ²⁵⁷ Patillo RA, Husa RO, Story MT, Ruckert ACF, Shalaby MR, Mattingly RF. Tumor antigen and human chorionic gonadotropin in CaSki cells: a new epidermoid cervical cancer cell line. *Science* 1997; **196**: 1456-8.
- ²⁵⁸ Jones HW Jr, McKusich VA, Harper PS, Wu KD. The HeLa cell line and a reappraisal of its origin. *Obstet & Gynecol* 1971; **38**: 945-59.
- ²⁵⁹ Resnick RM, Cornelissen MT, Wright DK, Eichinger GH, Fox HS, ter Schegget J, Mannos MM. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. *J Nat Cancer Inst* 1990; **82**: 1477-84.

-
- ²⁶⁰ de Roda Husman AM, Walboomers JM, van den Brule Aj, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J General Virol* 1995; **76**: 1057-62.
- ²⁶¹ Ha PK, Pai SI, Westra WH, Gillison ML, Tong BC, Sidransky D, Califano JA. Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. *Clin Cancer Res* 2002; **8**: 1203-9.
- ²⁶² Jimenez C, Correnti M, Salma N, Cavazza ME, Perrone M. Detection of human papillomavirus DNA in benign oral squamous epithelial lesions in Venezuela. *J Oral Pathol & Med* 2001; **30**: 385-8.
- ²⁶³ Sand L, Jalouli J, Larsson PA, Hirsch JM. Human papillomavirus in oral lesions. *Anticancer Res* 2000; **20**: 1183-8.
- ²⁶⁴ Nielsen H, Norrild B, Vedtofte P, Praetorius F, Reiel J, Holmstrup P. Human papillomavirus in oral premalignant lesions. *Eur J Canc Pt B, Oral Oncol* 1996; **32**: 264-70.
- ²⁶⁵ Cox M, Maitland N, Scully C. Human herpes simplex-1 and papillomavirus type 16 homologous DNA sequences in normal, potentially malignant and malignant oral mucosa. *Eur J Canc Pt B Oral Oncol* 1993; **29**: 215-9.
- ²⁶⁶ Miller CS, White DK, Royse DD. In situ hybridization analysis of human papillomavirus in orofacial lesions using a consensus biotinylated probe. *Am J Dermatopathol* 1993; **15**: 256-9.
- ²⁶⁷ Young SK, Min KW. In situ DNA hybridization analysis of oral papillomas, leukoplakias and carcinomas for human papillomavirus. *Oral Surg Oral Med Oral Pathol* 1991; **71**: 726-9.

²⁶⁸ Jontell M, Watts S, Wallstrom M, Levin L, Sloberg K. Human papillomavirus in erosive oral lichen planus. *J Oral Pathol & Med* 1990; **19**: 273-7.

²⁶⁹ Maitland NJ, Cox MF, Lynas C, Prime SS, Meanwell CA, Scully C. Detection of human papillomavirus DNA in biopsies of human oral tissue. *Br J Cancer* 1987; **56**: 245-50.

²⁷⁰ Syrjänen SM, Syrjänen KJ, Lamberg MA. Detection of human papillomavirus DNA in oral mucosal lesions using in situ -- DNA hybridization applied on paraffin sections. *Oral Surg Oral Med Oral Pathol* 1986; **62**: 660-7.

²⁷¹ Bouquot JC, Young S, Fisher M. HPV-16 and -18 in oral leukoplakia. A comparative PCR analysis of 341 cases and controls. Abstract from the Proceedings from the International Association of Oral Pathologists, International Congress of Oral Pathology and Medicine, 11th Biennial Meeting August 2002.

CASE PRESENTATIONS

By

ANASTASIA FOTINI GEORGIUO BDS

Presented for the degree of Masters of Dental Science
(Oral Medicine Oral Pathology) of the University of Sydney, New South Wales

2002

Department of Oral Pathology, Faculty of Dentistry,
The University of Sydney, New South Wales

INVESTIGATION OF A PAROTID SWELLING

A Case Presentation

INVESTIGATION OF A PAROTID SWELLING

SYNOPSIS

A case report of a patient who presented with a swelling involving the left angle of the mandible.

CASE PRESENTATION

Name: MR
Gender: Female
Age: 68 years (15/11/1933)
MRN: 081 69 93

CHIEF COMPLAINT

Mrs MR presented to the Westmead Centre for Oral Health in September 2001 with a loose fixed prosthesis of the maxillary right quadrant. This 3-unit cantilever bridge, with the maxillary right canine as the abutment was recemented. At the time of examination a swelling was noted in the area of the left angle of mandible. No dental cause for this swelling was identified at the time of examination and the patient was referred to the Oral Medicine Unit for further investigation.

HISTORY OF CHIEF COMPLAINT

The patient reported that she has been aware of the swelling visually, and thought that it had been present for “a couple of years”. She stated that the swelling had not changed in size or shape, and that it was asymptomatic. She could not recall an associated trauma with the onset of this swelling. No aggravating or relieving factors were reported by the patient.

MEDICAL HISTORY

Mrs. MR had a transient ischaemic attack in 1994, with no residual neurological deficit. She has well-controlled hypertension, which is managed with Avapro™, an angiotensin II receptor antagonist. She has psoriasis which has previously been managed with topical *Celestone*™ (betamethasone 0.02%) and methotrexate. Currently she is being well managed with Celebrex™ (celecoxib), a cox-II inhibitor. She reported no known allergies. The patient reports that she does not imbibe alcohol.

She has smoked for 45-years, at a current level of approximately 10 cigarettes per day.

DENTAL HISTORY

The patient attends Westmead Centre for Oral Health for her general dental care. She is a partially dentate and does not have any dental prosthesis.

SOCIAL HISTORY

Mrs MR was born in Australia. She is now retired. She is married with two grown children. She occasionally takes care of her grandchildren.

She attended her appointments with her husband.

EXTRA ORAL EXAMINATION

On extra oral examination a swelling was noted in the pre-auricular region overlying the angle of the mandible. This mass was firm and not tender to palpation. The mass was not fixed to the underlying tissues or overlying skin, and measured approximately 3 x 5 cm.

It was quite separate from the left sternocleidomastoid muscle. Displacement of the left earlobe was apparent.

There was no regional lymphadenopathy, and no limitation in oral opening. There was no swelling of the submandibular and sublingual spaces. There was no sensory loss or hyperaesthesia of the skin in the vicinity of the swelling. She was able to discriminate between two points during extra-oral examination. Facial movements were intact and speech was unimpaired.

INTRA ORAL EXAMINATION

The patient was partially dentate, with no removable dental prosthesis. Clinical and radiographical examination failed to demonstrate a dental cause for this swelling. The mass was not palpable intra-orally. The soft tissues were non-remarkable.

Minimal clear saliva was expressed from the left parotid duct in comparison with the right duct where clear saliva was freely expressed.

PROVISIONAL DIAGNOSIS

- LEFT PAROTID MASS

INVESTIGATIONS ARRANGED

- Fine needle aspiration biopsy of the left parotid mass – November 2001
- Computed tomography with contrast, from the skull base to the thoracic inlet

INTERPRETATION OF RESULTS - FNA

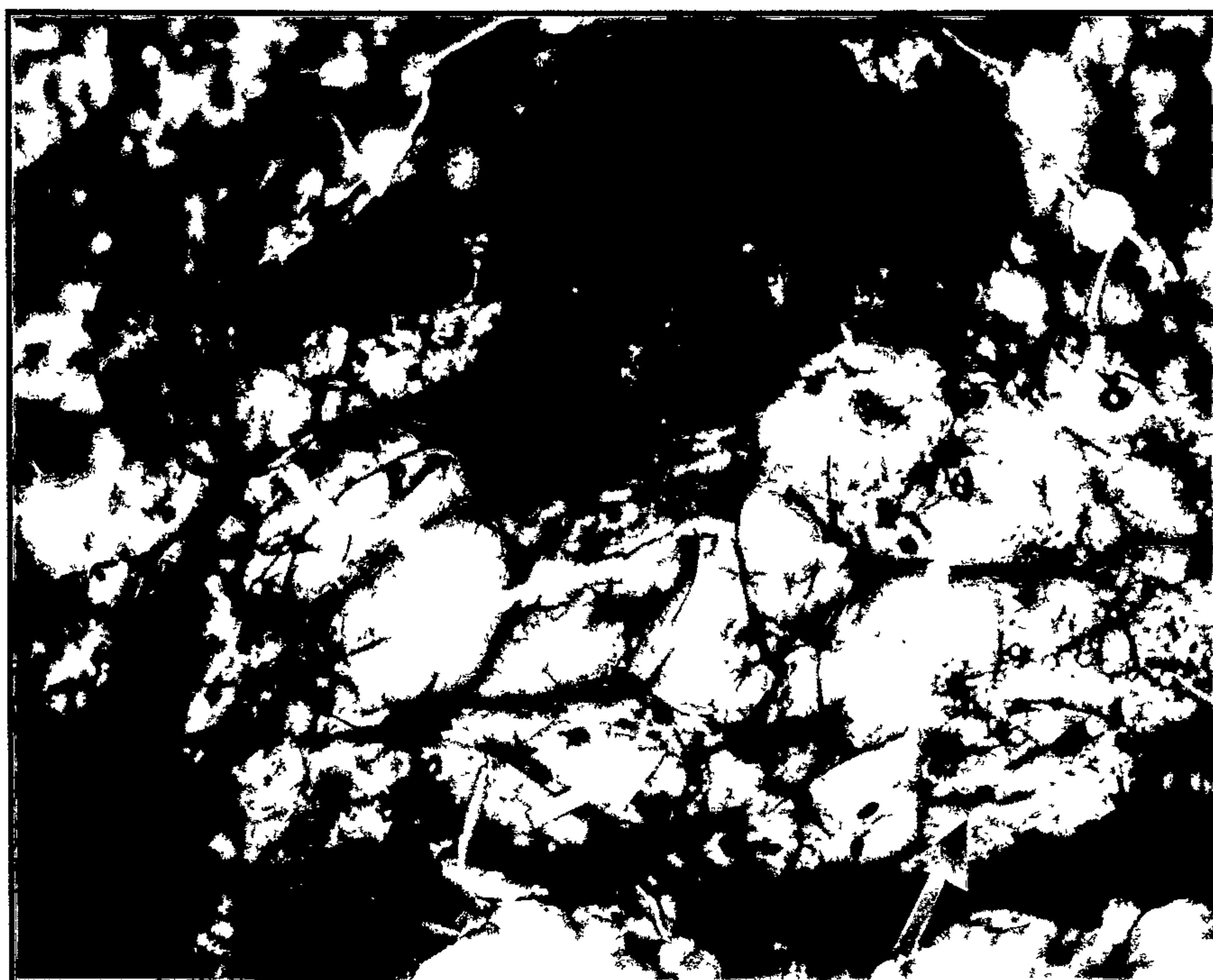


Figure 1. Wet-fixed slide of material obtained from fine needle aspirate of left parotid mass (November 2001). Yellow arrow demonstrates acini, and blue arrow identifies ductal epithelium (Papanicolau stain, x40 magnification).

The cellular smears from the FNA of the left parotid mass in November 2001, demonstrated numerous intact acini, some in clusters. The acini cells were uniform with a low nuclear to cytoplasmic ratio. Ductal epithelium was also noted. The background shows abundant blood contamination and a small amount of amorphous granular material and cell debris. Material was also collected and placed in Hank's medium for a cell block preparation. The sample was centrifuged and the resultant pellet was embedded. Haematoxylin and eosin stain sections only showed blood.

The comments made from the fine needle aspiration biopsy were that the cytological appearances are those of “benign parotid acini and ducts only”, and that these may not be representative of the lesion.

INTERPRETATION OF COMPUTED TOMOGRAPHY SCANS

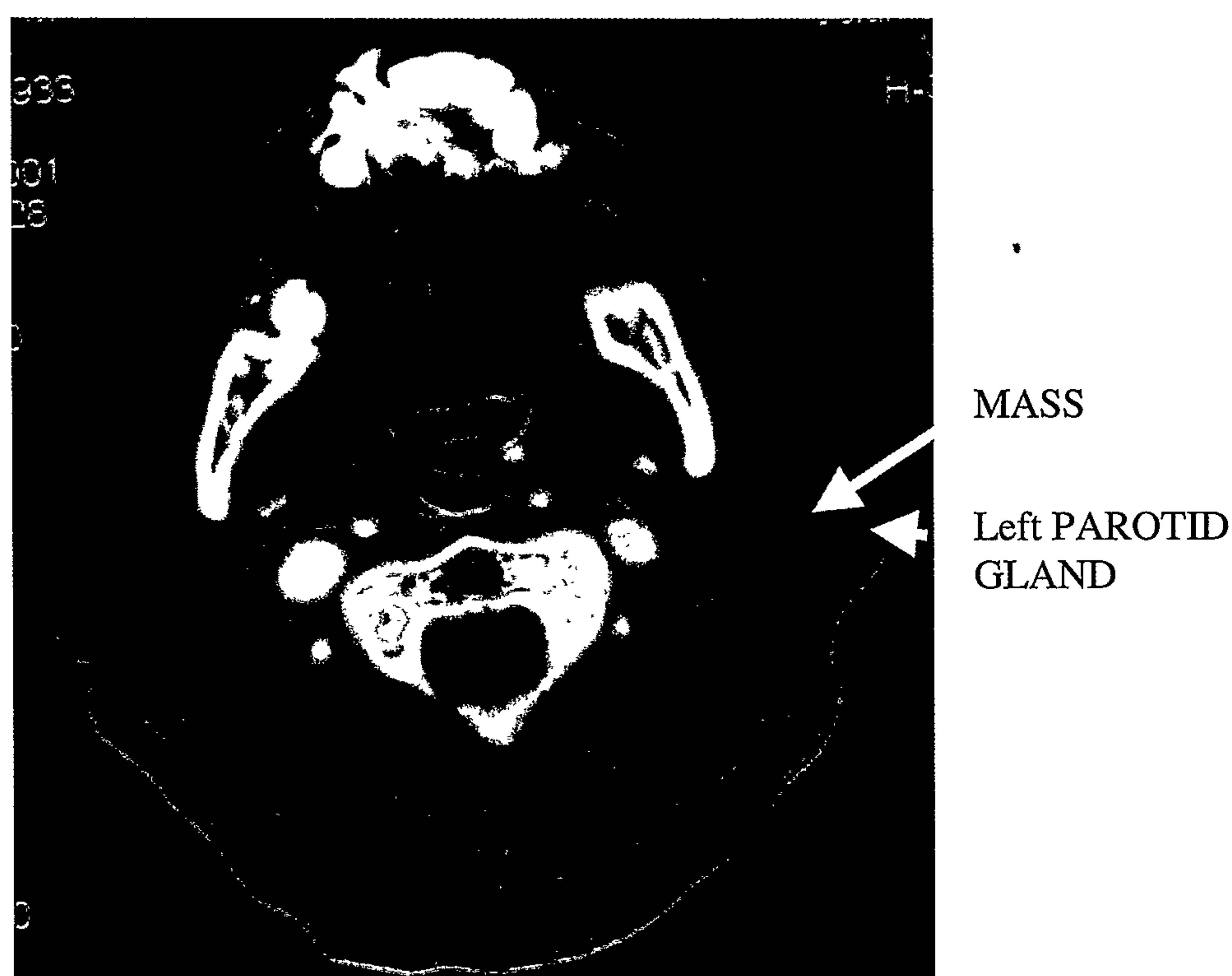


Figure 2. Computed tomography with contrast, axial section demonstrating left mass involving the left parotid gland.

In the computed tomography a well circumscribed mass was seen in the inferior aspect of the left parotid gland measuring 3x3x3 cm (*Figure 2*). The remainder of the left parotid gland outlined normally. The right parotid gland was normal and there was no cervical lymphadenopathy.

The sections extended to the thoracic inlet which revealed a second lesion. A large mass was seen 8x7x7 cm adjacent to the mediastinum a large mass (*Figure 3*), with mediastinal nodes noted. No bone destruction was seen.

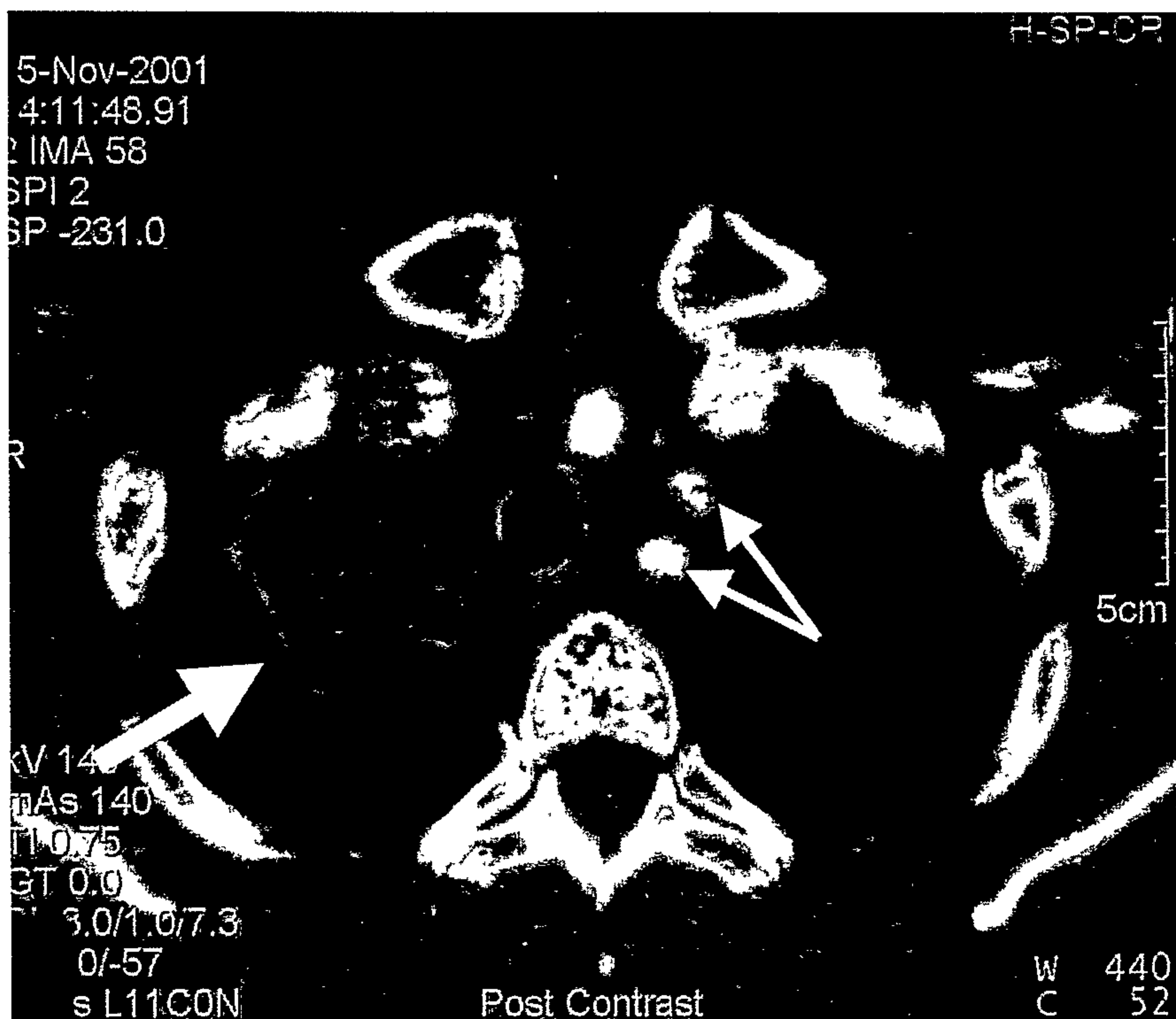


Figure 3. Axial section of computed tomography scan post contrast showing mass right of the mediastinum (wide arrow) with mediastinal nodes (thinner arrows).

INTERPRETATION OF RESULTS

Computed tomography confirmed the presence of a mass involving the inferior pole of the left parotid gland. The fine needle aspiration biopsy cytology was most probably not representative of this lesion. The limitations of aspiration cytology will be discussed in the second part of this case report. Further investigations were required to identify the nature of this lesion.

The finding of a lung mass was most unexpected and required urgent attention. There were several alternative possible explanations for the findings:-

- Lung primary with a metastasis in the parotid gland
- Primary in the parotid gland with a secondary lung deposit
- Unrelated pathology

FURTHER INVESTIGATIONS

The patient was reviewed and the findings from the initial investigations discussed with her. Regarding the lung mass, Mrs. MR reported that she has had symptoms of “whooping cough” for approximately 4 months. In consultation with the Head and Neck Cancer Unit, Westmead Hospital it was decided that a computed tomography scan of the chest should be performed as an initial investigation with referral to the Lung Cancer Clinic. Following the initial consultation at the Lung Cancer Clinic, further fine needle aspiration biopsies with imaging guidance, were arranged, this time.

- Computed Tomography – CHEST

Right upper lobe mass (5cm) which involves the right hilum, abuts and invades the right side of the mediastinum and extends to the apex. No evidence of any distant metastases in the liver or adrenals.

- Fine needle aspiration (ultrasound guidance) – RIGHT UPPER LOBE LUNG

Highly atypical cells were seen on a background of inflammation, scattered histiocytes and abundant necrotic debris. These cells contain large nuclei with coarse chromatin, irregular nuclear membranes and distinct nucleoli. Small to moderate amounts of cytoplasm and distinct cell borders were reported. Abundant atypical and highly atypical squamous cells were present, many of which showed cytoplasmic keratinization and many of which were degenerate. These appearances favoured a malignant process with features consistent with a well differentiating squamous cell carcinoma.

- Second fine needle aspiration (computed tomography guidance) of the LEFT PAROTID MASS in December 2001

Smears from the left parotid mass showed scattered cohesive sheets and clusters of oncocytic eosinophilic cells (*Figure 4*). Occasional clusters of oncocytic cells show mild anisonucleosis and slight crowding. Numerous mixed lymphocytes, scattered polymorphonuclear leucocytes and occasional histiocytes were noted. These appearances were suggestive of a Warthin's tumour of the parotid gland. No diagnostic malignant cells were found.

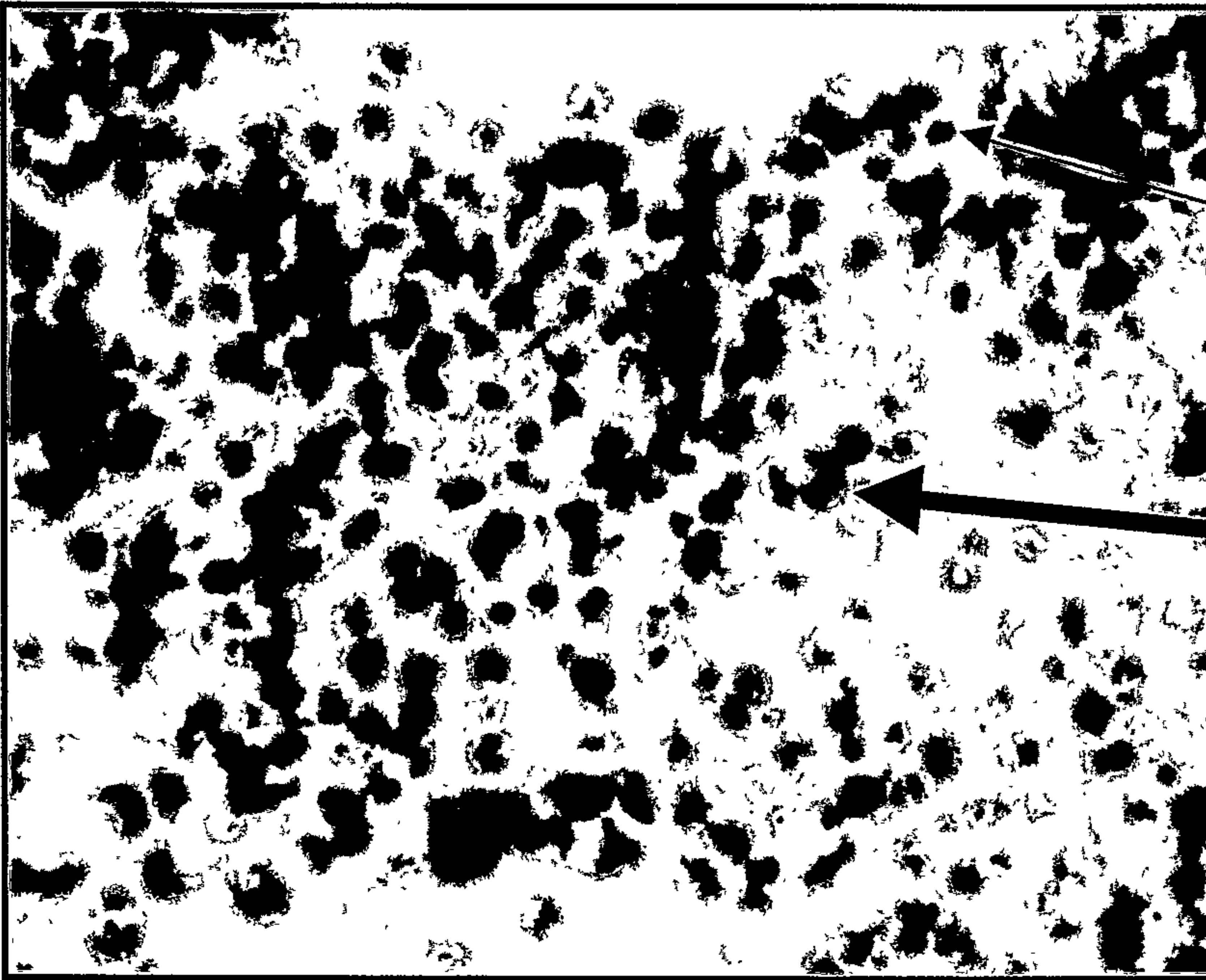


Figure 4. Second fine needle aspirate of the left parotid gland in December 2001. The aspirate shows sheets of oncocytic cells (thick arrow) and lymphocytes (thin arrow). Differential quick stain, x40 magnification.

