STUDIES ON SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Declaration

Twenty-three publications are submitted in this section. Study (1) was initiated by Ligthelm. I was responsible for case selections while all the co-authors participated in the preparation of the manuscript. I initiated studies (2 and 3) and prepared the manuscripts for publication. Study (4), a review on the role of Langerhans cells and Human papillomavirus infection in aerodigestive squamous cell carcinoma was initiated by van Rensburg. I was involved in the literature retrieval and review and participated in preparation of the manuscript. I initiated studies (5 and 6), was responsible for case selection and provided all the histological material. Raubenheimer and I were responsible for interpretation of the histological slides and participated in preparation of the manuscript. I initiated study (7) with equal participation of Postma in the design of the study. The data collection and statistical interpretation were performed by Postma. We equally contributed towards preparation of the manuscript. I initiated studies (8 and 9) with valuable assistance from van Rensburg and Engelbrecht in the study design. I was responsible for case selection and provided all the material while Raubenheimer and I were responsible for evaluation of the histological slides. I initiated study (10) and did the study design although interpretation of the data was mainly done by co-authors van der Hoven and Swart. Study (11) was initiated by van Rensburg as an extension of

study (10), using the same cases. I initiated studies (12 and 13) and was involved in case selection, ploidy interpretation and did the grading in collaboration with Raubenheimer. Studies (14, 15 and 16) were initiated by Hemmer. I was involved in preparation of the manuscripts. I initiated and designed study (17), did the ploidy analyses and histological interpretations and was responsible for the manuscript preparation. I initiated studies (18 and 19) with equal participation of van Rensburg in the study design. Engelbrecht and van Rensburg were responsible for the molecular analyses while I did the case selection, DNA extraction and immunohistochemical analysis. I took responsibility of the manuscript preparation for study (19). Study (20) was initiated by co-author van Heerden. I was responsible for the study design, histological evaluation of the special stains and participated in the manuscript preparation. I initiated studies (21, 22 and 23) with valuable participation by Huebner in the study design. I was responsible for case selections, immunohistochemical analyses and manuscript preparations.

Abstract

The early detection of premalignant and early intraoral squamous cell carcinoma lesions are important to reduce the mortality and morbidity of oral squamous cell carcinoma (OSCC). This is especially relevant in South Africa where OSCC is one of the most common malignancies. The purpose of study (1) was to describe the clinical and histological diagnostic criteria necessary for the early diagnosis of premalignant lesions and

OSCC. The role of stomatoscopy, toluidine blue staining and exfoliative cytology were discussed. It was however concluded that histological examination was still the method of choice to assess the nature of OSCC and its precursors. The purpose of study (2) was to give an overview on the epidemiology, aetiology and clinical presentation of OSCC and premalignant lesions and conditions in South Africa. This study was aimed towards the general medical practitioner. Study (3) focussed on the role of the general dental practitioner in the prevention and early diagnosis of OSCC. It discussed the identification of high-risk patients, recognition and diagnostic criteria of premalignant lesions and conditions as well as the management principles relevant to the general dental practitioner.

Human papillomavirus (HPV) infection is implicated in squamous cell carcinogenesis. The possible role of HPV in head and neck squamous cell carcinogenesis was the topic of several studies. Oesophageal carcinoma is the most common malignancy in Black males in South Africa. Study (4) was a review about the role of HPV and Langerhans cells (LCs) in the development and behaviour of oesophageal and laryngeal carcinomas. Oesophageal carcinoma shows a remarkable geographical distribution with a high incidence in South African Black males. Conflicting results on HPV infection suggest a role for other environmental factors *e.g.* fungal infestation of corn, alcohol and tobacco and/or vitamin and trace element deficiencies in the carcinogenesis process. A common factor for all high-risk areas is a low socio-economic status of the affected population. HPV DNA, especially HPV 16, has been detected in laryngeal carcinomas,

especially verrucous carcinomas. Patients with a marked infiltration of LCs in both oesophageal and laryngeal carcinoma have a better survival rate than those with a low density of LCs.