FINDINGS & TREATMENT

From the further investigations it was concluded that the salivary gland mass and lung lesions were not related. The second fine needle aspiration of the left parotid which was performed under guidance demonstrated features suggestive of a Warthin's tumour. Mrs. MR also had been shown to have a locally advanced non-small cell lung cancer with mediastinal involvement. Lung function tests performed demonstrated relatively poor function. Given these findings and a bulky primary tumour with mediastinal involvement, surgery was no longer an option. A more aggressive approach was agreed upon at the Joint Lung Cancer Clinic (Department of Medical Oncology and Palliative Care, Westmead Hospital) involving concurrent chemoradiotherapy as compared to palliative

dose radiation followed by chemotherapy. It was felt that Mrs. MR was otherwise fairly fit and was a candidate for this more aggressive approach.

TREATMENT

- Two cycles of induction chemotherapy with Carboplatin and Taxol
- Followed by weekly chemotherapy with concurrent radical dose radiation to the lung tumour

Unfortunately Mrs. MR did not tolerate the treatment well. She developed an acute skin reaction quite early during the treatment. Following the third week of weekly chemotherapy she became thrombocytopaenic. The last three weeks of concurrent chemotherapy were withheld and radiation was continued alone.

A progress computer tomography scan of her chest in June 2002 (6 months post diagnosis) showed a reduction in size of the right upper lobe mass now measuring 3cm in maximum diameter,

FUTURE

Consolidation chemotherapy was not considered to be a wise option given the side effects from her previous treatment. It was decided to review Mrs. MR in three months and to make an assessment at that stage. No treatment has been carried out at this stage for the Warthin's tumour involving the left inferior lobe of the parotid gland.

DISCUSSION

In this section of the case presentation, the clinical and histological features of Warthin's tumour will be reviewed. The value of fine needle aspiration biopsy cytology and the limitations will also be discussed.

Warthin's tumour (papillary cystadenoma lymphomatosum) almost exclusively involves the parotid gland (95% of cases) *Eveson & Cawson* 1986¹. The parotid gland has the most prominent lymphoid component of the three major salivary glands which may explain the site predilection. After pleomorphic adenoma, Warthin's tumour is the second most common benign parenchymal salivary neoplasm (*Aguirre et al.*, 1998²).

The pathogenesis of these tumours is uncertain. The traditional hypothesis proposed that they arise from heteropic salivary gland tissue found within intra- and paraparotid lymph nodes (Neisse-Nicholson rests). It has also been suggested that these tumours may develop from a proliferation of salivary gland ductal epithelium that is associated with secondary formation of lymphoid tissue. Following evaluation of histopathological features of 79 Warthin's tumours in 63 patients *Aguirre et al.*, 1998² suggested that this tumour initially develops as an adenomatous epithelial proliferation followed by lymphocytic infiltration.

A strong association between the development of this tumour and smoking has been described (*Kotwall* 1992)³. *Yu et al.*, 1998⁴ reviewed 160 consecutive patients with Warthin's tumour to investigate the relationship with smoking.

These patients were compared with patients with pleomorphic adenoma involving the parotid gland and an age-matched control group. The Warthin's group had the highest proportion of tobacco smokers (96.3% in comparison to 26.4% and 25.5% respectively). The smoking history was also longer in patients with Warthin's tumour and the tobacco consumption greater. An immune pathogenesis has also been suggested. A retrospective study during a 25-year period which analysed 140 patients with Warthin's tumours compared with 380 patients with pleomorphic adenoma revealed a higher incidence of autoimmune disorders in patients with Warthin's tumours (*Gallo & Bocciolini 1997*⁵). Furthermore, 87% of patients with Warthin's tumour were tobacco smokers compared to only 38% of patients with pleomorphic adenoma, lending more support for a role of tobacco smoking.

Epstein-Barr virus (EBV) has also been implicated in the pathogenesis of this tumour (*Gallo 1995*)⁶. This is not widely supported in the literature. *van Heerden et al., 1999*⁷ did not find EBV DNA in any of the 20 Warthin's tumours they investigated by in situ hybridization, nor in the adjacent normal parotid tissue.

Clinically the tumour usually presents as a slowly growing painless mass of the parotid gland. It may be firm or fluctuant to palpation. It most frequently occurs in the inferior pole of the parotid gland near the angle of the mandible. Warthin's tumour can present bilaterally or be multifocal in a single gland. *Lam et al., 1994*⁸ found multiple tumours in 50% of cases by step-serial whole organ sectioning (13/26 cases) and that bilateral tumours were present in 12.5% of patients (3/24).

Quite often these bilateral tumours are metachronous. Warthin's tumour most often presents in older adults with a highest incidence in the sixth and seventh decade of life¹. Most early studies show a male predilection (10:1), but recent studies show a more balanced sex distribution of 1.6:1¹. This may reflect the changing smoking habits seen in the population with more young females commencing smoking.

Early descriptions of this entity were reported by *Hildebrand* 1895⁹, *Albrecht & Artz* 1910⁹ in German and *Nicholson* 1923⁹ before the American pathologist *Aldred Scott Warthin* described two unusual tumours of the parotid gland which he called papillary cystadenomas. This tumour described by Warthin in 1929¹⁰ has the most distinct histopathological pattern. The terminology "*papillary cystadenoma lymphomatosum*" is most suitable as it accurately describes the histology. Warthin tumour is also referred to as "adenolymphoma" however it is thought that this term overemphasizes the lymphoid component and may imply misleadingly that this lesion is a type of malignant lymphoma rather than a benign epithelial salivary gland tumour.

This tumour is composed of epithelial and lymphoid tissue. The epithelium is arranged as a double layer of oncocytic cells with abundant eosinophilic granules in their cytoplasm reflecting the multiple distended mitochondria seen by transmission electron microscopy, forming papillary processes which protrude into cystic spaces (*Figure 5*). The inner luminal layer is composed of tall columnar cells with centrally placed, palisading nuclei with slightly hyperchromatic nuclei. Stroma-poor variants with little lymphoid tissue can cause diagnostic problems for the pathologist.

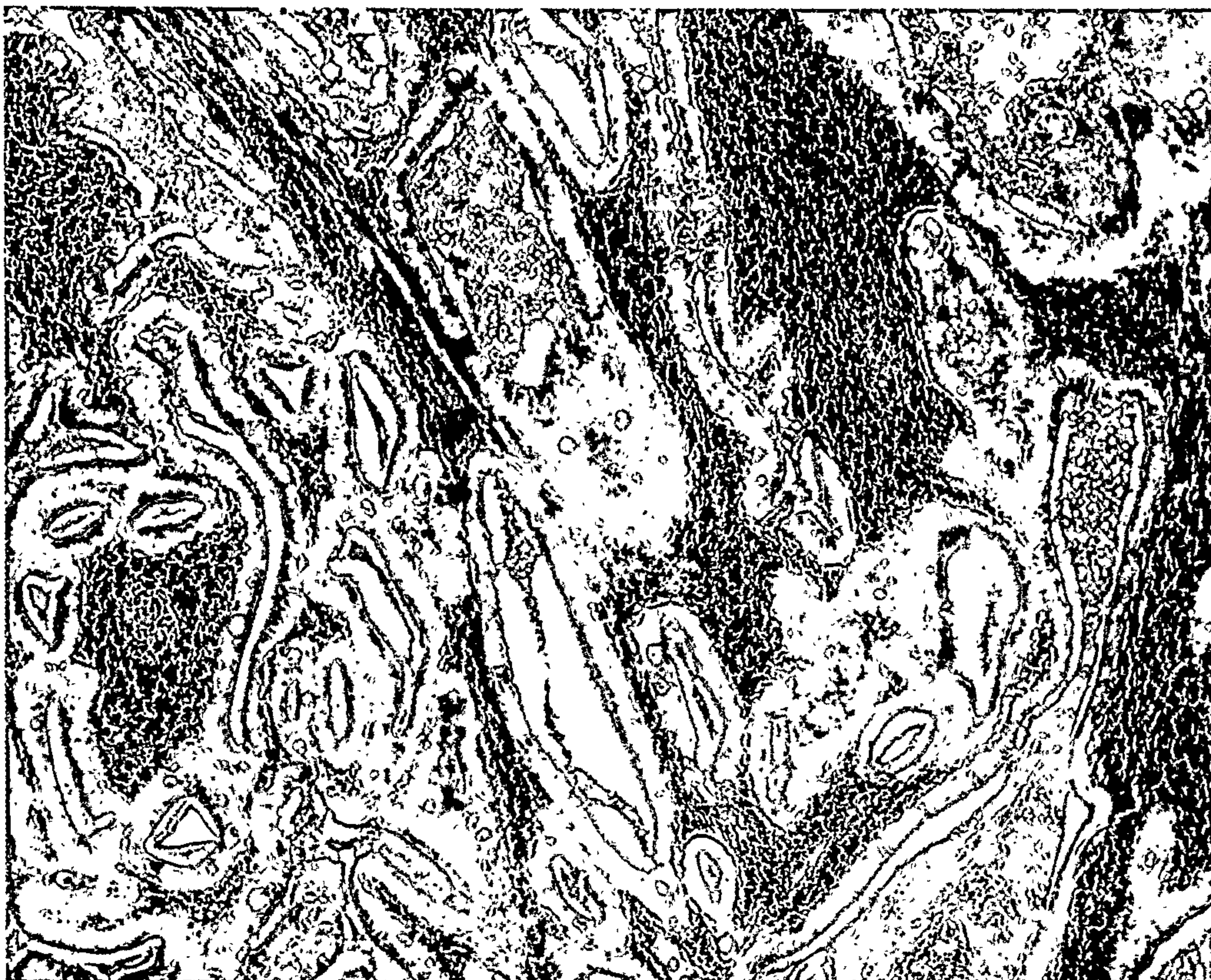


Figure 5. Low power magnification demonstrates the characteristic papillary spaces lined by eosinophilic epithelium supported by a lymphoid stroma (H&E stain, x8 magnification)

The second layer is composed of cuboidal or polygonal cells with more vesicular nuclei. The lining epithelium demonstrates multiple papillary infoldings that protrude into the cystic spaces. This epithelium is supported by a lymphoid stroma (*Figure 6*), which may show germinal center formation. The amounts of epithelial and lymphoid stroma are highly variable and either may be predominant. Areas of necrosis may be seen with formation of epithelioid granulomas. *Eveson & Cawson* reported this in 20 of the 278 tumours reviewed^{1,11}. Squamous and mucous metaplasia may be seen. A capsule may be identified at the tumour periphery. Electron microscopy studies reveal that the eosinophilic oncocytes contain abundant mitochondria. These react with a **PTAH** stain.

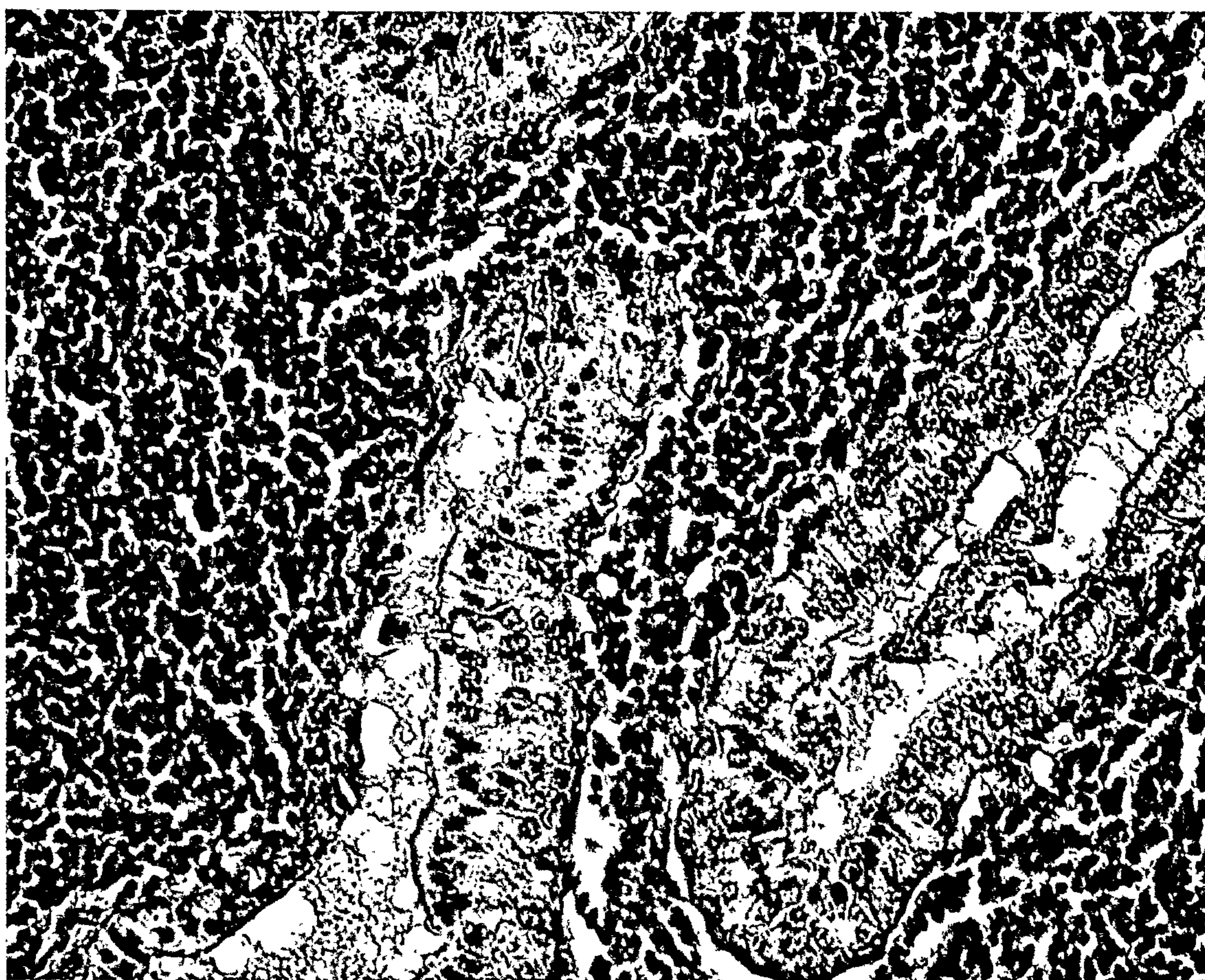


Figure 6. High power magnification of epithelium lining cystic spaces demonstrating double row of oncocytes with supporting lymphoid stroma (H&E section x80 magnification)

This tumour can be safely left with regular follow-up if there are no symptoms and the patient is not suitable for surgery. Surgical removal is generally recommended for the treatment of this tumour. This is usually achieved with a superficial parotidectomy, which is considered appropriate as most of the parotid lymph nodes lie in the superficial lobe. This method also spares the facial nerve. A 6-12% recurrence rate has been reported. This tumour is thought to be multicentric in nature which may account for some of the recurrences.

Carcinomatous^{12,13} or lymphomatous change have been reported but this is a rare occurrence.

Fine needle aspiration biopsy cytology is the study of cells which have been obtained by a small-gauge needle penetrating the lesion being investigated. Ferguson in 1937¹⁴ (pioneer of prostate aspiration) wrote "*the only function of aspiration biopsy is to differentiate neoplastic from non-neoplastic tissue*". Today, this still remains the primary goal of aspiration biopsy. On some occasions, benign tumours may be distinguished from malignant neoplasms and on others a definitive diagnosis can be reached, for example a metastatic squamous cell carcinoma in a lymph node. In addition, there are a number of other indications for such a procedure which include differentiation of a salivary gland mass from lymphadenopathy or from a soft tissue lesion.

The advantages of this relatively safe procedure are that it is usually performed "in-office" which does not require patient preparation, where both the procedure and its interpretation may be completed in a short time period. The constituent tissue can usually be identified (lymph nodes or salivary gland) with sampling of multiple masses without scar formation. This technique can also facilitate early evaluation of recurrent neoplasms.

The limitations of needle aspiration biopsy are well known. This technique does not replace an open biopsy in many situations. However there are examples, such as a salivary gland pleomorphic adenoma or a metastatic squamous cell carcinoma in a cervical lymph node, where a fine needle aspirate is preferable to an open biopsy, which

is associated with an increased rate of local recurrence. In comparison, needle aspiration biopsy can often complement an open biopsy by assisting surgical planning (remove entire gland or portion, or in a patient with a high operative risk). Frequently inappropriate or insufficient biopsy specimens are obtained. This should be recognized and reported as such rather than attempt a diagnosis. Quite often it is difficult to classify neoplasms by needle aspiration biopsy.

Few complications result from fine needle aspiration biopsy when compared with traditional incisional or excisional biopsy. The most frequently discussed complication of this procedure is seeding of tumour cells. The actual incidence is quite low - only three recurrences were reported in 157 cases of pleomorphic adenoma aspirated and treated surgically after a follow-up period of ten years¹⁵. Other complications include bleeding, haematoma, transient discomfort, and infection. *Boccatto et al.*, 1998¹⁶ reported no complications in a series of 841 salivary gland lesions investigated with fine needle aspiration biopsy. *Mavec et al.*, 1964¹⁷ reported one haematoma in a series of 652 aspirations.

The needle aspiration biopsy cytology of Warthin's tumour is characterized by oncocytes and lymphocytes. The columnar oncocytes appear in monolayered large or small sheets in "picket-fence" rows, or rarely are isolated. These cells are identified by their eosinophilic, finely granular cytoplasm (approximately 12-25 μ m in diameter)¹⁸.

The central vesicular nuclei often show prominent nucleoli. Oncocytic ghost cells, also referred to as “amorphous debris” are invariably present. These are rounded or irregular anucleated cell remnants measuring up to 30µm in diameter¹⁸. Variable numbers of lymphocytes are present.

The overall frequency of inadequate or unsatisfactory aspirates has been reported as low as 3%¹⁶. The overall correlation between cytology and histology was reported by *Boccato et al.*, 1998 was 98% in malignant salivary gland neoplasms and 100% in benign neoplasms¹⁶. The expertise of the cytopathologist and a satisfactory sample representative of the lesion play a crucial role in the agreement between cytology and histology.

The sensitivity of FNA diagnosis of Warthin’s tumour can be adversely effected if the tumour is missed by the needle sampling or poorly sampled. Errors can arise in four situations:-

- i. Mature lymphocytes
- ii. Fluid specimens
- iii. Well differentiated malignant tumours
- iv. Squamous cells due to squamous metaplasia, these may be atypical and lead to error in diagnosis of a metastatic squamous cell carcinoma

i. Mature lymphocytes

Lymphocytes from a Warthin's tumour may be mistaken for sialadenitis or benign lymphoepithelial lesion. The presence of characteristic oncocytes with lymphocytes is supportive of a Warthin's tumour, other cases must also be screened for features of lymphoma.

ii. Fluid specimens

Cysts are often associated with Warthin's tumours and mucoepidermoid tumour. If fluid is obtained it is essential that the residual mass is re-aspirated. This method will decrease the false-negative rate.

iii. Well differentiated malignant tumours

Several well-differentiated malignant neoplasms (adenoid cystic carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma) exhibit only some of the criteria for malignant disease (cellularity, dyshesion, monomorphism, nuclear membrane irregularity etc).

iv. Squamous cells

Squamous cells which may be components of well-differentiated mucoepidermoid carcinoma can be mistaken for benign cells from an epidermal inclusion or branchial cleft cyst. In this case investigation for the presence of mucinous metaplastic and or columnar cells is essential. At all times correlation with the clinical history is most important.

False positives reducing the specificity occur frequently due to the misinterpretation of;

- Pleomorphic adenomas
- Mucin
- Basaloid cells
- Degeneration

Pleomorphic cells from a pleomorphic adenoma are the most common diagnostic pitfall of aspiration biopsy cytology from salivary gland lesions. Mucin is prevalent in both salivary gland neoplasms and non-neoplastic lesions (mucoepidermoid tumour was misdiagnosed from aspiration biopsy cytology of a retention cyst with this feature¹⁹).

Basaloid cells are a feature of adenoid cystic carcinoma and basal cell adenoma.

Degenerated cells and inflammation can lead to diagnostic errors.

The first fine needle aspiration biopsy performed on our patient yielded a false negative result due to a sampling error. This was quickly identified when correlated with both the clinical features of a parotid mass and the computed tomography imaging. It was essential that the nature of the left parotid mass was diagnosed to assess firstly if there was a relationship to the lung mass and secondly for treatment planning and prognostic information. The second fine needle aspiration biopsy was performed with imaging modality assistance (computed tomography). This provided sectional images that enabled localization of the lesion in the parotid gland, and demonstration of the needle tip in the mass improving sample collection. This aspiration yielded cells from the center of the capsulated mass with the eventual diagnosis of a Warthin's tumour.

This benign tumour was unrelated to the squamous cell carcinoma of the right upper lobe. Given the current circumstances the Warthin's tumour has not been surgically removed at this stage.

This case highlights the importance of a thorough medical history and clinical examination. Not only is this important in regard to the specific reason for referral, but should also consider the patient holistically. The recommendation by her primary carer that the long-standing parotid swelling be further investigated led to the incidental finding of a lung mass and the ultimate diagnosis of a pulmonary squamous cell carcinoma.

Although this finding was serendipitous, the diagnosis may have been suggested by a recent history of respiratory symptoms on a long-standing background of cigarette smoking.

¹ Eveson JW, Cawson RA. Warthin's tumour (cystadenolymphoma) of salivary glands. A clinicopathologic investigation of 278 cases. *Oral Surg Oral Med Oral Pathol* 1986; **61**: 256-62.

² Aguirre JM, Echebarria MA, Martinez-Conde R, Rodriguez C, Burgos JJ, Rivera JM. Warthin tumor. A new hypothesis concerning its development. *Oral Surg Oral Med Oral Pathol Oral Radiol & Endod* 1998; **85**: 60-3.

³ Kotwall CA. Smoking as an aetiological factor in the development of warthin's tumour of the parotid gland. *Am J Surg* 1992; **164**: 646-7.

⁴ Yu Gy, Liu XB, Li ZL, Peng X. Smoking and the development of Warthin's tumour of the parotid gland. *Br J Oral Maxillofac Surg* 1998; **36**: 183-5.

⁵ Gallo O, Bocciolini C. Warthin's tumour associated with autoimmune diseases and tobacco use. *Acta Oto-Laryngologica*. 1997; **117**: 623-7.

⁶ Gallo O. New insights into the pathogenesis of Warthin's tumour. *Eur J Cancer B: Oral Oncol* 1995; **31**: 211-5.

⁷ van Heerden WF, Kraft K, Hemmer J, Swart TJ, van Heerden MB, van Rensburg EJ. Warthin's tumour is not an Epstein-Barr virus related disease. *Anticancer Res* 1999; **19**: 2881-3.

⁸ Lam KH, Ho HC, Wei WI. Multifocal nature of adenolymphoma of the parotid. *Br J Surg* 1994; **81**: 1612-4.

⁹ Leverstein H, van der Wal JE, Tiwari RM, van der Waal I, Snow GB. Results of the surgical management and histopathological evaluation of 88 parotid gland Warthin's tumours. *Clin Otolaryngol & Allied Sc* 1997; **22**: 500-3.

¹⁰ Warthin AS. Papillary cystadenoma lymphomatosum. *J Cancer Res* 1929; **13**: 116-25.

¹¹ Eveson JW, Cawson RA. Infarcted ("infected") adenolymphomas. A clinicopathological study of 20 cases. *Clin Otolaryngol* 1989; **14**: 205-10.

¹² Bengoechea O, Sanchez F, Larringa B, Martinez-Penueal JM. Oncocytic adenocarcinoma arising in a Warthin's tumour. *Pathol Res Pract* 1989; **185**: 907-11.

¹³ Batsakis JG. Carcinoma ex papillary cystadenoma lymphomatosum. Malignant warthin's tumour. *Ann Oto Rhinol, Laryngol* 1987; **96**: 223-35.

¹⁴ Ferguson RS. Diagnosis and treatment of early carcinoma of the prostate. *J Urol* 1937; **37**: 744-82.

¹⁵ Engzell U, Esposti PL, Rubio C. Investigation on tumour spread in connection with aspiration biopsy. *Acta Radiol* 1971; **10**: 385-98.

¹⁶ Boccato P, Altavilla G, Blandamura S. Fine needle aspiration biopsy of salivary gland lesions. A reappraisal of pitfalls and problems. *Acta Cytol* 1998; **42**: 888-98.

¹⁷ Mavec P, Eneroth CM, Franzén S. Aspiration biopsy of salivary gland tumours: I. Correlation of cytologic reports from 652 aspiration biopsies with clinical and histologic findings. *Acta Otolaryngol* 1964; **58**: 472-84.

¹⁸ Kline TS. Salivary glands (Chapter 5) in Handbook of fine needle aspiration biopsy cytology. Second Edition. 1988 Churchill Livingstone USA 129-30.

¹⁹ Persson PS, Zettergren L. Cytologic diagnosis of salivary gland tumours by aspiration biopsy. *Act Cytol* 1973; **17**: 351-4.

PEMPHIGUS VULGARIS

A Case Presentation

PEMPHIGUS VULGARIS

SYNOPSIS

A case report of a patient presenting with pemphigus vulgaris limited to the oral mucosa.

CASE PRESENTATION

Name : Ms PC
Gender : Female
Age : 49
Marital Status : Single
MRN : 1224437

CHIEF COMPLAINT

The patient was referred to the Oral Medicine Unit by a periodontist in private practice for assessment of recurring mouth ulcers.

HISTORY OF PRESENTING COMPLAINT

The patient presented with a three-month history of recurrent, painful ulceration involving the buccal mucosa bilaterally, and the gingivae. The recurrent ulceration was without any remission, with no apparent initiating factors. The patient reported some symptomatic relief with the use of DifflamTM (benzydamine).

MEDICAL HISTORY

The patient occasionally suffers from mild asthma, which is well controlled with the use of a salbutamol inhaler. She also experiences seasonal hayfever. The patient attends her local medical practitioner for regular Papanicolaou (Pap) smears. A colposcopy was performed in 1999 following a positive Pap smear. She is reviewed regularly by her gynaecologist. Ms PC is a non smoker and does not imbibe alcohol.

DENTAL HISTORY

The patient attends her dentist on a regular basis for recall examinations.

EXTRA ORAL EXAMINATION

- There were no extra-oral abnormal clinical findings.
- No eye involvement, no nasopharyngeal lesions, no skin lesions and the patient reported no genital involvement.
- There was tender bilateral submandibular lymphadenopathy, clinically reactive.

INTRA ORAL EXAMINATION

Hard tissues: Ms PC was a dentate patient with extensive restorations present.

No active carious lesions present.

Soft tissues: Desquamative gingivitis especially affecting mandibular lower anterior teeth, in particular the mandibular left central incisor (see *Figure 1*).

There was bilateral severe ulceration of the buccal mucosa with an extensive covering slough (see *Figure 2*).

There was loss of the buccal sulcus depth, and the tissues were extremely tender.

There was possibly a positive Nikolsky sign.



Figure 1. Desquamative appearance of mandibular gingivae



Figure 2. Ulcerative lesions involving the left buccal mucosa

PROVISIONAL DIAGNOSIS

Based on the history, the age of the patient (> 40 years), her female gender and the clinical appearances the differential clinical diagnoses included:

- Benign mucous membrane (cicatrising) pemphigoid
- Pemphigus vulgaris
- Erosive lichen planus

LABORATORY INVESTIGATIONS

The haematological investigations ordered included full blood count, differential white cell count and indirect immuno-fluorescence for circulating auto-antibodies.

INITIAL TREATMENT

Commencement of dexamethasone mouthwash (0.1mg/ml), as a therapeutic trial to assess the clinical response. The dexamethasone mouthwash was discontinued 1 week prior to biopsy to enable an intact bullae and unmodified histology to evolve for a biopsy.

BIOPSY

Three representative tissue samples were taken under local anaesthesia. The specimens were subdivided and one half fixed overnight in 10% neutral buffered formalin for routine light microscopic examination and the other half wrapped in saline-soaked gauze for immediate transport to the laboratory for direct immunofluorescence.

The biopsy sites were:-

1. Left retromolar trigone
2. Left buccal mucosa
3. Left buccal surface of mandibular gingivae (premolar region)

RESULTS of INVESTIGATIONS

Indirect immunofluorescence demonstrated a high titre of intercellular substance antibody 1:160 (a titre greater than 10 is considered positive). This is a serological marker for pemphigus.

Basement membrane substance antibody, a serological marker for pemphigoid was negative. This supports a diagnosis of pemphigus. The patient's low haemoglobin level (94 g/L, normal range 115-165) and ferritin level (10µg/L, normal range 15-150) indicated an iron deficiency. Ferritin levels are considered to reflect bone marrow iron stores.

The packed cell volume (haematocrit) is reduced in anaemia, a microcytosis and hypochromasia (reduced mean cell volume) is found in iron deficiency. These haematological and serological tests suggested an iron deficiency anaemia.

Table 1. Haematological and serological results

	<i>Results:</i>	<i>Normal range</i>
Iron	5.6	7.0 – 29.0 umol/L
Transferrin saturation	0.07	0.08 – 0.44
Ferritin	10	15 – 150 µg/L
Transferrin	3.4	1.8 – 3.3 g/L
White cell count	10.6	3.9 – 11.1 x 10 ⁹ /L
Red cell count	4.8	3.9 – 5.0 x 10 ¹² /L
Haemoglobin	94	115 – 165 g /L
Haematocrit	0.33	0.36 – 0.44
Mean cell volume	68	82 – 98 fL
Platelets	442	150 – 400 x 10 ⁹ /L
Mean cell haemoglobin	19	27 – 32 pg
Film comment	Microcytes present Hypochromasia present	
Neutrophils absolute	5.9	2.0 – 8.0 x 10 ⁹ /L
Lymphocytes absolute	3.8	1.0 – 4.0 x 10 ⁹ /L
Monocytes absolute	0.5	0.2 – 1.0 x 10 ⁹ /L
Eosinophils absolute	0.1	0.0 – 0.5 x 10 ⁹ /L
Basophils absolute	0.1	0.0 – 0.1 x 10 ⁹ /L
Neutrophil	56	
Lymphocyte	36	
Monocytes	5	
Eosinophil	1	
Basophils	1	
Vitamin B12	389	125 – 780 pmol/L
Red cell folate	1017	370 – 1050 nmol/L
Serum folate	7	5 – 21 nmol/L
Intercellular substance antibody	160	
Basement membrane substance antibody	Negative	

BIOPSY RESULTS

The histopathology showed suprabasal clefting with the presence of acantholytic “Tzank” spinous cells (see Figures 3 & 4), some of which were surrounded with polymorphonuclear leucocytes

Tzank cells are acantholytic spinous cells lying free in the suprabasal bulla. Direct immunofluorescence stained with antibody to IgG showed fluorescence along lines of inter-epithelial attachment of tissue.



Figure 3. Left buccal mucosa histopathology, H&E x8 magnification

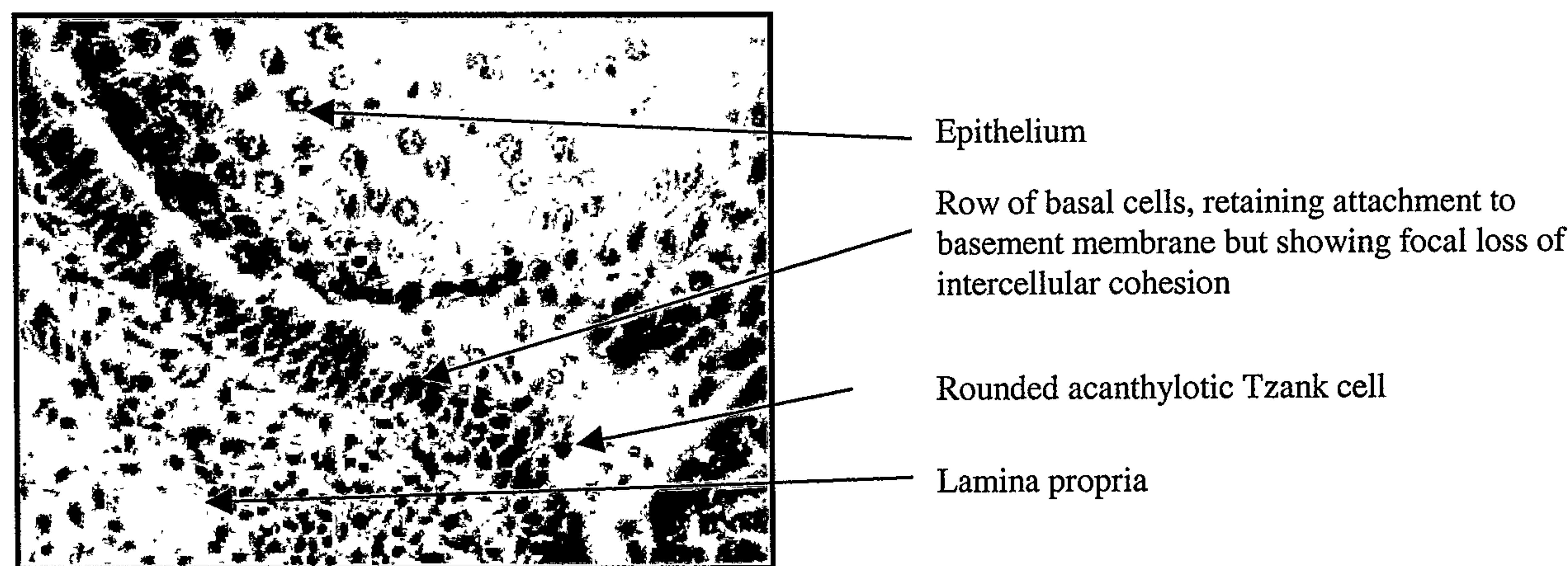


Figure 4. Suprabasal cleft, H&E x80 magnification

DEFINITIVE DIAGNOSIS

- PEMPHIGUS VULGARIS

MANAGEMENT

Following confirmation of the diagnosis Ms PC was referred to the Dermatology Outpatient Clinic at Westmead Hospital for consultation and conjoint management. Ms PC was commenced on glucocorticosteroids (prednisone 25 mg/day) by the Dermatologist, in conjunction with the use of topical dexamethasone mouthwash 0.1mg/mL.

FURTHER INVESTIGATIONS & INITIAL MANAGEMENT

Any patient that requires high doses or long term use of glucocorticosteroids requires ongoing assessment to monitor the adverse effects such as hypertension, diabetes mellitus and increased risk of osteoporosis. Table 2 summarises the investigations carried out with the initial prescription of glucocorticosteroids.

<i>Blood sugar levels</i>	Steroid induced diabetes mellitus ➤ Weekly check by general medical practitioner
<i>Blood pressure</i>	Hypertension ➤ Weekly check by general medical practitioner
<i>Chest radiograph</i>	Reactivation of tuberculosis ➤ Assess for past history of tuberculosis
<i>Bone density scan</i>	Risk of osteoporosis ➤ Baseline levels at onset of glucocorticosteroids

Table 2. Investigations prior to systemic glucocorticosteroids

The history of a positive Pap smear and colposcopy in 1999 was concerning, particularly with the requirement of immunosuppressants for management of pemphigus vulgaris. The results from 1999 demonstrated cervical intraepithelial neoplasia (CIN) grade III: severe dysplasia but with no evidence of the human papillomavirus. Human papillomavirus (types 16 and 18) are associated with the aetiology of cervical dysplasia and cervical carcinoma. In 1992, HPV was recognised by the World Health Organisation as the major cause of cervical cancer. The absence of HPV infection excluded the need for elective gynaecological surgery and ongoing review with her gynaecologist was organised.

ONGOING MANAGEMENT

A marked reduction in the severity of the oral lesions was noted after 3 weeks of glucocorticosteroids (25mg/day) and no cutaneous lesions developed. Of interest, the circulating auto-antibody titre (intercellular cement substance antibody) decreased from 160 to 40 one week after commencement of glucocorticosteroids. After two months the dose of glucocorticosteroids was reduced by 2.5mg every four weeks with no new lesions developing.

After 3 months minimal involvement of the oral cavity was noted, however the posterior left buccal mucosa continued to be affected. Examination demonstrated that the posterior teeth may have been contributing to the persistence of these lesions (Koebner phenomenon). The maxillary left second molar and mandibular left third molar were extracted under local anaesthesia with no complications. These teeth were the most posterior teeth on the left side. Review one week post-extractions demonstrated a 95% objective improvement of mucosal lesions. There was no active area of ulceration present at the time of examination. This demonstrates that the Koebner phenomenon associated with trauma from these teeth contributed to persisting lesions involving the left buccal mucosa.

Seven months after the diagnosis of Pemphigus Vulgaris, Ms PC had no mucosal involvement on clinical examination. She was managed with the use of systemic glucocorticosteroids and topical dexamethasone. Her dose of glucocorticosteroids could be reduced in a relatively short time period with clinical improvement of lesions. A steroid-sparing immunosuppressant did not need to be introduced during her management. I have been reviewing Ms PC on a regular basis, 20-months from the initial diagnosis she presents with no evidence of further mucosal involvement. The left buccal mucosa demonstrates a slight reduction of the mandibular buccal sulcus as a result of healing with fibrosis, however she is able to access the buccal surfaces of the teeth for oral hygiene. Clinical photos were taken 16-months following the diagnosis of the intra-oral sites initially affected (see *Figures 5 & 6*). She has never developed cutaneous lesions. She had a second bone density scan with no change demonstrated from the original scan and she experienced minimal adverse effects from the glucocorticosteroids.



Figure 5. Clinical photo A demonstrates resolution of desquamative gingivitis 16 months following initial presentation (clinical photo B) with systemic and topical glucocorticosteroid therapy.

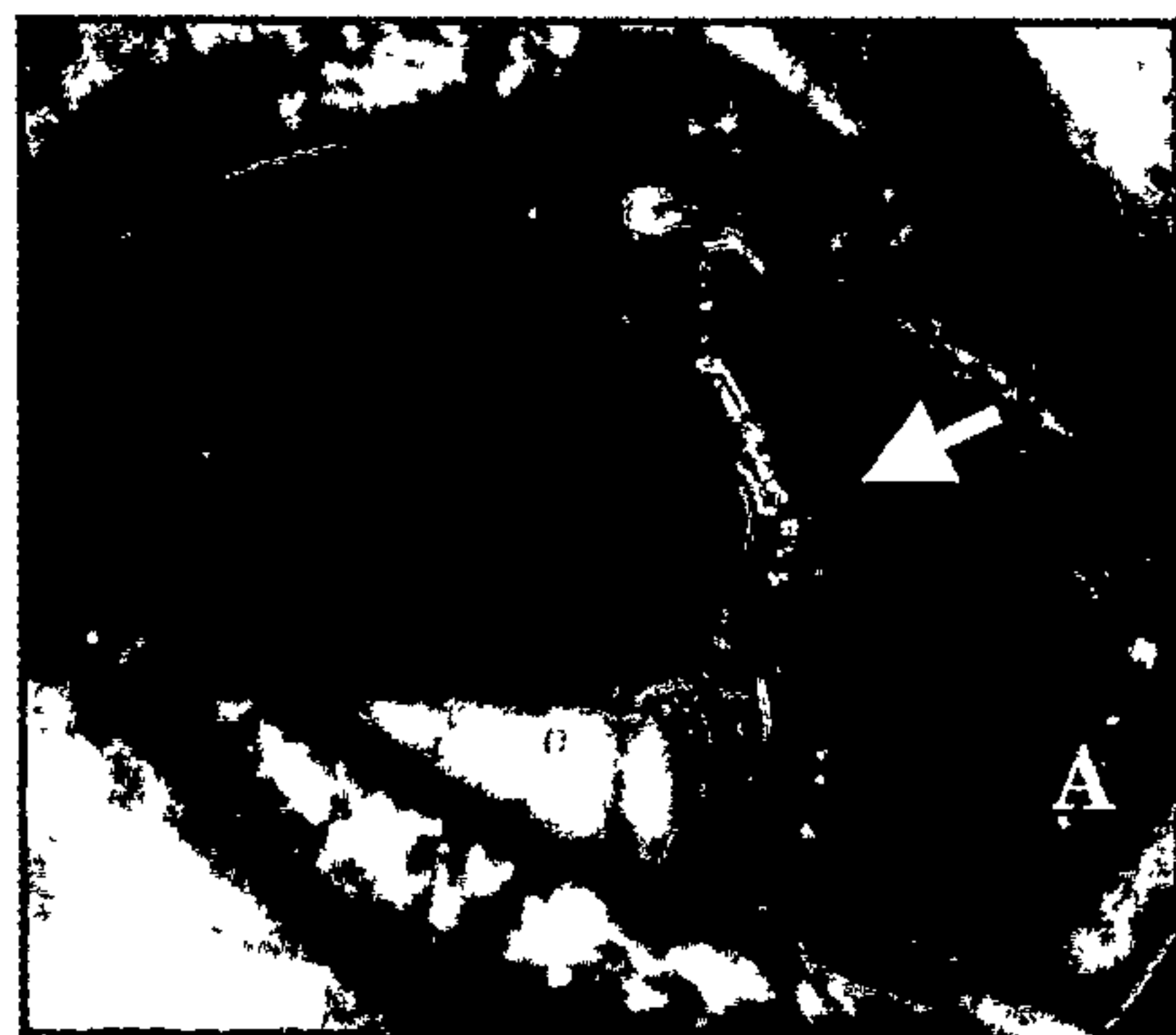


Figure 6. Clinical photo A demonstrates resolution of left buccal mucosa lesions 16 months after initial diagnosis (clinical photo B), following corticosteroid therapy and extraction of 27 and 28.

This case report demonstrates an effective multidisciplinary approach to the care of a patient.

Working together with the Dermatologist we were able to provide a management programme with an excellent outcome. The role of Oral Medicine is highlighted with the identification of the teeth which continued to aggravate the mucosa. Extraction of these teeth resulted in prompt resolution of the mucosal lesions with subsequent reduction of systemic immunosuppressants and eliminating risk of adverse effects of long-term glucocorticosteroids.

DISCUSSION

The term “Pemphigus” comprises a group of chronic auto-immune diseases that have in common the following features:

1. Loss of cell to cell adhesion :

The disruption of cell to cell adhesion manifests clinically with vesiculo-bullous presentation of epithelial surfaces.

2. Presence of auto-antibodies :

Autoantibodies bound *in vivo* around plasma membranes of affected epithelial cells in a characteristic pattern demonstrated with direct immunofluorescence.

Circulating auto-antibodies detected in serum with indirect immunofluorescence.

3. Pathogenic circulating auto-antibodies (generally of IgG isotype) that can reproduce clinical features of diseases *in vivo* and histological features *in vitro* and *in vivo*¹

Classically, three major forms have been described; pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus. Since the early 1970's, new clinical variants of pemphigus have been reported, namely IgA pemphigus and pemphigus herpetiformis². The major forms of pemphigus and their variants are outlined in *Table 3*.

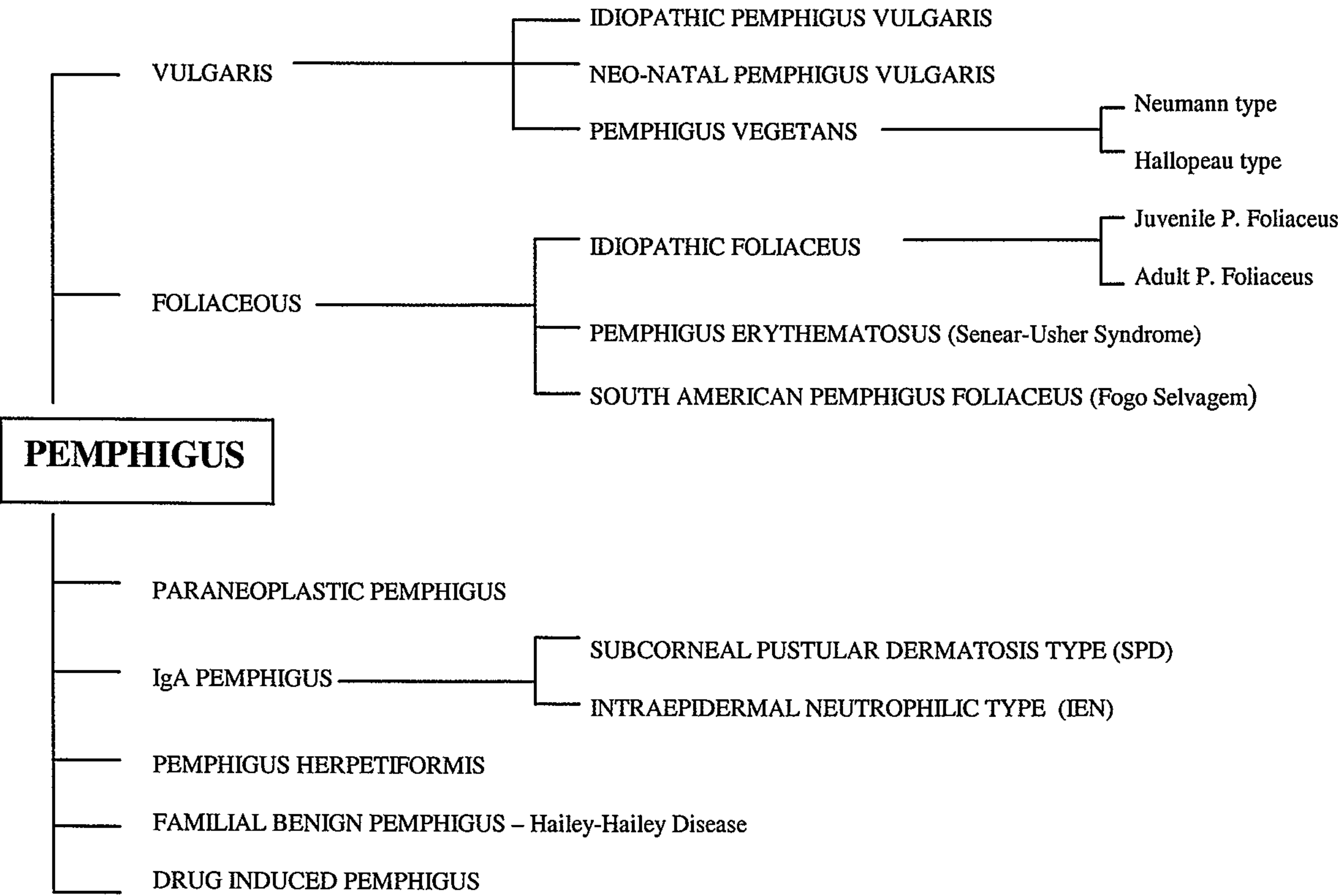


Table 1. Pemphigus variants

Pemphigus Foliaceus

This is a less severe variant of Pemphigus. The clinical manifestations of adult and juvenile pemphigus foliaceus are similar, however juvenile pemphigus foliaceus is rare³. Pemphigus foliaceus is characterised by acantholysis in the stratum granulosum, therefore being more superficial than pemphigus vulgaris. Lesions rupture quickly and present as erosions with crusting margins and surrounding erythema usually involving the face, scalp, chest and back (sun-exposed areas). Oral lesions are not common, however when they occur they are less severe than those of pemphigus vulgaris³.

Pemphigus Erythematosus (Senear-Usher Syndrome)

This is a variant of Pemphigus Foliaceus with lesions confined to head, neck and upper trunk.

Senear-Usher Syndrome shows features of both pemphigus foliaceus and systemic lupus erythematosus⁴.

South American Pemphigus Foliaceus

This is an endemic form of pemphigus foliaceus known as Fogo Selvagem (Portuguese for “*wild fire*”). This form of pemphigus affects children and young adults in rural areas of Brazil and Columbia, with a suggested environmental cause (such as an insect vector)⁵ and aggravated by ultra-violet B radiation exposure. Clinical features consist of superficial blisters and erosions of sun-exposed areas of skin.