The presence of HPV DNA in OSCC was determined in study (5). *In situ* hybridisation with radiolabelled probes for HPV-6, 11, 16 and 18 along with immunohistochemistry for HPV common viral antigen was used on paraffin blocks from 66 cases of OSCC. It was the largest series of OSCC to be investigated for HPV DNA to that date. Radiolabelled, instead of biotinylated probes were used because of their reported superior sensitivity. HPV-18 DNA was detected by *in situ* hybridisation in only one case. This low prevalence was most likely due to the method not being sensitive enough to detect low viral copy numbers. Alternatively, the possibility that the transformed tumour cells contained altered viral DNA not detectable by the probes used or that other HPV types might have been involved, could also explain the low prevalence.

Study (6) was a follow-up of the previous study in order to utilise the sensitive polymerase chain reaction (PCR) technique to detect HPV DNA in OSCC. One hundred and forty six cases of OSCC were included in this study. It was the largest study of its kind to that date. A segment of the E6 region of HPV 6, 11, 16 and 18 was amplified using type specific primers. Southern blotting was used to confirm the PCR results. The case selection included OSCC in young patients as well as OSCC containing normal epithelium. Only two cases showed HPV DNA (HPV 11 and 16

respectively). The sensitivity of the technique was such that one copy of control plasmid DNA could be detected. The suitability of the specimens for DNA amplification was evaluated with human β-globin primers in all cases. It was concluded that the HPV types investigated were probably not important in the development of OSCC in the population sample studied. It however subsequently came to light that DNA extraction from formalin-fixed paraffin-embedded tissue for HPV DNA detection was in all likelihood responsible for the low positivity rate in the sample studied.

Cancer of the cervix is the most common malignancy of Black, Coloured and Asian women in South Africa. Infection with oncogenic HPV types is the most important risk factor in its aetiology. An epidemiological study (7) was undertaken to determine if a correlation could be found between the incidence of cervical SCC and OSCC in females and OSCC in males in South Africa. The raw data for the ten-year period 1986 to 1995 were obtained from the National Cancer Institute. The study demonstrated several strong correlations between the incidences of cervical SSC and OSCC in the Black and Coloured populations, supporting the concept of systemic susceptibility and infection through a common agent, such as HPV, to be involved in the carcinogenesis process.

Epstein-Barr virus (EBV) is a double stranded virus and causes widespread infection. It is closely associated with Burkitt's lymphoma, nasopharyngeal carcinoma and a link to other epithelial tumours has been suggested. Study (8) was undertaken to determine the presence of EBV

DNA in OSCC using the PCR technique with primers for the *Bam* HI W-fragment of the EBV genome. The presence of EBV DNA in the OSCC groups was not dependant on the presence of histologically normal adjacent epithelium and its presence in the OSCC groups was lower compared to the control group. Over 90% of the adult population worldwide is infected with EBV implying the presence of viral DNA. EBV was considered to be merely a passenger when neoplastic change occurs in a latently infected epithelial cell.

Study (9) was a follow-up of the previous study to determine the presence of EBV DNA in OSCC in patients from two age categories and to evaluate the possible role of EBV as an aetiological agent in carcinogenesis in young patients. EBV DNA was demonstrated in 24% of the OSCCs in both the group younger than 40 years and in the older patient group suggesting that the prevalence of EBV in OSCC was not influenced by the age of the patient.

No data was available on the prevalence and incidence of nasopharyngeal carcinoma (NPC) or its association with EBV in South Africa. The purpose of study (10) was to determine the prevalence of EBV in the different types of NPCs. *In situ* hybridisation using probes to detect expression of the smaller nuclear RNAs (EBER-1 and EBER-2) was used. Use of the more sensitive PCR technique was not applicable due to the close association of the tumour cells with surrounding lymphoid tissue. All the non-keratinising NPCs, both the differentiated and undifferentiated groups

yielded EBV signals in the tumour cells indicating an important role in its pathogenesis while none of the squamous cell NPCs showed any positivity. No positive signals were detected in the dysplastic epithelium found adjacent to positive primary tumours, suggesting a late role for EBV in the carcinogenesis process of NPCs.