Familial Benign Pemphigus (Hailey – Hailey Disease)

Familial benign pemphigus was first described by the dermatologist brothers Hailey and Hailey in 1939⁶. This is an autosomal dominant inherited disorder which begins in the second to third decades of life. Lesions appear as vesicles on an inflammatory base that spread peripherally with a serpiginous border. Intertriginous areas are typically affected, such as the axillae, side of the neck and groin. A characteristic feature is spontaneous remission with exacerbation during the warm season. Histological examination reveals suprabasal acantholytic lesions similar to pemphigus vulgaris. However, in contrast there are no auto-antibodies detected by immunofluorescence.

Drug-induced Pemphigus

This is rare, and is most often associated with drugs that have terminal sulfhydryl groups (such as penicillamine and captopril). These terminal groups resemble the molecular structure of desmoglein 3 which results in cross-reactivity⁷. Clinical features resemble those of pemphigus foliaceus most frequently⁷.

Paraneoplastic Pemphigus

This entity was described formally in 1990 by Anhalt *et al*⁸. This autoimmune condition presents with polymorphous blistering eruptions, muco-cutaneous ulcerations and an underlying neoplasm. Camisa & Helm (1993)⁹ proposed a modification to the set of diagnostic criteria outlined by Anhalt *et al.*, (1990) and proposed the term “*Neoplasia-induced Pemphigus*” following an observation that muco-cutaneous changes may persist after the tumour is in complete remission. The three major criteria of paraneoplastic pemphigus proposed by Camisa and Helm are as follows;

1. Polymorphous muco-cutaneous eruption
2. Concurrent neoplasia
3. Characteristic serum immunoprecipitation findings.

Various neoplasms have been associated with paraneoplastic pemphigus, with non-Hodgkin's lymphoma being the most common. Other neoplasms are chronic lymphocytic leukaemia, Castleman's tumour, thymoma (benign and malignant), poorly differentiated sarcoma, Waldenstrom's macroglobulinaemia, inflammatory sarcoma, bronchogenic squamous cell carcinoma, round cell liposarcoma, Hodgkin's disease and T-cell lymphoma (in decreasing order of frequency)⁹.

The most frequently observed clinical features are oral manifestations with painful erosions and ulcerations involving the oropharynx and vermilion borders of the lips⁸. Most patients also have a severe pseudomembranous conjunctivitis.

Pemphigus Herpetiformis

Jablonska *et al.*, (1975)¹⁰ originally described pemphigus herpetiformis, a condition which combines the clinical features of dermatitis herpetiformis with immunological and histological features of pemphigus. Most patients experience pruritus and an eruption characterized by superficial vesicles and inflammatory plaques. Clinical presentation and histology are often atypical, employment of direct immunofluorescence being a more reliable basis for diagnosis. Direct immunofluorescence shows IgG deposition at epidermal cell surfaces, sparing the basal layers.

IgA Pemphigus

This was first described in 1982 by Wallach, Foldes, & Cottenot with the title “Subcorneal pustular dermatosis and monoclonal IgA”².

There are two distinct types of IgA pemphigus;

- i. Subcorneal pustular dermatosis
- ii. Intraepidermal neutrophilic IgA pemphigus

Patients with both types present clinically with flaccid pustules or vesicles, or both, on either erythematous or normal skin. Typical sites affected are axillary and groin areas. Mucous membrane involvement is rare¹¹.

Pemphigus Vegetans

This is a rare variant which constitutes 1% to 2% of all pemphigus cases¹². There are two types of pemphigus vegetans;

- i. Neumann type
- ii. Hallopeau type

In the Neumann type, the disease begins and ends as pemphigus vulgaris, however differing by having many denuded areas heal with verrucous vegetations instead of normal skin. The Hallopeau type involves pustules as the primary lesions instead of bullae. Their occurrence is followed by formation of gradually extending verrucous vegetations, especially in the intertriginous areas. Oral lesions present almost invariably in both types of pemphigus vegetans¹², with characteristic involvement of tongue referred to “cerebriform tongue”.

Neonatal Pemphigus Vulgaris

This form is caused by transplacental transfer of IgG antibodies. Intra-uterine deaths have been reported although the cause is not clear. Maternal titres of pemphigus auto-antibodies were high (between 1:160 and 1:320) and in addition, the mothers had disease which was difficult to control, requiring high doses of prednisone as part of their management (100-300mg/day). Three out of the four cases of intra-uterine deaths had an autopsy. One case indicated possible placental failure secondary to corticosteroids¹³ and the other suggested fatal cytomegalovirus infection¹⁴. The cause of death was undetermined in one case¹⁵.

In neonatal pemphigus neither the antibody titres nor clinical presentation of the mother predict severity of the neonatal disease¹⁶. Close foetal monitoring is required for severe maternal pemphigus vulgaris. Routine caesarean section is of no benefit as blisters and increased rates of infection may occur in the surgical wound¹⁷. Neonatal pemphigus has not been reported as progressing to adult disease¹⁶.

Pemphigus Vulgaris

This is the most common form of Pemphigus observed in North America and Europe¹⁸. Pemphigus is a rare condition itself with a global incidence of 0.1 – 0.5 cases per 100,000 persons per year¹². It has been documented in all races and ethnic groups, particularly seen in Ashkenazi Jews¹⁹. Pemphigus vulgaris commonly presents between the fourth and sixth decades of life, and affects both sexes equally, though some studies suggest a predilection for females²⁰. Parks *et al.*, (1979)²¹ have demonstrated an association between pemphigus and the major histocompatibility complex (MHC) – specifically Human Leukocyte Antigen DR4 in a Jewish population.

In most cases the disease develops slowly, usually taking up to seven months from the time of onset to the time of diagnosis²². The diagnosis is based on clinical presentation and confirmed by histological and direct immunofluorescence examination. Other auto-immune disorders which may be associated with pemphigus and they include lupus erythematosus, rheumatoid arthritis and Sjögren's Syndrome.

Pemphigus vulgaris most commonly affects the oral mucosa but can involve any area of stratified squamous epithelium. In the majority of cases, oral lesions "limited pemphigus" precede skin lesions (60%) by periods of six months to one year²³.

Clinically, the lesions are fragile, flaccid bullae or vesicles which rupture almost immediately, presenting as irregular, ragged, mucosal ulcers, with a yellowish grey-pseudomembrane and an erythematous base. Any part of the oral mucosa may be involved, most frequently the soft palate, buccal mucosa and gingiva.

A positive Nikolsky's sign is demonstrated by gentle lateral pressure on a clinically unaffected area causing stripping of epithelium. Patients with extra-oral mucosal involvement may have involvement of the pharynx, larynx, oesophagus, conjunctiva, vaginal, penile and anal mucosa.

The most common cutaneous areas involved are the face, trunk, pressure points, groin and axillae.

HISTOLOGY

The earliest histological feature in pemphigus vulgaris is intercellular oedema and loss of adhesion between plasma membranes of adjacent epithelial cells at desmosomes (macula adherens). Electron microscopic examination of lesions demonstrates the intercellular cement substance (glycocalyx) is partially or entirely dissolved where acantholysis begins²⁴. This is observed as a widening of the intercellular spaces while intact desmosomes are still present. Immunoelectron microscopy, in which peroxidase or gold rather than fluorescein is coupled to anti-human IgG, demonstrates the location of pemphigus auto-antibodies at the intercellular space.

The resultant acantholysis (loss of cell to cell adhesion) leads to formation of clefts, resulting in bullae predominantly at the suprabasal level¹⁹. Basal cells, although separated by loss of intercellular bridges, remain attached to the basement membrane (lamina densa) giving a characteristic "row of tombstones" appearance. This is due to the preservation of structures connecting the basal cells with underlying connective tissue (lamina propria), namely the hemi-

desmosomes (lamina lucida to lamina densa) and anchoring fibrils (lamina densa to lamina propria). The connection between basal cells and lamina propria contains no intercellular cement substance, therefore remaining intact even at a stage where basal cells show severe damage.

During the early phase of bullae formation there is usually little evidence of inflammation present.

In some cases, however, there are eosinophils producing “eosinophilic spongiosis”²⁵. Fully developed bullae contain single (or grouped clusters) of epithelial cells known as “Tzank cells”.

Tzank cells are free epithelial cells present within a cleft due to loss of intercellular adhesion.

These cells appear rounded as a result of retraction of tonofilaments from their insertion into the desmosomal plaque perinuclear region. Tzank cells have a large hyperchromatic nucleus with a thin rim of homogenous cytoplasm, and they may be associated with polymorphonuclear leukocytes in a “rosette” formation, binding presumably by their IgG F_c membrane receptors to the IgG autoantibody attached to the epithelial cells.

In chronic lesions, the number of inflammatory cells (including eosinophils and plasma cells) may be considerable.

PEMPHIGUS ANTIGEN

The glycoprotein recognized by the pemphigus vulgaris autoantibody (IgG) is 130 kD (desmoglein 3). It is referred to as the pemphigus vulgaris antigen and was initially demonstrated by immunoprecipitation. The pemphigus antigen is a member of the adhesion glycoproteins called cadherins (calcium dependent adhesion molecules). Desmogleins are transmembrane glycoproteins that span the plasma membrane and are critical in cell to cell adhesion.

PATHOGENICITY OF PEMPHIGUS AUTOANTIBODY

Animal model investigations provided evidence for the pathogenicity for pemphigus antibody. Neonatal mice injected parenterally with pemphigus vulgaris IgG developed pemphigus-like lesions within 18-72 hours of the first injection²⁶. Immunofluorescence revealed IgG binding to lesional and peri-lesional epidermis. Clinical and histological findings consistent with pemphigus vulgaris were evident.

In vitro studies have demonstrated induction of acantholytic lesions in explants of normal skin incubated with pemphigus IgG²⁷. Autoantibodies can cross the placenta and affect the foetus providing further support for their involvement in the pathogenesis of pemphigus.

IMMUNOPATHOGENESIS

Beutner & Jordan²⁸ demonstrated in 1964 that serum from patients with pemphigus contained autoantibodies that bind to the intercellular substance of skin and mucosa with indirect immunofluorescence. Subsequent skin biopsies revealed *in vitro* deposition of autoantibodies in the epithelium of pemphigus patients²⁹. Direct immunofluorescence of pemphigus vulgaris shows a diffuse deposition of immunoglobulins (principally IgG) and complement (C3) in the intercellular substance.

Mechanism of Acantholysis:

1. Enzymatic Mechanism
2. Complement Activation
3. Plasminogen Activator

1. Concept of enzymatic mechanism.

In vitro studies provide evidence for enzymatic activation of acantholysis by pemphigus autoantibody IgG. Cultured mouse epidermal cells were incubated with IgG preparations from pemphigus patients inducing detachment of 60% of cells. Proteinase inhibitors (trypsin inhibitor and α_2 macroglobulin) introduced into cultures blocked the cell detachment induced by pemphigus IgG³⁰. Schiltz *et al.*, (1979)³¹ reported the presence of a proteolytic enzyme in the culture medium of the explants incubated with pemphigus IgG, but not from those incubated with normal IgG. These results lead to the concept that interaction of the pemphigus autoantibody with the epithelial cell surface causes loss of cellular adhesion and results in acantholysis¹.

2. Concept of complement activation.

The role of complement in the development of pemphigus lesions has been conflicting. Complement deposition (C3 plus the early complement components C1q and C4) is observed using direct immunofluorescence in acantholytic lesions of pemphigus³². Patients undergoing corticosteroid therapy showed no detectable complement deposition within lesions³³. Complement activation may be responsible for the influx of inflammatory cells that occurs in developing lesion. Studies indicate that complement activation occurs *in vivo* after binding of pemphigus IgG to the epithelial cell surface, augmenting acantholysis. If there are sufficient autoantibodies binding to the cell surface, cells lose their adhesion in the absence of activation of the complement system.

3. Role of Plasminogen Activator in acantholysis.

Becker *et al.*, (1981)³⁴ reported that the production of plasminogen activator by two different cell lines could be stimulated by rabbit antibodies raised against the cells. It was suggested that plasminogen activator might be the proteinase stimulated in epithelial cells after binding of pemphigus antibody. Plasminogen activators are serine proteinases that catalyse the conversion of inactive plasminogen into active plasmin by cleaving a single peptide bond³⁵.

Two major types of human plasminogen activators are distinguished biochemically and immunologically, namely the “*urokinase* type” and “*tissue* type”. Studies by Singer *et al.*, (1985)¹ to determine which type of plasminogen activator is produced by human epithelial cells indicated that it is the urokinase-type enzyme. The same group showed that pemphigus antibody stimulates the production of plasminogen activator by cultured human epithelial cells.

Binding of the pemphigus antibody to the surface of human epithelial cells stimulates production of plasminogen activator. Increased levels of plasminogen activator in the presence of plasminogen, results in conversion of plasminogen to plasmin (*Figure 7*). Plasmin is then responsible for loss of cellular adhesion.

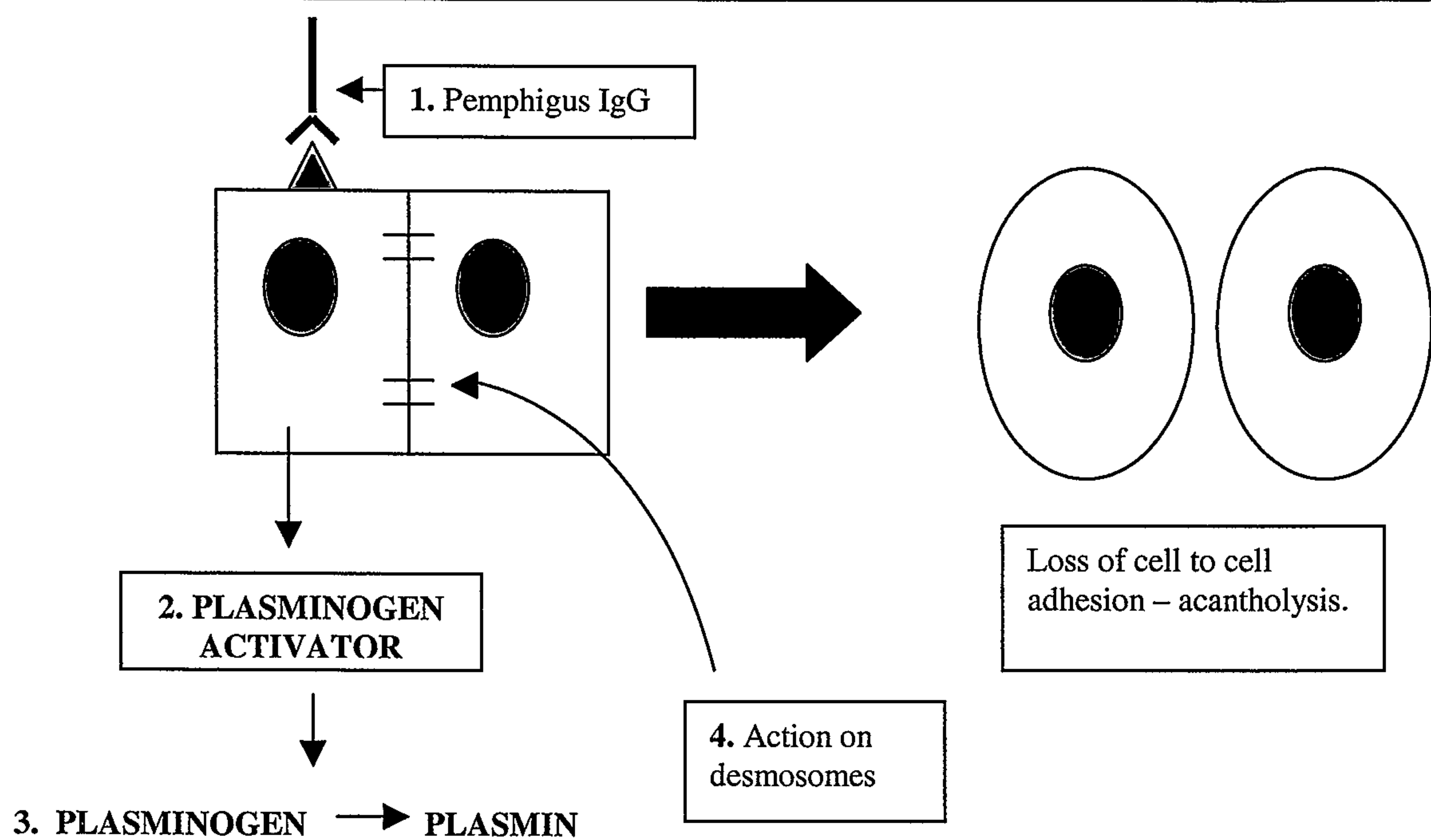


Figure 7. Plasminogen Activator³⁶

MANAGEMENT

The conventional basis of treatment of pemphigus is to induce immunosuppression to decrease antibody synthesis. The oral cavity may involve extensive, denuded mucosa resulting in poor oral intake leading to malnutrition. Secondary infection and fluid loss can also occur if skin lesions are left untreated.

Before glucocorticosteroids became available in the 1950's, the mortality rate from most types of pemphigus ranged from 60% to 90%³⁷. With the use of immunosuppressive agents and corticosteroids, this has decreased to 5% to 10%, but with a corresponding increase in morbidity and mortality associated with complications of the medication.

Treatment regimes are dictated by several factors, including;

- i. age of patient
- ii. degree of involvement
- iii. rate of disease progression
- iv. subtype of pemphigus

Treatment of pemphigus can be divided into two phases, namely the initial or induction phase and the maintenance phase.

The objective of the initial phase is to control the disease progression preventing new eruptions.

This initial phase employs the use of high dose glucocorticosteroids. The aim of the maintenance phase is disease control with the lowest dose of medication. Maintenance therapy may require long term steroid administration. Glucocorticosteroids have numerous side effects, so steroid-sparing immunosuppressive agents may be indicated.

Depending on disease severity, pemphigus is initially treated with prednisone at doses of 1 to 1.5 mg/kg. Studies indicate that prednisone doses of 60-80 mg daily are usually as effective as higher doses (greater than 100 mg daily) in pemphigus³⁶. If there is no response, and the patient remains stable, a second immunosuppressive agent is introduced rather than increasing the dose of glucocorticosteroid. Oral pemphigus vulgaris can be resistant to therapy so topical steroids (eg dexamethasone mouthwash 0.1mg/ml) and topical cyclosporine can be of value.

Once the disease comes under control, prednisone can be introduced as an alternate day therapy with the immunosuppressive agent maintained at full dose.

When the indirect immunofluorescence study is negative and the clinical features have resolved, prednisone can be tapered, at a rate and level to prevent disease recurrence. When patients remain clinically clear without prednisone therapy, the dosage of the immunosuppressive agent is lowered.

Successful treatment of pemphigus has been achieved with prednisone in combination with steroid sparing agents described below and summarised in *Table 4*.

Gold, given orally (Auranofin™) or intramuscularly, or oral Dapsone™ are first choice agents for young patients with pemphigus vulgaris³⁷. Gold therapy can decrease circulating antibody titres by inhibiting immunoglobulin synthesis. It can also have an anti-inflammatory action on the complement cascade.

Auranofin™ can be initiated at a dose of 3mg three times a day after four to six weeks of high dose corticosteroids³⁸ and will take effect while the corticosteroids are tapered. The effect of gold therapy may take six to twelve weeks to be noticeable.

Gold toxicity may result in diarrhoea, nephrotoxicity, leukopenia, thrombocytopenia and pulmonary fibrosis. A full blood count (including differential white cell count) and a urine analysis should be performed every month during gold treatment.

Steroid sparing agent	Dose (Adults)	Adverse effects	Clinical & laboratory surveillance
<i>Gold (Auranofin™)</i>	• 3mg tds • 3mg daily once disease control attained	Diarrhoea Nephrotoxicity Leukopenia, Thrombocytopenia, Eosinophilia Aplastic anaemia (rare cases)	➤ Complete blood count & differential ➤ Urinalysis ➤ Liver function tests
<i>Dapsone</i>	• 200-300mg/day	Haemolysis Leukopenia Hepatitis Nephrotoxicity Peripheral neuropathy Not tolerated in individuals with glucose-6-phosphate dehydrogenase deficiency	➤ Complete blood count & differential ➤ Serum chemistry profile ➤ Liver function tests ➤ Urinalysis ➤ Neurological screening
<i>Cyclophosphamide</i>	• 2mg/kg/day	Carcinogenicity (lymphoma, leukaemia, bladder carcinoma) Leukopenia Thrombocytopenia Haemorrhagic cystitis	➤ Complete blood count & differential ➤ Serum chemistry profile ➤ Urinalysis ➤ Periodic complete physical examination & lymph node examination, chest radiography and pap smear
<i>Azathioprine</i>	• 1.0mg/kg/day	Carcinogenicity (non-Hodgkin's lymphoma, cutaneous squamous cell carcinoma) Bone marrow suppression Nausea & vomiting Hepatotoxicity Reduced fertility	➤ Complete blood count & differential ➤ Serum chemistry profile ➤ Urinalysis ➤ Tuberculin skin test (initially) ➤ Complete physical examination

Table 4. Steroid sparing immunosuppressant agents used in the treatment of pemphigus vulgaris.

A clinical trial has evaluated dapsone as a steroid sparing agent in the treatment of pemphigus vulgaris³⁹. Dapsone side effects include haemolysis, leukopenia, dapsone hypersensitivity syndrome, hepatitis, nephrotoxicity and peripheral neuropathy. Weekly haematological investigation, monthly neurological examination and periodic renal and hepatic monitoring are advised.

Other immunosuppressive agents commonly used as steroid sparing agents include cyclophosphamide⁴⁰ and azathioprine. Cyclophosphamide appears to be the more effective of the two agents but has numerous adverse effects³⁸, including bone marrow suppression, haemorrhagic cystitis, bladder fibrosis, and an increased risk of malignancy (non-Hodgkin's lymphoma and bladder carcinoma). Major side effects of azathioprine include bone marrow suppression, hepatotoxicity and a possible increased risk of malignancy.

Mycophenolate mofetil (Cellcept™) has recently been used with success in patients initially treated with prednisone and azathioprine who relapsed with tapering of the prednisone. These patients were treated with mycophenolate mofetil 2g daily and prednisone and did not relapse with tapering of prednisone⁴¹. Mycophenolate mofetil inhibits monophosphate dehydrogenase and is used as an anti-tumour agent. It is a potent and selective inhibitor of lymphocyte proliferation⁴².

As previously mentioned, plasminogen activator may have a role in the aetiology of pemphigus. Drugs that competitively inhibit the conversion of plasminogen to plasmin, such as tranexamic acid might be expected to be effective in treating pemphigus, but initial studies on the use of tranexamic acid have yielded disappointing results⁴³.

Patients with severe disease unresponsive to the above therapies may respond to treatments that lower antibody titres. These include the following:

- Extracorporeal photopheresis (photochemotherapy)
- Plasmapheresis
- Immunoapheresis
- Intravenous gammaglobulin

Extracorporeal photopheresis

This utilises 8-methoxypsoralen and ultraviolet A radiation. Rook *et al.*, (1990)⁴⁴ reported a series of four patients who were treated with photopheresis. At initiation of treatment, all four patients had high initial titres of pemphigus antibody, ranging from 1:320 to 1:1280 and were being treated with prednisone and either cyclophosphamide or azathioprine. All patients benefited from photopheresis as demonstrated by a decrease in antibody titre (1:80) and improvement of their clinical disease. One patient achieved clinical resolution with a non-detectable titre. All patients relapsed when photopheresis was ceased, yet responded to further treatment.

Plasmapheresis

This therapy involves removal of blood from the patient which is then passed through a centrifuge to separate the plasma (which contains the pemphigus antibodies) from the cellular elements. Concurrent use of low dose corticosteroids is not sufficient to suppress the rebound antibody formation seen after plasmapheresis and most likely accounts for the disappointing results from the first controlled study conducted by Guillaume *et al*⁴⁵. For plasmapheresis to be effective, B-cell antibody synthesis must also be suppressed (by concurrent use of immunosuppressive drugs) and the procedure regularly repeated to prevent re-accumulation of antibody.

Serum antibody titres must be monitored regularly during treatments. If titres do not decline, then treatment should be performed more regularly, dosage of immunosuppressive drugs increased or therapy should be aborted³⁷. Complications of plasmapheresis include depletion of clotting factors, electrolyte imbalance, fluid overload and infection.

Immunoapheresis

This is a process by which human immunoglobulin is separated and selectively removed from the remaining plasma and blood elements by absorption of plasma onto columns containing anti-human immunoglobulin (IgG). Schoen *et al.*, (1998)⁴⁶ described the use of immunoapheresis for paraneoplastic pemphigus in a sixteen year old boy with a previously resected inflammatory myofibroblastic tumour. Immunoapheresis was employed following unresponsiveness to corticosteroid therapy. This modality must be employed in combination with therapies to prevent antibody resynthesis, such as immunoglobulin or immunosuppressive agents.

Intravenous gammaglobulin

Intravenous gammaglobulin is a blood product prepared from pooled plasma. It works through the Fc portion of IgG. It creates a functional blockade of Fc receptors on macrophages, inhibits complement and complement-mediated damage and neutralises circulating autoantibodies by anti-idiotypic antibodies⁴⁷. Side effects usually occur thirty to sixty minutes after infusion and include headache, flushing, myalgia, fever, chills, low back pain, vomiting, chest tightness, tachycardia, or changes in blood pressure. Many of these can be avoided with administration of acetaminophen and an anti-histamine before infusion. Rare serious complications include renal failure, haemolysis and anaphylaxis, therefore patients should be screened prior to treatment for renal disease, liver disease, immunoglobulin A deficiency and cryoglobulinemia.

-
- ¹ Singer K H, Hashimoto K, Jensen P J, Morioka S, Lazarus GS. Pathogenesis of autoimmunity in pemphigus. *Ann Rev Immunol* 1985; **3**: 87-108.
- ² Robinson N D, Hashimoto T K, Amagai M D, Chan LS. The new pemphigus variants. *J Am Acad Derm* 1999; **40**: 649-71.
- ³ Mehravaran M, Morvay M, Molnar K, Olah J, Korom I, Husz S, Dobozy A. Juvenile pemphigus foliaceus. *Br J Derm* 1998; **139**: 496-9.
- ⁴ Provost TT. Pemphigus. *N Engl J Med* 1982; **306**: 1224-5.
- ⁵ Diaz LA Sampaio SA Rivitti EA, Martins CR, Cunha PR, Lombardi C, Almeida FA, Castro CM, Macca ML, Lavrado C. Endemic pemphigus foliaceus (fogo selvagem): clinical features and immunopathology. *J Am Acad Derm* 1989; **20**: 647.
- ⁶ Hailey H, Hailey H. Familial benign chronic pemphigus. Report of 13 cases in 4 generations of a family and report of 9 additional cases in 4 generations of a family. *Arch Derm Syph* 1939; **39**: 679-85.
- ⁷ Korman NJ, Eyre RW, Zone J, Stanley JR. Drug-induced pemphigus; auto-antibodies directed against pemphigus antigen complexes are present in penicillamine and captopril-induced pemphigus. *J Invest Dermatol* 1991; **96**: 273-6.
- ⁸ Anhalt GJ, Kim SC, Stanley JR, Korman NJ, Jabs DA, Kory M, Izycki H, Rathi H 3rd, Mutasim D, Ariss-Abdo L, Labib RS. Paraneoplastic pemphigus: an autoimmune mucocutaneous disease associated with neoplasia. *N Eng. J Med* 1991; **323**: 1729-35.
- ⁹ Camisa C, Helm TN. Paraneoplastic pemphigus is a distinct neoplastic induced autoimmune disease. *Arch Derm* 1993; **129** : 883-6.
- ¹⁰ Jablonska S, Chorzelski T, Beutner E, Chorzelska J. Herpetiform pemphigus; a variable pattern of pemphigus. *Int J Dermatol* 1975; **14**: 353-7.
- ¹¹ Wallach D. Intraepidermal IgA pustulosis. *J Am Acad Dermatol* 1992; **27**: 429-52.
- ¹² Becker BA, Gaspari AA. Pemphigus vulgaris and vegetans. *Dermatol Clin* 1993; **11**: 453-62.

-
- ¹³ Terpestra H, deJong MC, Klokke AH. In vivo bound pemphigus antibodies in a stillborn infant: passive intrauterine transfer of Pemphigus Vulgaris? *Arch Derm* 1979; **115**: 316-9.
- ¹⁴ Ross MG, Kane B, Frieder R, Gurevitch A, Hayashi R. Pemphigus in pregnancy, a re-evaluation of fetal risk. *Am J Obstet Gynaecol* 1986; **155**: 30-3.
- ¹⁵ Wasserstrum N, Laros RK Jr. Transplacental transmission of pemphigus. *JAMA* 1983; **249**: 1480-2.
- ¹⁶ Chowdhury MM, Natarajan S. Neonatal pemphigus vulgaris associated with mild oral pemphigus vulgaris in the mother during pregnancy. *Br J Derm* 1998; **139**: 500-3.
- ¹⁷ Goldberg NS, DeFeo C, Kirshenbaum N. Pemphigus vulgaris and pregnancy: risk factors and recommendations. *J Am Acad Dermatol* 1993; **28**: 877-9.
- ¹⁸ Lever WP. Pemphigus and pemphigoid: a review of the advances made since 1964. *J Am Acad Dermatol* 1979; **1**: 2-31.
- ¹⁹ Pisanti S, Sharau Y, Kaufman E, Posner LN. Pemphigus vulgaris – incidence in Jews of different ethnic groups according to age, sex and initial lesion. *Oral Surg Oral Med Oral Pathol* 1974; **38**: 382-7.
- ²⁰ Lamey PJ, Rees TD, Binnie WH, Wright JM, Rankin KV, Simpson NB. Oral presentation of pemphigus vulgaris and its response to systemic steroid therapy. *Oral Surg Oral Med Oral Pathol* 1992; **74**: 54-7.
- ²¹ Park MS, Tersaki PI, Ahmed AR, Tiwari JL. HLA-DRW4 in 91% of Jewish pemphigus vulgaris patients. *Lancet* 1979; **2**: 441-2.
- ²² Zegarelli DJ, Zegarelli EV. Intraoral pemphigus vulgaris. *Oral Surg Oral Med Oral Pathol* 1977; **44**: 384-93.
- ²³ Siegel MA, Balciunas BA, Kelly M, Serio FG. Diagnosis and management of commonly occurring oral vesiculoerosive disorders. *Cutis* 1991; **47**: 39-43.

-
- ²⁴ Hashimoto K, Lever WF. An electron microscopic study on pemphigus vulgaris of the mouth and the skin with special reference to the intercellular cement. *J Invest Dermatol* 1967; 48: 540-52.
- ²⁵ Wilson Jones E. Eosinophilic spongiosis in pemphigus. *Arch Dermatol* 1965; 97: 252-7.
- ²⁶ Anhalt GJ, Labib RS, Voorhees JJ, Beals TJ, Diaz LA. Induction of Pemphigus in neonatal mice by passive transfer of IgG from patients with disease. *N Engl J Med* 1982; 306: 1189-96.
- ²⁷ Schiltz JR, Michael B. Production of epidermal acantholysis in normal human skin invitro by Ig G fraction from pemphigus serum. *J Invest Dermatol* 1976; 67: 254-60.
- ²⁸ Beutner EH, Jordon RE. Demonstration of skin antibodies in sera of pemphigus patients by indirect immunofluorescent staining. *Proc Soc Exp Biol Med* 1964; 117: 505.
- ²⁹ Jordon RE, Triftshauser CT, Schroeter AL. Direct Immunofluorescence studies of pemphigus and benign pemphigoid. *Arch Dermatol* 1971; 103: 486-91.
- ³⁰ Farb R, Dykes, Lazarus GS. Anti epithelial cell-surface pemphigus detaches viable epidermal cells from culture plates by activation of proteinase. *Proc Natl Acad Sci* 1978; 75: 459-63.
- ³¹ Schiltz JR, Michel B, Papay R. Appearance of "pemphigus acantholysis factor" in human skin cultured with pemphigus antibody. *J Invest Dermatol* 1979; 72: 575.
- ³² Van Joost TH, Cormane RH, Pondman KW. Direct immunofluorescence study of skin on occurrence of complement in pemphigus. *Br J Derm* 1972; 87: 466-74.
- ³³ Jordon RE, Sams WM Jr, Diaz G, Beutner EH. Negative complement immunofluorescence in pemphigus. *J Invest Dermatol* 1971; 57: 407-10.
- ³⁴ Becker D, Ossowski L, Reich E. Induction of plasminogen activator synthesis by antibodies. *J Exp Med* 1981; 154: 386.
- ³⁵ Reich E. Activation of plasminogen: A widespread mechanism for generating extracellular proteolysis. In Ruddon R W (ed) *Biological markers of neoplasia: Basic and applied aspects* 1978; 491-8.

-
- ³⁶ Mourellou O, Chademenos GC, Koussidou TH, Kapetis E. The treatment of pemphigus vulgaris. Experience with 48 patients seen over eleven year period. *Br J Dermatol* 1995; **133**: 83-7.
- ³⁷ Korman N J. New and emerging therapies in the treatment of blistering diseases. *Dermatol Clin* 2000; **18**: 127-37.
- ³⁸ Fivenson D P. Nonsteroidal treatment of autoimmune skin diseases. *Dermatol Clin* 2000; **15**: 695-05.
- ³⁹ Basset N, Guillot B, Michel B, Giujou JJ. Dapsone as initial treatment in superficial pemphigus. Report of 9 cases. *Arch Dermatol* 1987; **123**: 783-5.
- ⁴⁰ Fellner M J, Katz J M, McCabe J B. Successful use of cyclophosphamide and prednisone for initial treatment of pemphigus. *Arch Dermatology* 1978; **114**: 889-94.
- ⁴¹ Enk A H, Knop J. Mycophenolate is effective in treatment of pemphigus vulgaris. *Arch Dermatology* 1999; **135**: 54-6.
- ⁴² Kitchen JE, Pomerantz KP, Pak G, Washenik K, Supack JL. Rediscovering mycophenolic acid – a review of its mechanisms, side effects and potential uses. *J Am Acad Dermatol* 1997; **37**: 445-9.
- ⁴³ Marsden J. Lack of response of pemphigus to tranexamic acid. *Br J Dermatol* 1987; **116**: 882-3.
- ⁴⁴ Rook AH, Jegasothy BV, Heald P, Amann G, Rappersberger K, Stingl G. Extracorporeal photochemotherapy for drug-resistant pemphigus vulgaris. *Ann Intern Med* 1990; **112**: 303-5.
- ⁴⁵ Guillaume JC, Roujeau JC, Morel P, Doutre MS, Guillot B, Lambert D, Lauret P, Lorette G, Prigent F, Triller R. Controlled study of plasma exchange in pemphigus. *Arch Dermatol* 1988; **124**: 1659-63.
- ⁴⁶ Schoen H, Foedinger D, Derfler K, Volv-Platzer B. Immunoapheresis in paraneoplastic pemphigus. *Arch Dermatol* 1998; **134**: 706-10.
- ⁴⁷ Jolles S, Hughes J, Whittaker S: Dermatological use of high dose intravenous immunoglobulin. *Arch Dermatol* 1998; **134**: 80-6.

RECURRENT APHTHOUS STOMATITIS

A Case Presentation

RECURRENT APHTHOUS STOMATITIS

SYNOPSIS

A case report of a patient with recurrent aphthous stomatitis

CASE PRESENTATION

Name: TS

Gender: Female

Age: 29 years

MRN: 0140972

CHIEF COMPLAINT

Long standing history of recurrent mouth ulcers.

HISTORY OF CHIEF COMPLAINT

The patient presented with a six-year history of crops of simultaneous recurrent, painful ulcerations involving the soft tissues of her mouth.

- i. She would have a minimum of 1 ulcer to a maximum of 10 ulcers present at one time.
- ii. Each ulcer would be present for approximately 10-14 days.
- iii. There was no significant remission period between ulcers. A new crop of ulcers would erupt following the resolution of previous ulcers.
- iv. In general, the non-keratinized mucosa was affected. The lateral and ventral surfaces of the tongue, the labial and buccal mucosa.
- v. These ulcers erupted spontaneously and were exacerbated by stress.
- vi. She had used many "over the counter" ulcer-relief topical preparations, with no success.

The patient had consulted many different specialists, including a dermatologist and a clinical immunologist, regarding her ulcers with no improvement of her symptoms. Skin patch tests demonstrated an allergy to nickel. The patient had had her amalgam dental restorations replaced with tooth-coloured adhesive materials by her dentist. There was no marked improvement of her symptoms following this procedure.

MEDICAL HISTORY

The patient suffers from allergic rhinitis. She denies any gastrointestinal complaints, and reports regular bowel movements. She did not report any weight loss, her weight was steady at 70kg.

She is a reformed non-smoker for the past four years, with a previous 11-year smoking history. She consumes alcohol on an infrequent social basis. She takes the contraceptive pill, and a multivitamin over-the-counter preparation regularly.

DENTAL HISTORY

The patient attends her dentist on a regular basis for recall examinations

SOCIAL HISTORY

The patient is married with two children whom she looks after. She is not a vegetarian and eats a nutritional diet including vegetables. She avoided certain fruit, such as oranges, when she had ulcers.

FAMILY HISTORY

There was no family history of ulcers.

EXTRA ORAL EXAMINATION

No clinical abnormality was evident on extra-oral examination. There was no regional lymphadenopathy. The patient had no history of skin lesions and her skin was clinically normal at the time of examination. No lesions were present in her nasal passages, by direct visual examination. The patient denied any eye or genital involvement. The patient was not clinically anaemic and neither obese nor anorexic in appearance (that is, no clinical evidence of malabsorption).

INTRA ORAL EXAMINATION

Hard tissues: TS was fully dentate, with the exception of her third molars which had been extracted. Her molar teeth had been restored with tooth-coloured restorations. There was no active caries.

Soft tissues: Two ulcers were present at the time of the first examination:-

1. An ulcer, 2mm in diameter on the labial mucosa of the left side of the lower lip. The crater was covered by a yellowish-white slough and had an erythematous halo.
2. There was a cluster of small ulcers of varying size, with features of coalescence with a grey slough on the left anterior ventral surface of the tongue.

There was also a slight “cobblestone” appearance, bilaterally in the buccal mucosa. There were localized scars on the oral mucosa (lateral and ventral surface of the tongue), presumably resulting from previous lesions. No bullae or vesicles were present.

PROVISIONAL DIAGNOSIS

1. Recurrent aphthous stomatitis
2. Oral manifestation a mucocutaneous disease – *pemphigus, pemphigoid*
3. Oral manifestation of a gastrointestinal disease – *Crohn's (in particular with the cobblestone appearance of buccal mucosa), coeliac disease, ulcerative colitis*
4. Recurrent viral infection – *herpes group*

INVESTIGATIONS

Initial investigations involved haematological and serological assessment. Haematinic deficiencies, markers of gastrointestinal disease and autoimmune vesicular bullous diseases were specifically checked (*Table 1*).

Table1. Haematological, biochemical investigations and autoantibody profile

	<i>Results:</i>	<i>Normal range</i>
Iron	15.5	7.0 – 29.0 umol/L
Transferrin saturation	0.23	0.08 – 0.44
Ferritin	24	15 – 150 ug/L
Transferrin	3.0	1.8 – 3.3 g/L
White cell count	6.9	3.9 – 11.1 x 10 ⁹ /L
Red cell count	4.0	3.9 – 5.0 x 10 ¹² /L
Haemoglobin	119	115 – 165 g /L
Haematocrit	0.36	0.36 – 0.44
Mean cell volume	91	82 – 98 fL
Platelets	363	150 – 400 x 10 ⁹ /L
Mean cell haemoglobin	30	27 – 32 pg
Film comment	Normal red cell morphology Slight eosinophilia Normal platelet count	
Neutrophils absolute	3.1	2.0 – 8.0 x 10 ⁹ /L
Lymphocytes absolute	2.5	1.0 – 4.0 x 10 ⁹ /L
Monocytes absolute	0.3	0.2 – 1.0 x 10 ⁹ /L
Eosinophils absolute	0.8	0.0 – 0.5 x 10 ⁹ /L
Basophils absolute	0.1	0.0 – 0.1 x 10 ⁹ /L
Neutrophil	46 %	
Lymphocyte	36 %	
Monocytes	4 %	
Eosinophil	11 %	
Basophils	1 %	
Erythrocyte sedimentation rate	18	0 – 20 mm/hr
Vitamin B12	278	125 – 780 pmol/L
Red cell folate	1097	370 – 1050 nmol/L
Serum folate	36	5 – 21 nmol/L
Intercellular substance antibody	<10	
Basement membrane substance antibody	<10	
Gliadin IgA	4	0 – 25 U
Gliadin IgG	16	0 – 25 U
Endomysial IgA	<5	<5
C-reactive protein	7	0 – 8 mg/L
Angiotensin converting enzyme	53	20 – 55 U/L

INTERPRETATION OF RESULTS

In summary, no haematological, biochemical or immunological abnormality was identified.

Intercellular substance antibody (ICSA) and basement membrane substance antibody (BMSA) are markers of circulating autoantibodies for pemphigus and bullous pemphigoid, respectively. These are expressed as antibody titres, determined by indirect immunofluorescence, a value less than 10 being considered negative. Therefore these autoimmune mucocutaneous diseases were excluded on clinical and serological grounds.

Erythrocyte sedimentation rate (ESR) is a non-specific indicator of inflammatory (and neoplastic) disease. The ESR can be increased in both acute and chronic inflammatory disease. The ESR increases with age, and is raised in pregnancy and anaemia.

C-reactive protein is a more sensitive early indicator of an acute phase response in inflammation. These results were both within the normal reference range.

Serology for gastrointestinal disorders included serum endomysial and gliadin antibodies.

A positive result for gliadin antibodies can be found in up to 10% of the normal population.

Endomysial antibodies are a more specific test with a specificity greater than 90% for the diagnosis of coeliac disease. These results did not support a diagnosis of coeliac disease.

Mild eosinophilia is a common finding and is often transient. Eosinophilia can be seen in a variety of situations, including drug reactions, atopic disease, skin disorders and also parasitic infections and malignancy. It is recommended if there is a mild eosinophilia to repeat the test after a few months.

Folate levels were slightly elevated. On direct questioning, the patient reported that she had taken folate supplements when she was pregnant with her second child and recently discontinued this treatment.

PLAN

The patient was to be reviewed when she experienced her next crop of ulcers, so that a clinical assessment could be made. The patient was requested to make note of certain features:

- Number of ulcers present at any one time
- Duration of ulcers
- Longest remission period between ulcers
- Which sites affected in the mouth
- Aggravating and precipitating factors
- Associations with food, menstrual cycle and life-style (stress)
- Relieving factors

REVIEW

On review, the patient presented reporting crop of mouth ulcers with multiple sites affected, for approximately ten days duration. She reported that these lesions were painful, with no aggravating factors identified.

On extra-oral examination there were no significant findings. Intra-oral examination demonstrated 8 small ulcers involving the non-keratinised mucosal of the soft palate, the buccal mucosa bilaterally, the right labial mucosa, and the ventral surface of the tip of the tongue. Broadly, the individual lesions appeared as ulcerated areas with an erythematous halo, covered by a grey pseudomembranous slough.

WORKING DIAGNOSIS:

Recurrent aphthous stomatitis

The diagnostic features were the history of recurrent ulceration confined to the mouth, with a typical appearance including an erythematous halo and a site predilection for non-keratinized mucosa. The blood tests performed one month previously at the initial examination failed to demonstrate any haematological or immunological abnormalities, or evidence of intestinal malabsorption. An exfoliative cytology smear was performed at this review appointment to exclude a viral aetiology (although unlikely on clinical grounds) by cytology. Wet fixed slides were prepared from lesional and perilesional tissue of two sites, namely the soft palate and right labial mucosa.

Topical steroids were commenced in the form of a mouthwash. This delivery method was chosen due to the widespread involvement of oral tissues. Prescription: Dexamethasone 0.1mg/ml (5ml held in mouth for 5 minutes then contents expelled, four times a day)

REVIEW 2

The patient was reviewed two weeks later to assess response to topical steroid preparation and to review the cytology results.

Exfoliative cytology from the soft palate and right labial mucosa, demonstrated scattered squamous cells, including groups of reactive metaplastic squamous cells in a background of inflammation, blood and bacteria. No viral inclusions were noted and no diagnostic malignant cells found.

The patient reported an 80% improvement of her symptoms since starting the topical steroid mouthwash. She reported that there was a significant decrease in the discomfort associated with the ulcers, and she had no new lesions.

Intra-oral examination demonstrated complete resolution of lesions affecting the soft palate, bilateral buccal mucosa, and right labial mucosa. There was a small group of healing lesions involving the ventral tip of the tongue.

The plan was to continue with this current treatment regime of topical steroids. The frequency of mouthwash use would be reduced when there were no ulcers present, to once a day. If the patient experienced an eruption of ulcers then the frequency of topical steroid use would be increased, to four times a day.

REVIEW 3

The patient was reviewed one month later to assess management. She was most pleased to report that her mouth ulceration had been very well controlled with the dexamethasone steroid mouthwash, and was using it once a day. The patient stated that she went away for four days and had failed to take her topical steroid mouthwash – subsequently she had an eruption of oral ulcers, which responded with the subsequent use of the mouthwash.

She stated that the only site where she occasionally still gets new ulcers was the anterior part of the tongue on the ventral surface.

Extra-oral examination was unremarkable. Intra-oral examination demonstrated an area involving the ventral surface of the anterior tongue, there were coalesced ulcers with a pseudomembranous slough and erythematous halo. It was thought that this might possibly be related to an access problem for that site with the use of a mouthwash. It was decided to supplement the use of Dexamethasone mouthwash with a topical steroid cream application to this particular site. Betamethasone-17-valerate 0.05% in orobase 50:50 was prescribed.

This lady was most relieved by the improvement in her oral ulceration which was of 6 years duration, for which she had consulted many specialists with no improvement. The possible value of a biopsy was discussed with the patient, with the findings likely to be that of non-specific ulceration. However the patient felt that she would like to exclude any other aetiology, such as granulomatous conditions with oral manifestations included in our provisional diagnosis.

It was decided that she would stop the use of topical steroids one-week prior to the planned biopsy, to allow for representative lesional tissue.

BIOPSY

At the biopsy appointment, multiple characteristic aphthae-like ulcers were present (*see Figures 1, 2 & 3*). Incisional biopsies were performed from lesional and perilesional tissue, under local anaesthesia with no complications. Tissue was taken from the left buccal mucosa and left maxillary alveolar mucosa, placed in 10% buffered formalin for histopathological diagnosis. The differential diagnosis at the time of biopsy included aphthae and orofacial granulomatosis.

The patient was reviewed one week postoperatively, with no reported complications.



Figure 1. Intra oral photograph of ulcerative lesion, left maxillary alveolus. Tissue was taken from this site for histopathological examination



Figure 2. Intra oral photograph of ulcerative lesions, left buccal mucosa. Tissue was also taken from this site for histopathological examination

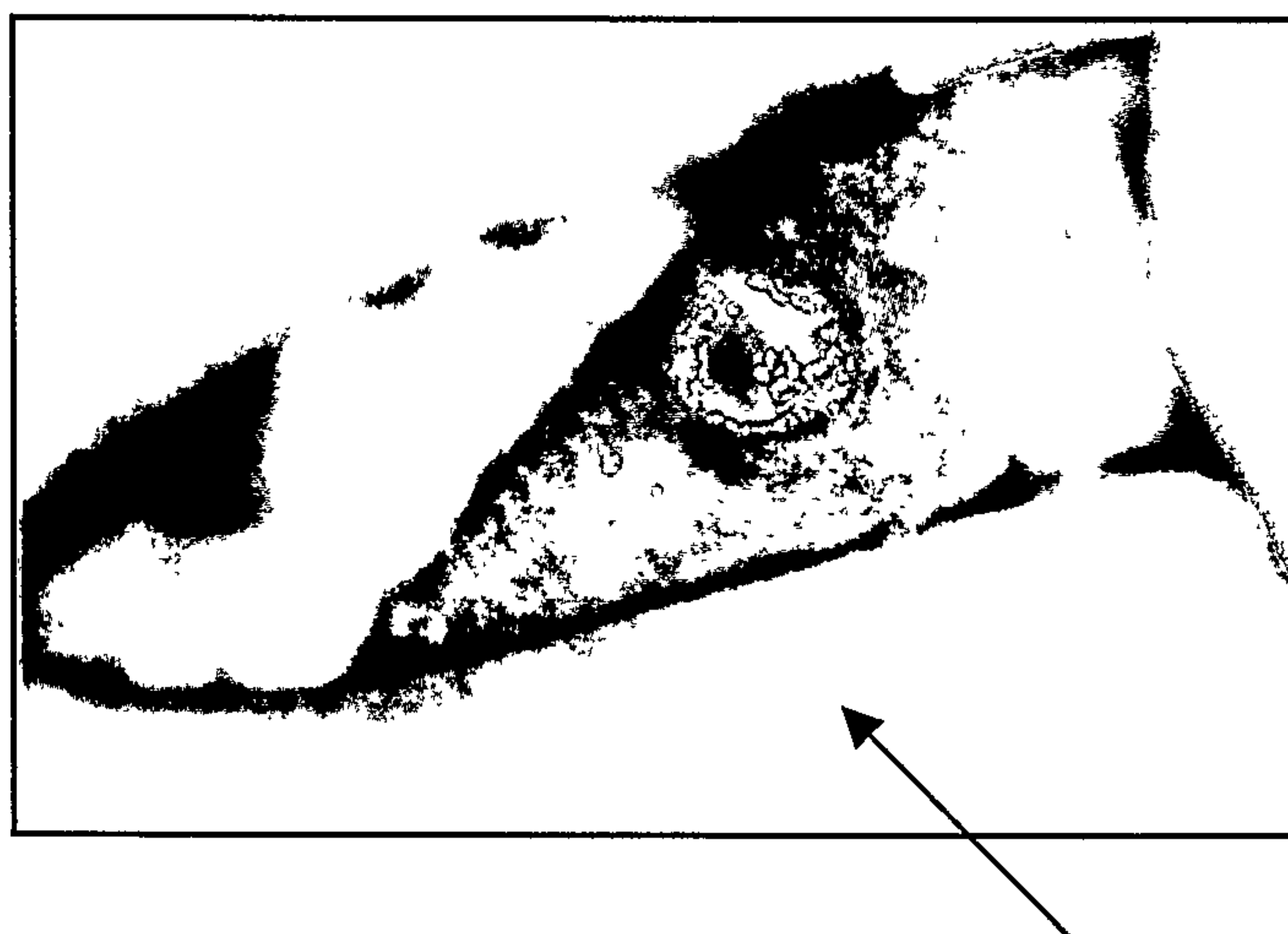


Figure 3. Intra oral photograph of typical ulcerated lesion. Right latero-ventral surface of anterior tongue.

HISTOPATHOLOGY

Left buccal mucosa: Sections examined demonstrated oral squamous mucosa with a heavy inflammatory infiltrate composed of polymorphonuclear neutrophils, lymphocytes and plasma cells. There was a moderate degree of basal hyperplasia. Ulceration was noted, which was covered by fibrin and blood clots (*Figure 4*). No multinucleated giant cells or viral inclusions were seen. There was no evidence of a vasculitis or formation of granulomas. No fungal elements were observed with PAS stain. There was no evidence of malignancy.

DIAGNOSIS: Active chronic inflammation.

Consistent with aphthous ulcer

Left maxillary alveolar mucosa: Sections showed a fragment of oral squamous mucosa. The oral epithelium demonstrates mild basal cell hyperplasia, with surface parakeratosis and hyperkeratosis. No granulomas were present in the sections examined. There were no multinucleated giant cells or viral inclusions. There was no evidence of vasculitis. There was no evidence of malignancy. No fungal elements were observed with PAS stain.

DIAGNOSIS: Mild chronic inflammation.

Although these histological features are non-specific and non-diagnostic, however there are no features to suggest Crohn's disease or a vasculitis.

These features are consistent with aphthous ulceration.



Figure 4. Left buccal mucosa biopsy shows ulcerated surface covered by fibrin, with heavy chronic inflammatory cell infiltrate (H&E section, x20 magnification).

REVIEW 4

At recall examination the patient reported that overall she was managing well, although still subject to occasional ulcers. She had identified aggravating factors, which included sugar and spicy foods. She had found the topical steroid preparations symptomatically beneficially.

She was planning to have another child, and is currently trying to get pregnant. There were no changes to her medical history.

Extra-oral examination was unremarkable. Intra-oral examination demonstrated two small ulcers, some 2mm in their largest dimension involving the ventral surface of the tongue.

These ulcers were consistent with the appearance of minor aphthae.

Management plan was as follows;

- * Use of betamethasone-17-valerate 0.05% with orabase to lesions (Category A- *no proven increase in the frequency of malformations or other direct or indirect harmful effects on the foetus observed* – Prescribing medicines in pregnancy: An Australian categorisation of risk of drug use in pregnancy 4th Edition. Australian Drug Evaluation Committee)
- * Vaseline was to be applied to the surfaces of the teeth in close proximity to ulcers, in attempt to reduce trauma.
- * Avoiding known aggravating factors, and minimizing microtrauma.

DISCUSSION

Recurrent aphthous stomatitis is a common oral mucosal disorder, and may affect 20% or more of the population. The diagnosis is made on clinical and historical features as there are no specific diagnostic laboratory tests.

Recurrent aphthous stomatitis is characterized by recurrent episodes of one or several rounded, shallow, painful oral ulcers at intervals of a few months to a few days. The ulcers can be classified depending on the size of the lesions, site affected and duration.

Assessment of healing with scarring can be of assistance. Three categories are described, namely minor, major and herpetiform ulceration (*Cooke BED 1960*)¹. The features of each type are summarized in *Table 1*.

Table 1. Classification of aphthous and herpetiform ulcers

	MINOR aphthae	MAJOR aphthae	HERPETIFORM
Prevalence	80%	10%	10%
Female : male ratio	1.3 : 1	0.8 : 1	2.6: 1
Number of lesions	1 to 5	1 to 3	10 to 100
Size of lesions	<10 mm	>10 mm	1-2 mm (may coalesce)
Common site of lesions	Lips, cheeks, tongue, floor of mouth	Lips, cheeks, tongue, palate and pharynx	Entire oral mucosa
Duration	4 - 14 days	>30 days	>30 days
Healing with scarring	10%	65%	30%
Rates of recurrence	1 to 4 months	< monthly	<monthly

Minor aphthae (*Mikulicz type*), are the most common form of aphthous ulceration. They are characterized by one or more small (usually less than 5mm) round or ovoid shallow ulcers. These ulcers have a well-defined depressed base, with a gray-white pseudomembrane surface and a peripheral zone of erythema, often referred to a “halo”. These ulcers frequently occur on labial and buccal mucosa and floor of mouth (non-keratinized oral mucosa), they are moderately painful and typically heal without scarring in 7-10 days (or within 10-14 days). These lesions can recur at 1- to 6- month intervals. Quite often they first occur during childhood or adolescence.

Major aphthae, also known as Sutton’s *disease* or *peradenitis mucosa necrotica recurrens*, are a more severe form which occurs less frequently. These present as round oval lesions with a clearly defined base, pseudomembranous slough covering and a peripheral erythematous halo. Compared with minor aphthae, major aphthous ulcers are larger (1-3 cm), present in smaller numbers and are more persistent, lasting 10 to 30 days. They frequently heal with scarring. Any site can be affected, but there is a predilection for involvement of the lips, soft palate and fauces. Severe discomfort, dysphagia and facial oedema may be experienced by the affected individual. These ulcers may also first occur during childhood or adolescence.

Herpetiform aphthae are the least common variety², with a female predisposition. The age of onset is later than that of minor and major, frequently commencing in the third decade. The lesions are akin to those of herpes simplex stomatitis, but are not caused by the herpes simplex virus. Multiple recurrent crops of small, painful ulcers that are widespread are seen. As many as 100 ulcers may be present at a given time, each measuring 2-3 mm in

diameter. These small ulcers tend to coalesce to form larger irregular ulcers. They have a variable healing time, from 7-30 days and may heal with scarring.

This traditional classification of recurrent aphthae is related to the morphology of clinical lesions. In North America the terms “simple” or “complex” are used to further describe the severity. Complex aphthosis is characterised by multiple large lesions, which are slow to heal. These may equate to major aphthae. Individuals may have continuous ulceration and remission is often short. Some patients with complex aphthosis develop Behçet’s disease (Rogers III 2002)³.

Stanley HR 1972⁴ divided the clinical features of aphthous stomatitis into four stages, outlined in Table 2.

Table 2: Stages of aphthae

Premonitory 1 st 24 hours of pathological process	• Prodrome – <i>tingling, paraesthesias, burning sensation</i> • Histologically there is spongiosis of the mucosa. • Infiltrate of neutrophils in the lamina propria. Plasma cells, eosinophils, mast cells may be seen.
Preulcerative 18 – 72 hours	• Macules and papules begin to appear • Superficial membrane with an erythematous halo • Pain – moderate intensity to quite severe
Ulcerative Days 1 -16	• Membrane becomes necrotic and sloughed • Resulting lesion- depressed base, fibrinous exudative centre, and erythematous halo • Pain intensity increases
Healing Days 4 – 35	• Granulation tissue forms, epithelial covering develops

AETIOLOGY

The aetiology of recurrent aphthous stomatitis is multifactorial. The lesions might arise as a consequence of immunologically mediated cytotoxicity of epithelial cells. Various predisposing factors including systemic, local, genetic and microbial will be discussed.

Predisposing systemic factors:

In general, most patients affected with RAS otherwise enjoy good health. However, there are various systemic disorders which may involve oral ulceration which should be considered in the differential diagnosis (*Table 3*)

Local factors:

Local physical trauma may initiate ulcers in persons who are predisposed to aphthous stomatitis⁵. Minor trauma can include dental manipulation (during treatment) local anaesthetic injections, tooth brushing (excessive), dental flossing, sharp foods, and dentures. Recurrent aphthous stomatitis is uncommon where mucosal keratinisation is present⁶, and is also less frequently seen in patients who smoke tobacco⁷. Indeed, the onset or a severe exacerbation of aphthous stomatitis often follows cessation of smoking.

Psychological stress can precipitate flare-ups in affected individuals. *Ship II et al., 1961*, noted an increase in incidence of ulcers in a postgraduate student group studying for examinations. There was a decrease in incidence during the vacation period.⁸ *Ship II 1972*, demonstrated predisposing personality traits, including anxiety, rigidity, inflexibility and repressed hostility in patients affected with recurrent aphthous stomatitis⁹.

Table 3. Systemic disorders predisposing to oral ulceration

Behçets syndrome	➤ Multisystem disease, affecting other mucocutaneous surfaces, eyes, musculoskeletal, neurological, haematological, gastrointestinal and other systems. ➤ Clinicopathologically an inflammatory disorder characterized by enhanced neutrophil chemotaxis, and neutrophil and platelet hyperfunction. ➤ Oral ulceration's seen in nearly all affected individuals (90%)¹⁰
Sweets syndrome	➤ Febrile neutrophilic dermatoses, setting in another illness (underlying malignancy, inflammatory bowel disease, infection (strep), medications, pregnancy
Cyclic neutropenia	➤ Agranulocytosis, characterized by periodic reduction of neutrophils from peripheral blood.
Benign familial neutropenia	➤ Periodic syndrome with fever and pharyngitis
Haematinic deficiencies	➤ Iron, folic acid, Vitamin B12 ➤ 2x common RAS patient cf controls^{11, 12, 13}
Nutritional deficiencies	➤ Zinc deficiency (apparent beneficial effect of zinc supplementation¹⁴)
Gastrointestinal disorders	➤ Coeliac disease (gluten-sensitive enteropathy) ➤ Crohns disease – aphthae may be sole manifestation¹⁵ ➤ Ulcerative colitis
Immunodeficiencies	➤ Primary ➤ Secondary
Psychological	➤ Psychological stress ➤ Personality traits
Drugs	➤ Non-steroidal anti-inflammatory agents
Hormonal	➤ Menstruation cycle^{16, 17} (luteal phase, with changing levels of progesterone) ➤ Aphthae may improve during pregnancy

Genetic factors:

In some individuals there is a familial basis. Patients with a positive family history often experience more severe symptoms than those of affected individuals with no family history. Those affected also may develop oral ulcers at an earlier age¹⁸. *Miller MF 1977*, demonstrated a high correlation of RAS identical twins¹⁹.

There are conflicting reports of an association with particular serologically determined HLA antigen or haplotype demonstrated in the literature. This may be as a result of inadequate patient numbers, and also variable ethnic backgrounds of investigated patients.

Microbiological factors:

Various organisms have with a possible role in the pathogenesis been isolated from lesions of RAS. These are summarized in *Table 4*.

Table 4. Microbiological associations with RAS

Streptococci	S. sanguis ¹⁷	Isolated from lesions, rapid rapid ↑ serum antibody titers following flare ups, initial form typed as S. Sanguis (type 2A) ²⁰
	S. mitis	Initial organism isolated, later typed as a strain of S. Sanguis
Helicobacter pylori		Detected in 71.8% of swabs from recurrent aphthae, using polymerase chain reaction, although there is uncertainty whether contributes to their aetiology ²¹ Frequency of serum IgG antibodies not increased in RAS
Viruses	Adenovirus	Association suggested ²² , (ubiquitous organism though)
	Herpes simplex	Complementary RNA detected in circulating mononuclear cells in some RAS pts ²³ Not successfully isolated from lesional material ²⁴
	Varicella Zoster	IgM and IgG antibodies may be elevated in some RAS patients, suggesting an association between reactivation and RAS ²⁵ . Conflicting findings: VZV DNA not detected in 21 RAS biopsy specimens by polymerase chain reaction (<i>Ghodratnama et al.</i> , 1997) ²⁶ whereas <i>Pederson et al.</i> , 1993 ²⁷ detected VZV DNA in RAS biopsies with polymerase chain reaction.
	Cytomegalovirus	Antibodies to CMV may be significantly elevated in some RAS patients ²⁵ CMV DNA detected in ill-defined oral ulcerations in non-HIV infected persons ²⁸
	HHV - 8	DNA present in HIV – related oral ulcers ²⁹

IMMUNOPATHOGENESIS

Numerous studies have examined circulating leukocytes and lesional infiltrating lymphocyte subsets. Cytokines and adhesion molecules, which govern leucocyte movement, have been suggested to play a role in the movement of leucocytes out of blood vessels and into the submucosa during outbreak of lesions.

The perilesional mucosal lymphocyte infiltrate is primarily composed of T-lymphocytes. Alterations in peripheral blood lymphocytes include selective activation of cytotoxic T cells and NK cells³⁰. There are specific T-lymphocyte subset variations reported³¹. T-inducer subsets were found to be depressed and natural killer (NK) cell activity increased when ulcers were present in major aphthae. NK cell activity decreased one-to-two weeks post-exacerbation of ulcers.

RAS can also be totally suppressed by high doses of steroids (not usually a clinically appropriate therapy) or responds at least partially to topical corticosteroids.

HISTOPATHOLOGY

Aphthae demonstrate non-specific inflammation. Typically, a mixed inflammatory infiltrate is seen. Perivascular cuffing is noted to be characteristic, however is infrequently seen on specimens. The main microscopic feature which may assist in distinguishing aphthae from traumatic ulcers is that the inflammatory infiltrate may be more intense and extend more deeply – however the clinical should also assist with this differentiation.

MANAGEMENT

1. **Diagnosis:**

Diagnosis is generally based on the patient's history and clinical findings. A biopsy is not usually undertaken as the histological features are usually non-specific, although the histology may occasionally be of use in excluding other disease processes. Haematological and serological investigations may reveal an accompanying deficiency, which is noted to be twice as common in patients who experience recurrent aphthae stomatitis^{5,6,7}. Conditions which are associated with oral ulceration need to be excluded, such as erythema

multiforme, pemphigus, pemphigoid, Reiter’s syndrome, lupus erythematosus and oral manifestations of gastrointestinal conditions. Behçets syndrome needs to be excluded, and a detailed history needs to be taken including ocular and genital symptoms. Atypical viral infections also need to be excluded.

2. **Therapy / Management:**

Management is generally directed at symptomatic relief. It is vital to identify and control contributing factors – working in co-operation with the patient to identify aggravating factors can be facilitated with the use of an ulcer and food diary. An ulcer diary should be kept for a period of 1 –3 months, with particular features recorded (see *Table 5*)

<i>Example</i>	<i>Date: 13th March 2002</i>	<i>Information that can be extracted</i>
Number of ulcers & duration	Three	<i>Want to identify number of ulcers that are present at a time, it is also crucial to note the duration of ulcers.</i>
Site	1. Left side of tongue 2. Right cheek 3. Right lower lip	<i>To identify if there is a site predilection</i>
Food	Salt and vinegar crisps	<i>Identify potential precipitating food types, low pH foods which are painful in RAS</i>
Social	Meetings at work Children’s exams	<i>Identify potential stresses that may play a role</i>
Menstruation	Day 2	<i>For females there can be an association with menstruation cycle</i>

Table 5: Example of an ulcer diary

If there is any secondary systemic disease identified during the examination and investigations, then the appropriate referral is required for management. There is no specific therapy for recurrent aphthae, but the symptoms can be reduced by appropriate therapy. Once precipitating factors are identified, then these can be avoided. Only controlled dietary exclusion can be relied upon to identify a dietary allergy and only gluten in coeliac disease is generally accepted as a proven factor in oral ulceration.

A number of therapies have been described – the choice of medication depends on the frequency of ulcers, the location and persistence of ulcers and also the level of associated orofacial pain. Broadly, treatment modalities can be divided into their mode of action, including analgesics, antibacterials and immunomodulators. The treatment therapies described in the literature are summarized in *Table 6*. Most frequently topical glucocorticosteroids are used, with analgesics for pain relief. Major aphthae remain a difficult problem for management. Systemic corticosteroids may give a short-lived relief but are not usually advisable in the long term due to their adverse effects. Thalidomide is likely to be more effective, but it is associated with significant risks of teratogenicity and peripheral neuropathy.

Scarring with fibrosis can present secondary problems, including limited opening, difficult access for both the patient and dentist for oral hygiene and restorations.

In summary, recurrent aphthae are a common presenting complaint by patients of both dentists and medical practitioners. Frequently these patients are referred to specialists, including oral medicine specialists, for investigation and management – with the request to exclude an underlying systemic problem. A comprehensive history in all areas, including

details of the ulceration pattern, family and social history, and a complete medical history is usually of diagnostic value to the informed clinician, with the clinical appearances confirming the provisional diagnosis. There are many systemic conditions associated with recurrent aphthae as described, and it is vital that these are investigated and excluded. Patients often present in some distress from their painful mouth ulceration which may interfere with eating, swallowing and speech. It is important to spend time with the patient, emphasizing that these ulcers are not transmissible, that they are only uncommonly an oral manifestation of systemic disease and that relief is available. With the use of an ulcer diary, precipitating and aggravating factors can be identified, and subsequently avoided. Generally, most patients report an improvement with the use of various therapies available, and become proficient in identifying the onset of an ulcer and in administering topical medications promptly.

Antimicrobial / Antibiotics	1. Chlorhexidine gluconate 2. Tetracycline hydrochloride 3. Minocycline hydrochloride	• Some benefit - ↓ ulcer days, ↑ ulcer free days. Cannot prevent the recurrence of ulcers². Reduce 2° infection • Used alone or combination with amphotericin can reduce the severity of ulceration, but do not alter the recurrence rate of RAS³². <i>250mg capsule dissolved in 180mg water, 3-minute rinse qid, 3-5 days.</i> • Found to be as effective as tetracycline, advantage bd cf qid³³ <i>100mg capsule bd</i>
Topical corticosteroids	1. Hydrocortisone hemisuccinate <i>2.5mg (slowly dissolving pellet)</i> 2. Triamcinolone acetonide 0.5% 3. Flucinonide 0.05% 4. Betamethasone valerate 5. Flumethasone pivalate 6. Beclomethasone dipropionate 7. Dexamethasone 0.1mg/ml	➤ Reduce symptoms, not rate of recurrence. ➤ Effective for single lesions ➤ Numerous widely distributed lesions use of rinse more effective. ➤ Steroid in combination with antibiotic and antifungal medication may be advantageous in some cases
Immunomodulators	1. Levamisole 2. Transfer factor 3. Colchicine 4. Gammaglobulins 5. Azathoprine 6. Dapsone 7. Thalidomide 8. Pentoxifylline 9. Prednisolone 10. Azelastine 11. Alpha-2-interferon 12. Cyclosporin 13. Amlexanox 14. Prostaglandin E2	• Antihelminthic agent, immune stimulant. Trials have demonstrated ↓ frequency & duration, and diminish pain³⁴. Administered onset prodrome – <i>50mg tds 3/7</i> adverse effects of nausea, hyperosmia, dysgeusia, agranulocytosis. • Suggested to be beneficial³⁵. • Significant reduction in pain scores and frequency of self-reported ulcers³⁶. Combined with thalidomine therapy may occasionally benefit recalcitrant RAS². Can produce infertility in young males. • Immunomodulatory agent – suggested some benefit in management of RAS³⁷ • Reported to ↓ oral lesions in pts with RAS-like lesions (clinical features of pt group not clearly described²) • Widely reported that may produce remission or ↓ symptoms of RAS³⁸. Teratogenicity, polyneuropathy and other side effects. Reported use in HIV-related ulceration (thalidomide hypersensitivity) <i>25mg day</i> • Immunosuppressive actions with minimal adverse effects. Notable ↓ in no. RAS episodes for up to 9 months after therapy, no side effects reported (400mg tds 30days)^{39,40}. • Persistent major aphthae with considerable tissue destruction, initiate with prodromal symptoms (steroid related S/E) • Suggested some benefit in management of RAS⁴¹. • Human alpha-2-interferon in a cream suggested benefit in RAS. • Suggested to be beneficial⁴² • 5% topical application suggested to be beneficial⁴³ • Applied as a gel, with suggested benefit⁴⁴ • Pain associated with aphthae can be effectively controlled³³ – Aspirin <i>650mg qid</i> Ibuprofen • May reduce severity of RAS⁴⁵ • Reduction in pain <i>5mg/ml with magnesium hydroxide 50:50</i> • Improves wound healing³³ • Low dose benzodiazepine medications prescribed daily for no more than 2 weeks effective adjunctive therapy³³ • Lozenges may provide mild symptomatic relief⁴⁶ <i>20mg lozenge, qid</i> • Transient relief of pain in severe RAS². No more benefit than a placebo reported⁴⁷ • Ablation of ulcerated surface, with pain relief⁴⁸ • Cases where RAS associated with menstrual cycle. Compounds / conjugated oestrogens available
Analgesics	1. Non-steroidal antinflammatory 2. Benzydamine hydrochloride 3. Diphenhydramine hydrochloride	
Others	1. Systemic zinc sulphate 2. Anti-anxiety drugs 3. Sodium cromoglycate 4. Carbenoxolone disodium 5. Laser 6. Oestrogen	

¹ Cooke BED. Br Dent J 1960

² Porter SR, Scully C, Pedersen A. Recurrent aphthous stomatitis. *Crit Rev Oral Biol Med* 1998; **9**: 306-21.

³ Rogers R III. Complex aphthosis. International Association of Oral Pathologists 11th Biennial Meeting 2002 (Free Paper Session).

⁴ Stanley HR: Aphthous lesions. *Oral Surg Oral Med Oral Pathol* 1972; **33**: 407-16.

⁵ Wray D, Graykowski EA, Notkins AL. Role of mucosal injury in initiating recurrent aphthous stomatitis. *Br Med J* 1981; **283**: 1569-70.

⁶ Sallay K, Bánóczy J. Remarks on the possibilities of the simultaneous occurrence of hyperkeratosis of the mucous membrane and recurrent aphthae. *Oral Surg, Oral Med, Oral Pathol* 1968; **25**: 171-5.

⁷ Axéll T, Henricsson V. Association between recurrent aphthous ulcers and tobacco habits. *J Dent Res* 1985; **93**: 239-42.

⁸ Ship II, Morris AL, Durocher RT et al. Recurrent ulcerations and recurrent herpes labialis in a professional school student population. *Oral Surg Oral Med Oral Pathol* 1961; **14**: 30-9.

⁹ Ship II. Epidemiological aspects of recurrent aphthous ulcerations. *Oral Surg Oral Med Oral Pathol* 1972; **33**: 400-6.

¹⁰ Rees TD, Binnie WH. Recurrent aphthous stomatitis. *Dermatologic Clinics* 1996; **14**: 243-56.

¹¹ Hutcheon AW, Wray D, Dagg JH, Ferguson MM, Mason DK, Lucie NP. Clinical and haematological screening in recurrent aphthae. *Postgrad Med J* 1978; **54**: 779-83.

¹² Challacombe SJ, Barkhan P, Lehner T. Haematological features and differentiation of recurrent oral ulceration. *Br J Oral Surg* 1977; **15**: 37-48.

¹³ Rogers RS, Hutton KP. Screening for haematinic deficiencies in patients with recurrent aphthous stomatitis. *Aust J Derm* 1986; **27**: 98-103.

¹⁴ Merchant HW, Gangarosa LP, Glassman AB, Sobel RE. Zinc Sulphate supplementation for treatment of recurring oral ulcers. *South Med J* 1977; **70**: 559-61.

-
- ¹⁵ Rehberger A, Püspök A, Stallmeister T, Jurecka W, Wolf K. Crohn's disease masquerading as aphthous ulcers. *Eur J Dermatol.* 1988; **8**: 274-6.
- ¹⁶ Ferguson MM, Carter J, Boyle P. An epidemiological study of factors associated with recurrent aphthae in women. *J Oral Med* 1984; **39**: 212-7.
- ¹⁷ Dolby AE. Recurrent Mikulicz's oral aphthae - their relationship to the menstrual cycle. *Br Dent J* 1968; **124**: 359-60.
- ¹⁸ Ship II. Inheritance of aphthous ulcers of the mouth. *J Dent Res* 1965; **44**: 837-44.
- ¹⁹ Miller MF, Garfunkel AA, Ram C, Ship II. Inheritance patterns on recurrent aphthous ulcers: twin and pedigree data. *Oral Surg Oral Med Oral Pathol* 1977; **43**: 886-91.
- ²⁰ Hutton KP, Rogers RS. Recurrent aphthous stomatitis. *Dermatologic Clinics* 1987; **5**: 761-68.
- ²¹ Birek C, Grandhi R, McNeill K, Singer D, Ficarra G, Bowden G. Detection of *Helicobacter pylori* in oral aphthous ulcers. *J Oral Path Med* 1999; **28**: 197-203.
- ²² Sallay K, Dan P, Geck P, Kulscar G, Nasz I. Immunofluorescent studies on circulating lymphocytes in oral mucosal diseases. *Arch Dermatol Forsch* 1971; **241**: 15-21.
- ²³ Eglin RP, Lehner T, Subak-Sharpe JH. Detection of RNA complementary to herpes simplex virus in mononuclear cells from patients with Behçet's syndrome and recurrent oral ulcers. *Lancet* 1982; **2**: 1356-61.
- ²⁴ Donatsky O, Justesen T, Lind K, Faber Vestergaard B. Microorganisms in recurrent aphthous ulcerations. *Scand J Dent Res* 1977; **85**: 426-33.
- ²⁵ Pedersen A, Hornsleth A. Recurrent aphthous ulceration: a possible clinical manifestation of reactivation of varicella zoster or cytomegalovirus infection. *J Oral Pathol Med* 1993; **22**: 64-8.
- ²⁶ Ghodrathnama F, Riggio MP, Wray D. Search for human herpesvirus 6, human cytomegalovirus and varicella zoster virus DNA in recurrent aphthous stomatitis tissue. *J Oral Pathol Med* 1997; **26**: 192-7.
- ²⁷ Pederson A, Madsen HO, Vestergaard BF, Ryder LP. Varicella-zoster virus DNA in recurrent aphthous ulcers. *Scand J Dent Res* 1993; **101**: 311-3.

-
- ²⁸ Leimola-Virtanen R, Happonen RP, Syrjänen S. Cytomegalovirus (CMVO and Helicobacter pylori (HP) found in oral mucosal ulcers. *J Oral Pathol Med* 1995; **24**: 14-7.
- ²⁹ Di Alberti L, Porter SR, Speight P, Scully C, Zakrewska JM, Williams I *et al.* Detection of human herpesvirus-8 DNA in oral ulcer tissues of HIV-infected individuals. *Oral Diseases* 1997; **3**: S133-4.
- ³⁰ Ratis G, Poccardi G, Rota Scalabrini D, Pomatto E, Vercellino V. The lymphocytogram in recurrent aphthous stomatitis, The etiopathogenic aspects. *Minerva Stomatol* 1991; **40**: 45.
- ³¹ Sun A, Chu CT, Wu YC, Yuan JH. Mechanisms of depressed natural killer cell activity in recurrent aphthous ulcers. *Clin Immunol Immunopathol* 1990; **60**: 83
- ³² Graykowski EA, Kingman A. Double-blind trial of tetracycline in recurrent aphthous ulceration. *J Oral Pathol* 1978; **7**: 376-82.
- ³³ Burgess JA, Johnson BD, Sommers E. Pharmacological management of recurrent oral mucosal ulceration. *Drugs* 1990; **39**: 56-65.
- ³⁴ Miller MF. Use of levamisole in recurrent aphthous stomatitis. *Drugs* 1980; **19**: 131-6.
- ³⁵ Schulkind ML, Heim LAR, South MA, Jeter WS, Small PA. A case report of the successful treatment of recurrent aphthous stomatitis with some preparations of orally administered transfer factor. *Cell Immunol* 1984; **84**: 415-21.
- ³⁶ Katz J, Langevitz P, Shema J, Barak S, Livneh A. Prevention of recurrent aphthous stomatitis with colchicine: an open trial. *J Am Acad Dermatol* 1994; **31**: 459-61.
- ³⁷ BrownRS, Bottomley WK. Combination immunosuppressant and topical steroid therapy for treatment of recurrent major aphthae. A case report. *Oral Surg Oral Med Oral Pathol* 1990; **69**: 420-4.
- ³⁸ Revuz J, Guillaume JC, Janier M, Hans P, Marchand C, Souteyrand P *et al.* Crossover study of thalidomide vs placebo in severe recurrent aphthous stomatitis. *Arch Dermatol* 1990; **126**: 923-7.
- ³⁹ Pizarro A, Navarro A, Fonseca E. Treatment of recurrent aphthous stomatitis with pentoxifylline. *Br J Dermatol* 1995; **133**: 659-60.
- ⁴⁰ Wahba-Yahav AV. Pentoxifylline in intractable recurrent aphthous stomatitis: An open trial. *J Am Acad Dermatol* 1995; **33**: 680-2.

-
- ⁴¹ Utea E, Osaki T, Yoneda K, Yamamoto T, Kato I. A clinical trial of Azelastine in recurrent aphthous ulceration, with an analysis of its actions on leucocytes. *J Oral Pathol Med* 1994; **23**: 123-9.
- ⁴² Eisen D, Ellis CN. Topical cyclosporine for oral mucosal disorders. *J Am Acad Dermatol* 1990; **23**: 1259-361.
- ⁴³ Greer RO Jr, Lindenmuth JE, Juarez T, Khandwala A. A double blind study of topically applied 5% amlexanox in the treatment of aphthous ulcers. *J Oral Maxillofac Surg* 1993; **51**: 243-8.
- ⁴⁴ Taylor LJ, Walker DM, Bagg J. A clinical trial of prostaglandin E2 in recurrent aphthous ulceration. *Br Dent J* 1993; **175**: 125-9.
- ⁴⁵ Poswillo D, Partridge M. Management of recurrent aphthous ulcers: a trial of carbenoxolone sodium mouthwash. *Br Dent J* 1984; **157**: 55-7.
- ⁴⁶ Dolby AE, Walker DM. A trial of cromoglycic acid in recurrent aphthous ulceration. *Br J Oral Surg* 1975; **12**: 292-5.
- ⁴⁷ Matthews RW, Scully GM, Levers BGH, Hislop WS. Clinical evaluation of benzydamine, chlorhexidine and placebo mouthwashes in the management of recurrent aphthous stomatitis. *Oral Surg, Oral Med, Oral Pathol* 1987; **63**: 189-91.
- ⁴⁸ Colvard M, Kuo P. Managing aphthous ulcers: Laser treatment applied. *J Am Dent Assoc* 1991; **122**: 51.

**PRIMARY SMALL CELL UNDIFFERENTIATED
(NEUROENDOCRINE CARCINOMA) OF MAXILLARY SINUS
MIMICKING AN ODONTOGENIC INFECTION**

Prepared for Submission

Oral Surgery Oral Medicine Oral Pathology Oral Radiology & Endodontics

**PRIMARY SMALL CELL UNDIFFERENTIATED
(NEUROENDOCRINE) CARCINOMA OF THE MAXILLARY SINUS
MIMICKING AN ODONTOGENIC INFECTION**

**Anastasia F Georgiou, BDS, FRACDS¹, Ann P Collins BDS, LDSRCS, MDS, FRACDS (OMS)²,
Gary J Morgan MB BS, FRACDS, FRACS³, Jennifer A Shannon MB BS (Hons) PhD FRACP³,
Michael J Veness MB BS, MMed FRANZCR³,
D Murray Walker MD, FDSRCS, FRCPath, FFOP, FRCPA¹.**

Acknowledgements:

Dr Ajivarudh Subarnbhesaj and Janice Mathews (Oral Pathology) for their assistance with the photomicrographs.

Mr Rudi Gottl (photographer) Audiovisual Department, Westmead Hospital.

Correspondence (and reprint requests):

Anastasia Georgiou; Department of Oral Pathology, University of Sydney, Westmead Hospital, Westmead, New South Wales 2145, Australia.

Email: AnaG@dental.wsahs.nsw.gov.au

Key words:

Neuroendocrine, carcinoma, paranasal, maxillary, sinus

¹ Oral Pathology & Oral Medicine University of Sydney / Anatomical Pathology, Institute of Clinical & Pathological Medical Research, Westmead NSW.

² Oral & Maxillofacial Surgery, Westmead Centre for Oral Health, Westmead NSW

³ Head & Neck Cancer Service, Westmead Hospital NSW.

Abstract

Primary small cell undifferentiated (neuroendocrine) carcinomas of the paranasal sinuses are extremely uncommon neoplasms. This tumour was first reported in this site in 1965, and since then there have been only 43 documented cases in the literature. The median age at presentation is 53 years without gender predilection. There is no reported association with tobacco use or occupation and outcome is usually poor. We report a case in a 25-year old man, initially treated as an odontogenic infection, which delayed the institution of the appropriate management. Further investigations identified a locally advanced neuroendocrine carcinoma of the left maxilla. Despite radiotherapy and chemotherapy he rapidly developed disseminated disease and died.

Introduction

Primary neuroendocrine carcinomas of the paranasal sinuses are rare compared with those in the lung. Excluding Merkel cell carcinomas of the skin, small cell undifferentiated (neuroendocrine) carcinomas in the head and neck region primarily arise in the major salivary glands (particularly the parotid), the larynx and rarely the sinonasal region and hard palate. The histological features of small cell undifferentiated (neuroendocrine) carcinomas arising in the head and neck region are, by definition indistinguishable from those of bronchogenic origin. The number of documented cases of true small cell undifferentiated (neuroendocrine) carcinoma presenting in this location is too small for a complete assessment of the clinical features but the tumours appear to be aggressive with destructive local recurrences. In comparison to small cell carcinomas of the lung and larynx distant spread appears to be less frequent. This case appears to be the first case to document how a primary neuroendocrine carcinoma may present as, and be treated as, an odontogenic infection.

Case Report

A 25-year old healthy Caucasian man, a non-smoker with no significant past medical history, presented with a two-week history of left facial swelling. He consulted his dentist who commenced root canal treatment of three teeth suspected as the cause of the swelling (maxillary left premolars and first molar). There was no improvement of his symptoms.

He presented to Westmead Hospital, Sydney, Australia, reporting an increase in the facial swelling with left peri-orbital involvement (*Figure 1*). He reported epistaxis from the left nostril, lacrimation of his left eye and paraesthesia in the distribution of the ophthalmic and maxillary division of the left trigeminal cranial nerve. Clinically, the patient had a 3cm lymph node in his left upper neck.

Radiographs demonstrated obliteration of the normal air shadow of the left maxillary antrum (*Figure 2*). Computed tomography (*Figure 3*) and magnetic resonance imaging demonstrated a soft tissue mass with invasion through the anterior, medial and inferior walls of the maxillary sinus, encroaching upon the left nasal cavity associated with destruction of the turbinates. Inferiorly, the tumour extended to the left maxillary alveolus and palate. There was direct tumour extension into the left ethmoid air cells. Superiorly, the tumour extended to the inferior wall of the orbit. There was no evidence of intracranial extension.

A biopsy of the left maxillary antrum lesion was carried out via a Caldwell-Luc approach. Light microscopic examination demonstrated a highly malignant epithelial tumour with extensive necrosis, haemorrhage and secondary infection (*Figure 4*). No antral mucosa was present and the origin of the tumour could not be demonstrated histologically. The tumour cells were intermediate in size, with finely stippled chromatin, prominent nucleoli and little cytoplasm. Widespread apoptosis was apparent and mitotic figures were present (4 per high power field), some of which were abnormal.

The tumour cells were loosely coherent, without intercellular bridges (*Figure 5*). There was no evidence of perineural invasion or intravascular spread.

Immunohistochemistry (*Table 1*) confirmed that the cells were epithelial in nature, with positive staining for pankeratin and AE1/AE3. The tumour cells also reacted for neuroendocrine markers such as synaptophysin, chromogranin and neuron specific enolase. The diagnosis was of a primary neuroendocrine carcinoma of the left maxillary sinus.

Surgery was not possible due to the advanced nature of the tumour. Platinum based chemotherapy was initiated but the tumour progressed on chemotherapy alone. A combination of weekly cisplatin and comprehensive radiotherapy was commenced with a total dose of 60Gy was prescribed. Radiotherapy was accelerated for the first two weeks and by the third week of radiotherapy tumour regression was noted. By completion of chemoradiotherapy, most of the clinical tumour had regressed.

Three and a half months after diagnosis the patient developed spinal metastases and presented with back pain and signs of spinal cord compression. Paraplegia soon developed despite palliative radiotherapy. The patient died, four months after diagnosis, from widespread dissemination and bone marrow failure.

Discussion

Primary small cell neuroendocrine carcinomas of the nasal cavity and paranasal sinuses are rare. Squamous cell carcinoma is the most common malignancy in this site, followed by adenocarcinoma. The first case of small cell carcinoma of the nasal cavity or paranasal sinuses was documented by Raychowdhuri in 1965¹, who found this tumour in the fronto-ethmoid sinuses on autopsy, in a comatosed young female who died of frontal lobe abscess and meningitis. Forty-three subsequent patients with this aggressive neoplasm of the nasal cavity or paranasal sinuses have been reported both in small series^{1,2,3,4,5,6} and isolated case reports^{7,8,9,10,11,12} (Table 2). To our knowledge, this is the fifteenth small cell carcinoma documented involving the maxillary sinus^{2,4,5,6,7,9,10,12}. In a retrospective study of extrapulmonary small cell carcinoma by the Mayo Clinic, 7 of the 81 tumours identified during a 20-year time period involved the sinuses¹³. In a recent prospective study, 9 of the 19 tumours were classified as neuroendocrine or mixed tumours involving the paranasal sinuses¹⁴. However, no information was given about individual cases to be able to include in our review. Our patient was young in comparison to previously documented cases, with most presenting in the sixth decade (range 26-77 years, median 53 years). There is no gender predilection. Nearly all patients presented with facial pain and epistaxis. Other symptoms described include swelling, nasal obstruction, proptosis, paraesthesia and anosmia. To our knowledge, this is the first case documented which was confused with an odontogenic infection. Small cell carcinoma of the sinonasal tract has not been reported to be associated with significant tobacco use, in contrast to the strong association with the tumours in laryngeal, tracheal and hypopharyngeal sites⁹. No correlation with occupation is described⁵.

The histogenesis of small cell carcinoma is debatable. The presence of neurosecretory granules and their affinity for silver stains is suggestive of neuroendocrine differentiation. Small cell undifferentiated (neuroendocrine) carcinoma of the head and neck has been described as an endocrine tumour of the Amine Precursor Uptake and Decarboxylation (APUD) system of Pearse¹⁵.

This term refers to cells of the diffuse neuro-endocrine system with characteristic cytochemical and functional properties, such as the argyrophilic Kulchitsky cells. Such cells have only been rarely described in the nasal cavity and paranasal sinuses however. Features common to APUD cells and APUD tumours are dense core cytoplasmic granules, biogenic amine production and production of neuron-specific enolase or other specific peptides. Small cell carcinomas may produce ectopic hormones, but clinical manifestations of hormone production are uncommon in head and neck small cell carcinomas⁹. The investigation of ectopic hormone production can be of value in diagnosis and treatment¹⁶. There is one reported case of small cell carcinoma of the head and neck associated with a paraneoplastic syndrome (syndrome of inappropriate antidiuretic hormone, SIADH)¹⁰.

It has also been asserted that tumours in the paranasal sinuses and nasal cavity may be of seromucinous origin² and head and neck small cell carcinomas (excluding Merkel cell carcinoma) are explained on the grounds that these tissues share a common foregut origin. Silva *et al*⁵ found that the tumour cells were associated with glands in all twenty cases of paranasal neuroendocrine carcinomas. It was suggested that the glandular epithelium belongs to the exocrine glands, which include Bowman's glands and the tumour cells derive from this epithelium. However, this association of tumour cells to glands is not consistently seen histologically nor reported in the literature.

Histologically, small cell undifferentiated (neuroendocrine) carcinomas arising in the head and neck are indistinguishable from those of bronchogenic origin. Typically there are sheets, cords and ribbons of small cells with little or no cytoplasm which appear undifferentiated by light microscopy. The nuclei have a uniformly stippled chromatin pattern. Mitotic figures are frequent.

Necrosis varies from scattered, individual cell death to irregular zones of infarct-like change. Nuclear moulding and encrustation of vessel walls by DNA from degenerating tumours (Azzopardi effect) may be present¹⁷. Intravascular and perineural invasion is common. A fibrovascular stroma is usually present with little or no inflammatory response.

Ultrastructurally, nuclei demonstrate condensation of chromatin along the nuclear margin. Cell junctions are present to a variable degree¹⁸. Membrane-bound cytoplasmic electron dense core granules, 80-250nm in diameter⁹ are present. These cannot be differentiated from normal endocrine-type small granules of polypeptide secretion or from endocrine-type granules in other neuroendocrine tumours. These granules are considered to contain polypeptides and / or biogenic amines².

Immunohistochemical findings are of assistance in differentiating between other small cell tumours. The pattern of staining appears to be similar to that observed in pulmonary small cell carcinoma⁶. Typically cytokeratins are positive, and may show the punctuate perinuclear positivity, a feature of small cell carcinomas arising in other locations. Neuroendocrine markers such as chromogranin, synaptophysin, neuron-specific enolase are also usually expressed.

Small cell undifferentiated (neuroendocrine) carcinoma of the nasal cavity and paranasal sinuses have a propensity for multiple local recurrences, with direct extension into the skull base and brain the most frequent route of spread. Dissemination however, is usually a late feature compared to small carcinomas arising in other sites in the head and neck. The most frequent sites for distant metastases are the lungs, liver and bone (the latter a terminal event in our patient). The extent of primary disease is the most important

prognostic indicator of survival. Small cell carcinoma metastatic to the head and neck region from other sites must also be excluded.

Treatment regimes described are combinations of modalities; surgery alone has few successes in the treatment of small cell carcinoma of the nasal cavity and paranasal sinuses. Galanis *et al.*, 1997¹⁹, reported a median time of relapse of 10 months, for five patients with paranasal tumours treated with surgery alone.

Tumour extent often limits the role of surgery. Small cell undifferentiated (neuroendocrine) carcinoma is radiosensitive and combinations of surgery and irradiation are likely to achieve the best outcome.

Neoadjuvant and concomitant chemotherapy may play a role, particularly since these are considered to be chemosensitive tumours. Commonly used adjuvant agents include cyclophosphamide, cisplatin, doxorubicin, vincristine and methotrexate⁹.

This is a highly aggressive neoplasm; extrapulmonary small cell carcinoma is usually a fatal disease with a 13% 5-year survival rate¹³. Galanis *et al*¹³ reported the median survival of 14 patients with extrapulmonary small cell carcinoma of the head and neck (including 7 paranasal cases) was 14.5 months. In our review (Table 2) the mean survival rate of 42 patients (1 patient unknown survival rate⁸) was 21 months. However recently, Fitzek *et al* 2002¹⁴ reported a 74% 5-year survival rate in a prospective study of 19 neuroendocrine tumours of the sinonasal tract treated with surgery (6 cases), neoadjuvant chemotherapy and high-dose precision radiotherapy.

In conclusion, small cell undifferentiated (neuroendocrine) carcinoma is an uncommon aggressive neoplasm where early diagnosis and local control may be important for improved prognosis. These patients frequently present with facial pain, swelling and epistaxis. Visual disturbances are also common. The present report is the first to document how the tumour can mimic and be treated initially as an odontogenic infection. Usually

a thorough clinically examination of the teeth for discolouration, gross caries, extensive restorations, mobility, tenderness to percussion, pulp vitality testing and radiographs to disclose any periapical bone rarefaction will exclude the possibility of a dental abscess from a non-vital tooth.

This aggressive disease responded poorly to neoadjuvant chemotherapy unlike the response which may be expected for small cell carcinomas of other sites, particularly lung. Though combined treatment achieved locoregional control, our patient quickly developed disseminated disease and died.

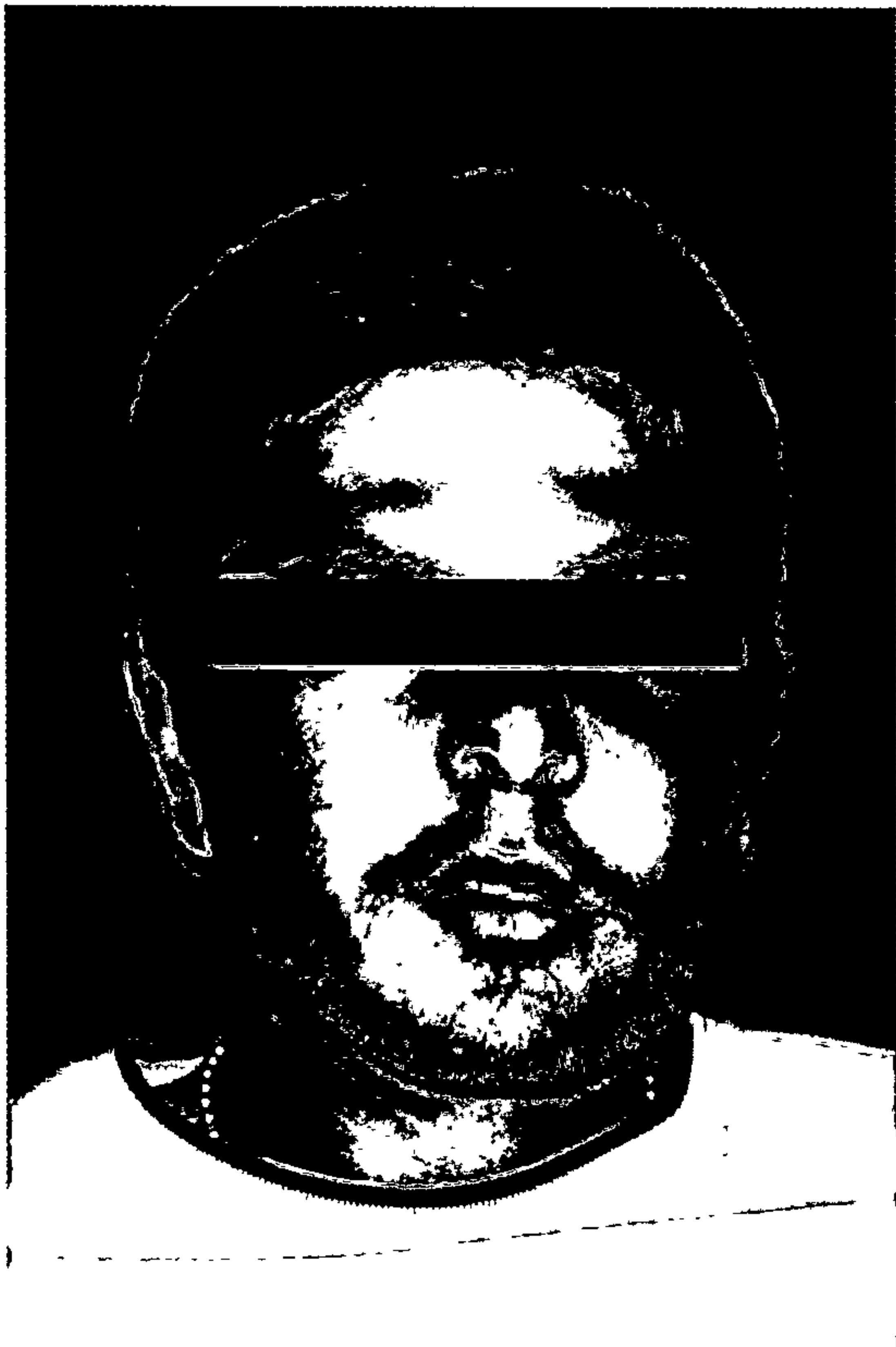


Figure 1. Left mid-facial swelling

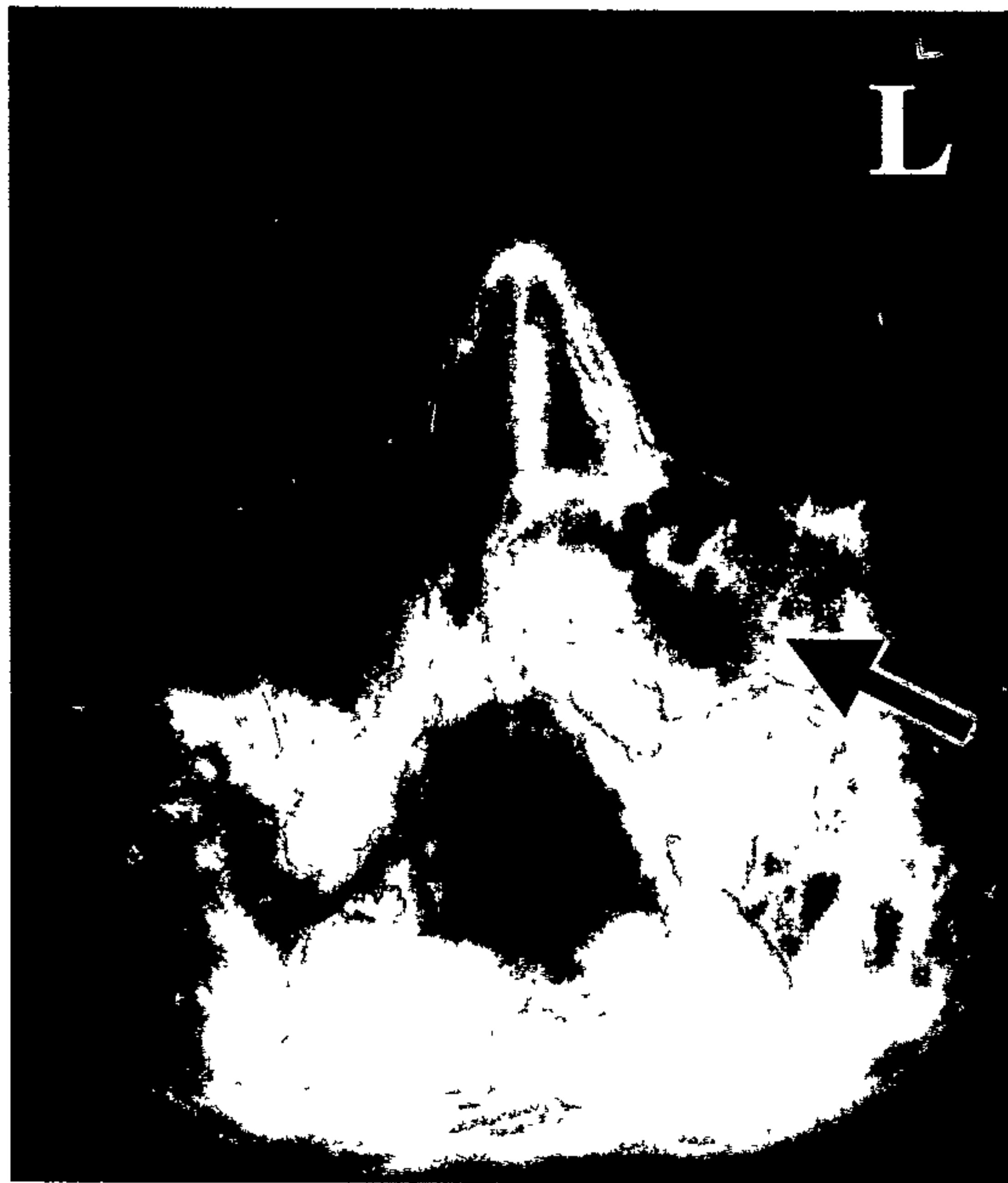


Figure 2. Occipitomental radiograph demonstrating obliteration of the air shadow of the left maxillary sinus and destruction of its lateral and nasal walls.

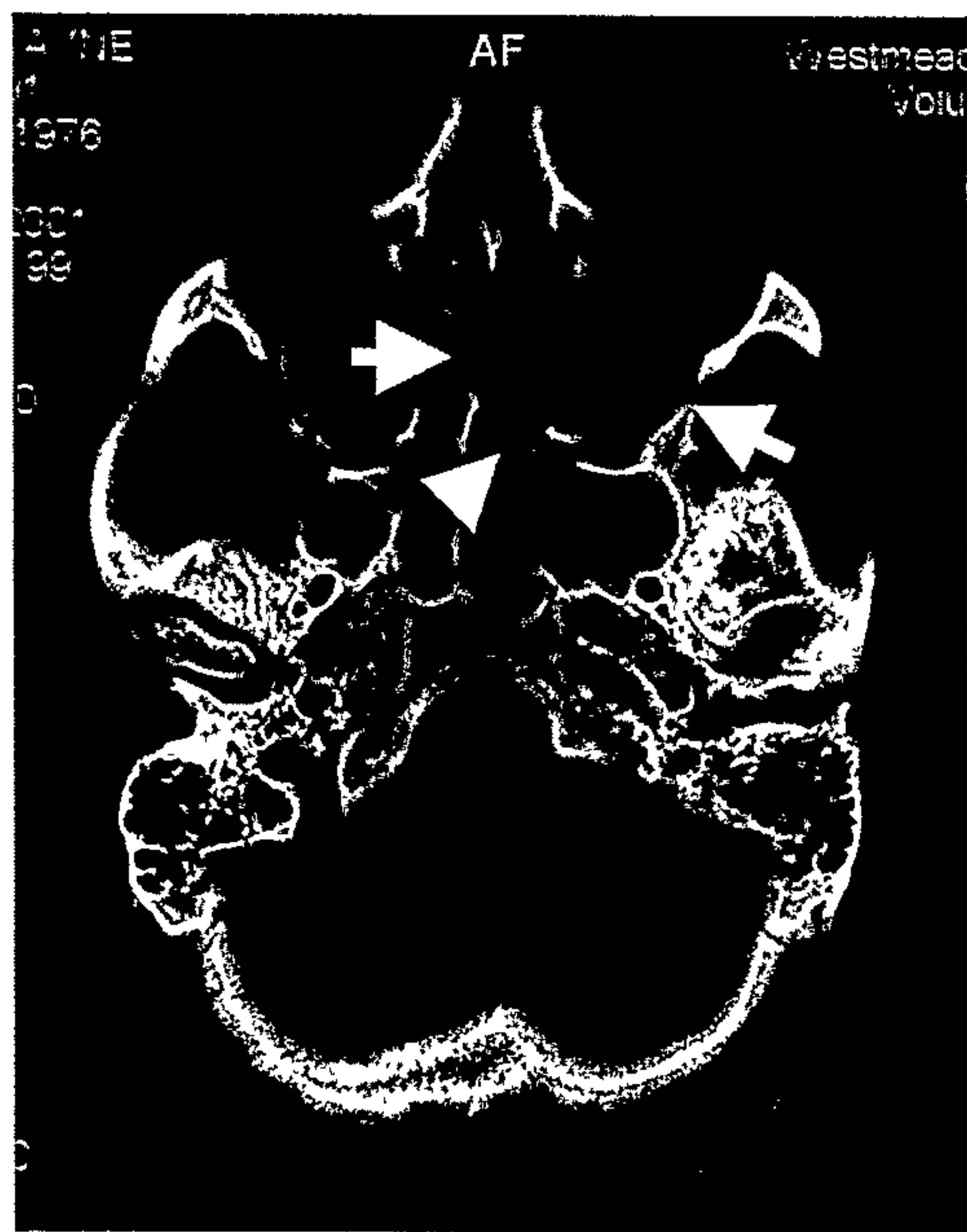


Figure 3. Computed tomography axial view demonstrating destructive lesion occupying left maxillary and ethmoid air sinuses and left nasal cavity with loss of medial, anterior and posterior bony walls of antrum.

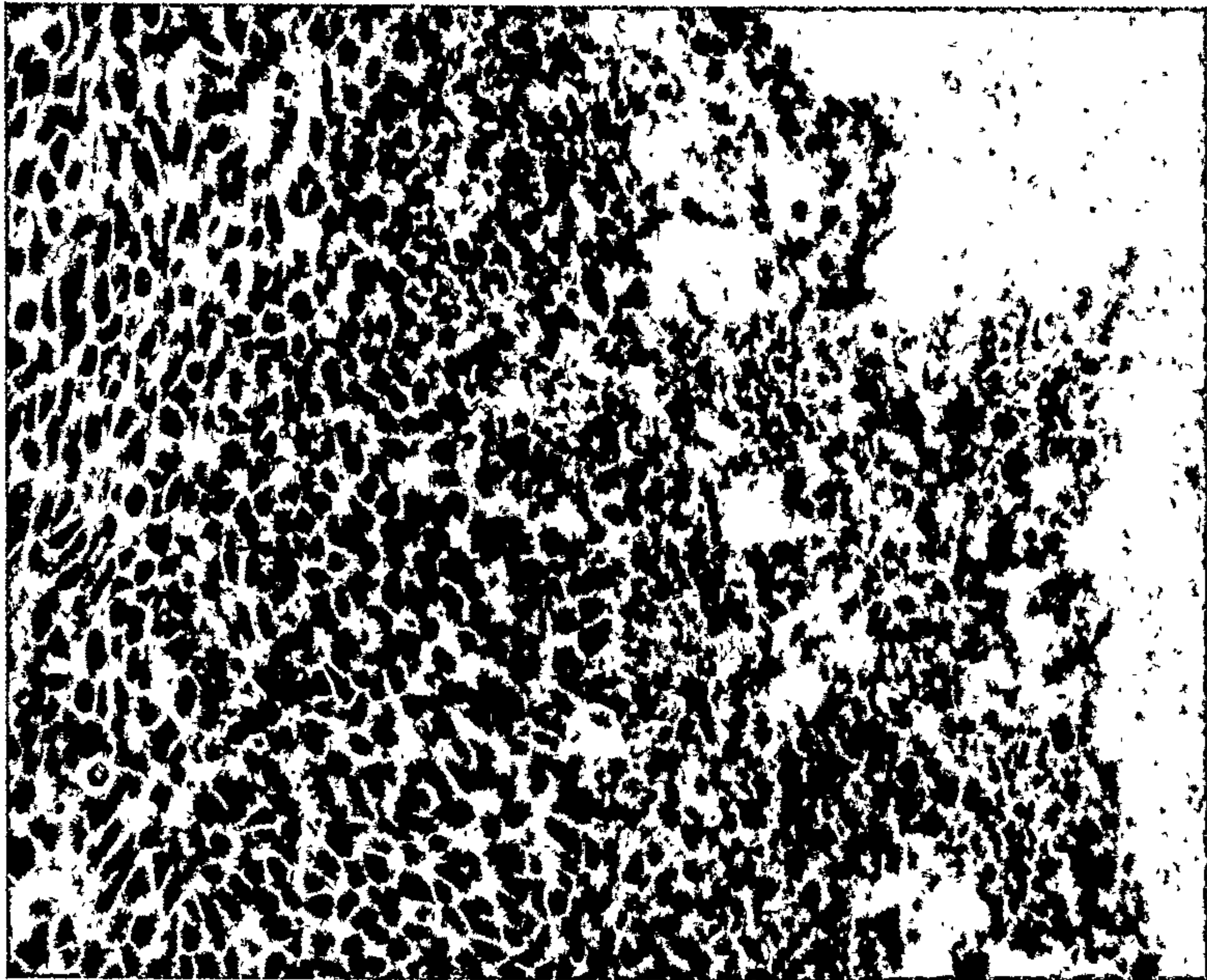
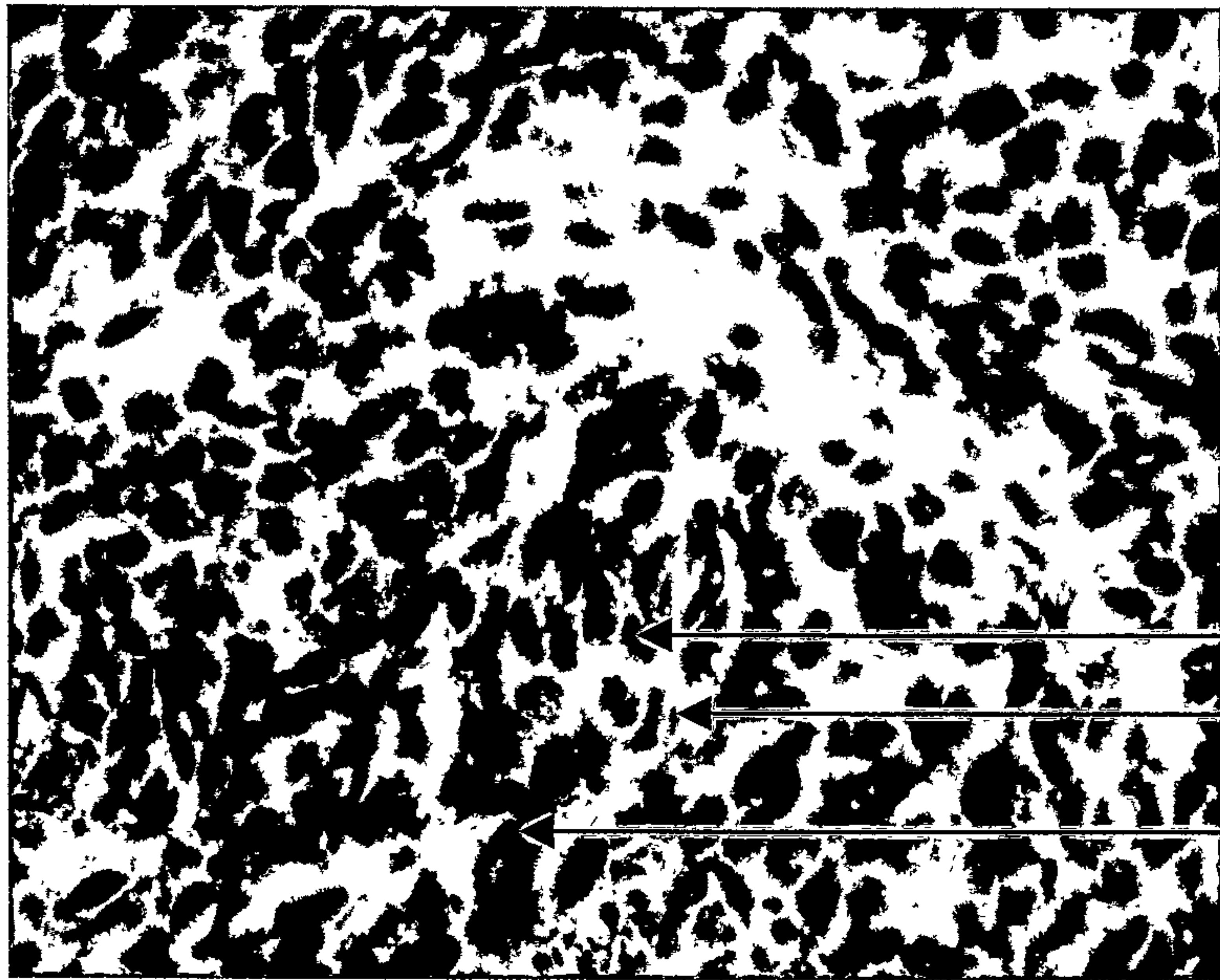


Figure 4. Light microscopic appearance of the tumour biopsy, showing sheets of tumour cells, necrosis and haemorrhage, H&E x40 magnification



- 1. Finely stippled chromatin
- 2. Frequent mitoses
- 3. Apoptosis

Figure 5. Small to intermediate sized tumour cells with high nuclear to cytoplasmic ratio, finely stippled chromatin (arrow 1), frequent mitoses (arrow 2) and apoptosis (arrow 3) H&E x80 magnification

Table 1. Summary of immunohistochemical investigations and other stains of the tumour

Positive	Negative
<i>Immunohistochemistry - Keratins</i>	<i>Immunohistochemistry</i>
PANKERATIN (<i>Biogenex</i>)	S-100 (<i>ID Lab Inc</i>)
AE1/AE3 (<i>ID Lab Inc</i>)	DESMIN (<i>Zymed Lab Inc</i>)
5D3 (<i>Novocastra Lab Ltd</i>)	SMOOTH MUSCLE ACTIN (<i>DAKO</i>)
	LEUCOCYTE COMMON ANTIGEN (<i>DAKO</i>)
<i>Immunohistochemistry -Neural</i>	MYOGLOBIN (<i>DAKO</i>)
NEURON SPECIFIC ENOLASE (<i>Neomarkers</i>)	CD99 (<i>Biogenex</i>)
CD56 (<i>Neomarkers</i>)	CK20 (<i>Neomarkers</i>)
SYNAPTOPHYSIN (<i>Zymed Lab Inc</i>)	
CHROMOGRANIN (<i>Biogenex</i>)	<i>Other stains</i>
	PERIODIC ACID SCHIFF
	PERIODIC ACID SCHIFF - DIASTASE

Table 2. Literature review of documented cases small cell undifferentiated carcinoma: paranasal sinuses & nasal cavity.
 Modified from *Baugh & McClatchey 1986*⁹

Author	Age	Sex	Location	Treatment	Recurrence	Outcome	Survival	
Raychowdhuri 1965 ¹	65	F	Upper nasal cavity	None	_____	DOD	1 mo	
Koss <i>et al</i> 1972 ²	26	M	Ethmoid sinus	S, RT	None	DOD	16 mo	
	39	F	<i>Maxillary</i> sinus	S	None	NED	8 yrs	
	42	F	Nasal cavity	S	x 1	DOD	11 mo	
	57	M	Nasal cavity	S	None	DOD	8 mo	
	50	M	<i>Max</i> & ethmoid (R)	S, RT	x 5 + mets	DUC	10 yrs	
Kameya <i>et al</i> 1980 ³	56	M	<i>Max</i> , ethmoid (R) & nasal cavity	S, RT	x 4 + mets	DOD	6 yrs	
	33	F	Ethmoid & frontal (R)	Sα	None	DOD	2 mo	
	70	M	<i>Maxillary</i> (R)	RT, CTx	x 1 + mets	DOD	6 mo	
	54	M	<i>Max</i> , ethmoid & sphenoid sinuses (L)	RT, CTx	None	NED	12 mo	
Rejowski <i>et al</i> 1982 ⁴	54	F	Between middle turbinate & septum (L)	S, RT	None	NED	12 mo	
Silva <i>et al</i> 1982 ⁵ † ‡				S, RT	x 1	NED	20 yrs	
				S, RT	None	DUC	15 yrs	
			<i>Precise site of</i>	S, RT	None	NED	13 yrs	
			<i>origin could not be</i>	S, RT	Mets	NED	10 yrs	
			<i>ascertained for</i>	S, RT	x 2	DUC	6 yrs	
			<i>14 of the tumours</i>	S, RT	x 1 + mets	DOD	6 yrs	
				S, RT	None	NED	2 yrs	
			<i>Remaining 6:</i>	S, RT	None	NED	2 yrs	
			<i>2 middle turbinate</i>	S, RT	None	NED	2 yrs	
			<i>2 middle turbinate &</i>	S, RT	x 1	NED	18 mo	
			<i>maxillary sinus</i>	S, RT	None	NED	12 mo	
			<i>2 superior turbinate</i>	S, RTα	None	TRD	2 mo	
			<i>& ethmoid sinus</i>	RT, CTx	x 1	NED	5 yrs	
				RT	x 5 + mets	DUC	19 yrs	
				S	x 2 + mets	DOD	13 yrs	
				S	x 3	NED	12 yrs	
				S	x 2	DOD	7 yrs	
				S	Mets	TRD	6 mo	
				S, CTx	None	NED	8 mo	
	Weiss <i>et al</i> 1983 ⁷	77	F	Ethmoid sinuses & <i>Max</i> sinus (R)	RT, Ctx	x 3 + mets	DOD	27 mo
	Baugh <i>et al</i> 1986 ⁹	64	M	<i>Maxillary</i> sinus & nasal cavity	RT, CTx	x 2 + mets	DOD	9 mo
Soussi <i>et al</i> 1990 ⁸	60	F	Ethmoid (L) & frontal sinuses	RT	None	DUC	MI	
Pierce <i>et al</i> 1994 ¹⁰	55	M	<i>Maxillary</i> sinus (L)	CTx	x 1 + mets	AWD	16 mo	
Lo Re <i>et al</i> 1994 ¹¹	49	M	Ethmoid sinus	CTx	None	NED	14.6 mo	
Perez-Ordenez <i>et al</i> 1998 ⁶	59	F	<i>Maxillary</i> sinus (L)	RT, CTx	Mets	DOD	21 mo	
	41	F	Lateral nasal wall (L)	S	Local	AWD	19 mo	
	42	M	<i>Maxillary</i> sinus (R)	S	Local	AWD	48 mo	
			ethmoid sinuses & nasal cavity					
	38	F	Ethmoid sinus (L)	S, RT, CTx	Local + mets	DOD	6 mo	
	68	M	Ethmoid sinus (L) & nose	S, RT	Mets	AWD	24 mo	
	52	M	Ethmoid sinus (L)	S, RT, CTx	Local	AWD	8 yrs	
Minetal <i>et al</i> 2001 ¹⁶	55	M	<i>Maxillary sinus</i>	RT, CTx	Mets	DOD	8 mo	
Noguchi <i>et al</i> 2002 ¹²	53	F	<i>Maxillary</i> sinus	S, RT, CTx	Mets	DOD	17 mo	
Georgiou <i>et al</i> 2002	25	M	<i>Maxillary</i> sinus	RT, CTx	Local + Mets	DOD	4 mo	

S, surgery; RT, radiotherapy; CTx, chemotherapy; AWD, alive with disease; DOD, dead of disease; DUC, died of unrelated causes; NED, no evidence of disease; TRD, tumour related death; MI, myocardial infarction during treatment ;*Surgical complication;† Mean 50 years; ‡ 10 male, 10 female; § died of myocardial infarction during treatment

-
- ¹ Raychowdhuri RN. Oat cell carcinoma and paranasal sinuses. *J Laryngol & Otology* 1965; **79**: 253-5.
- ² Koss LG, Spiro RH, Hajdu S. Small cell (oat cell) carcinoma of minor salivary gland origin. *Cancer* 1972; **30**: 737-41
- ³ Kameya T, Shimosato Y, Adachi I, Abe K, Ebihara S, Ono S. Neuroendocrine carcinoma of the paranasal sinus: A morphological and endocrinological study. *Cancer* 1980; **45**: 330-9.
- ⁴ Rejowski JE, Campanella RS, Block LJ. Small cell carcinoma of the nose and paranasal sinuses. *Otolaryngol Head Neck Surg* 1982; **90**: 516-7.
- ⁵ Silva EG, Butler JJ, Mackay B, Goepfert H. Neuroblastoma and neuroendocrine carcinomas of the nasal cavity. A proposed new classification. *Cancer* 1982; **50**: 2388-405.
- ⁶ Perez-Ordóñez B, Caruana SM, Huvos AG, Shah JP. Small cell neuroendocrine carcinoma of the nasal cavity and paranasal sinuses. *Hum Pathol* 1998; **29**: 826-32.
- ⁷ Weiss MD, defies HO, Taxy JB, Braine H. Primary small cell carcinoma of the paranasal sinuses. *Arch Otolaryngol* 1983; **109**: 341-3.
- ⁸ Soussi AC, Benghiat A, Holgate CS, Majumdar B. Neuro-endocrine tumours of the head and neck. *J Laryngol & Otology* 1990; **104**: 504-7.
- ⁹ Baugh RF, Wolf GT, McClatchey KD. Small cell carcinoma of the head and neck. *Head & Neck* 1986; **8**: 343-54.
- ¹⁰ Pierce ST, Cibull ML, Metcalfe MS, Sloan D. Bone marrow metastases from small cell cancer of the head and neck. *Head & Neck* 1994; **16**: 266-71.
- ¹¹ Lo Re G, Vanzonieri V, Veronesi A, Dal Bo V, Barzan L, Zancanaro C, Trovò M. Extrapulmonary small cell carcinoma: A single-institution experience and review of the literature. *Ann Oncol* 1994; **5**: 909-13.
- ¹² Noguchi K, Urade M, Sakurai K, Nishimura N, Hashitani, Kishimoto H. Small cell neuroendocrine carcinoma of the maxillary sinus – a case report and nude mouse transplantable model. *Head & Neck* 2002; **24**: 491-6.
- ¹³ Galanis E, Frytak S, Lloyd RV. Extrapulmonary small cell carcinoma. *Cancer* 1997; **79**: 1729-36.

¹⁴ Fitzek MM, Thornton AF, Varvares M, Ancukiewicz M, McIntyre J, Adams J, Rosenthal S, Joseph M, Amrein P. Neuroendocrine tumours of the sinonasal tract. *Cancer* 2002; **94**: 2623-34.

¹⁵ Pearse, AGE. The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series and the embryologic, physiologic and pathologic implications of this concept. *J Histochem Cytochem* 1968; **17**: 303-13.

¹⁶ Mineta H, Miura K, Takebayashi S, Araki K, Ueda Y, Harada H, Misawa K. Immunohistochemical analysis of small cell carcinoma of the head and neck: a report of four patients and a review of sixteen patients in the literature with ectopic hormone production. *Ann Otol Rhinol Laryngol* 2001; **110**: 76-82.

¹⁷ WHO. The World Health Organization histological typing of lung tumours. *Am J Clin Pathol* 1982; **77**: 123-36.

¹⁸ Mills SE, Gaffey MJ, Frierson Jr HF. Atlas of tumour pathology: Tumours of the upper aerodigestive tract & ear. Third edition. Washington DC: *Armed Forces Institute of Pathology*; 2000 179-180p.