Study (11) was a follow-up on the previous study to determine the subtype distribution of EBV DNA in South African NPCs. This was done by amplifying and sequencing the EBNA-2A and EBER regions of the EBV genome. The results demonstrated that the consensus genotype was A/B. Type A virus strains dominated with the EBNA-2 analysis while the EBER region showed a distinct combination of mutations belonging to type B, indicating that the EBV strains had arisen by recombination between viral types A and B. The impact of the HIV epidemic on EBV strain evolution and recombination should be investigated. This was the first study to type EBV strains from Southern Africa.

The search for the ultimate grading system for OSCC is an ongoing process. The different histopathological grading systems are based on phenotypical features of tumour cells and associated cell populations. Any biological data of tumour cells should ideally be incorporated into a grading system. Study (12) was performed to determine the inter-observer reproducibility of the invasive cell grading method on OSCC and to correlate this with the DNA ploidy status of the tumours. This grading method was reproducible but no correlation was found between the

grading results and ploidy status. The energy source of the flow cytometer used for ploidy analysis was an Argon ion laser and therefore propidium iodide had to be used to stain the nuclei. This did not allow for high resolution DNA flow cytometry and could have been responsible to possible "false diploid" classification of tumours.

Study (13) was an extension of the previous study to investigate a possible correlation between the DNA ploidy status, LC population in the tumour and adjacent epithelium and invasive cell grading method. No such a correlation could be demonstrated.

The advantages of high-resolution flow cytometry were demonstrated in the following studies (14-17). The flow cytometer used was equipped with a high-pressure mercury lamp allowing the use of more sensitive DNA staining with DAPI (4'6 diamidino 2 phenylindole) or ethidium bromide. This improved sensitivity was reflected by the low coefficient of variation (cv) of the measurements compared to those obtained from standard flow cytometers. This allows for the detection of tumour cells with small DNA abnormalities. Study (14), done on 386 consecutive patients with OSCC showed that DNA flow cytometry could be used to screen primary biopsies of OSCC to determine the relative risk of metastases. Lymph node metastases at admission were present in 18% of patients with diploid primary tumours compared to 52% of those with aneuploid tumours. A delay between aneuploidy formation and expression of the metastatic phenotype was postulated to explain the lack of cervical lymph node

involvement in all the aneuploid carcinomas. The 5-year survival rate of 90% in the diploid N0 group suggested that tumour progression could be prevented by local surgery alone if treated before the emergence of aneuploid cell lines. In contrast, a 5-year survival rate of only 52% was found in the aneuploid N0 patients.

A follow-up study (15) on 348 patients with OSCC who underwent radical surgery established a definite correlation between DNA ploidy of the primary tumour and the risk of local recurrence development. Recurrences were found in 9% of patients with diploid tumours compared to 46% of those with aneuploid primary tumours over the same period. Clinical staging and histological grading of the primary tumour did not have an effect on this finding. This study provided support that cytogenetic events responsible for aneuploidy formation from diploid progenitor cells are linked to the development of tumour cell populations with the potential to metastasise.

Study (16) evaluated the ploidy status of 93 primary OSCC and their subsequent recurrences. Thirteen of the primary tumours were diploid of which five recurred with aneuploid cell lines. All the recurrences of the 80 aneuploid tumours had aneuploid DNA content with a different DNA content aberration observed in 21 cases. The 5-year overall survival rate of patients who underwent radical surgery of diploid recurrent tumour was 87% while three of the 5 patients who developed aneuploid recurrences from diploid primary tumours died of cancer within 22 months. Only 31% of

those with aneuploid recurrence were 5-year survivors. This study confirmed the importance of a complete resection during initial treatment and the treatment of local recurrences before the emergence of aneuploid cell lines.

In a retrospective study (17) using archival material, DNA flow cytometry was the only parameter that could be used to predict the presence of regional metastases. This was possible regardless of the shortcomings of using paraffin-embedded blocks instead of fresh tissue. DNA flow cytometry is not used by clinicians to its full potential due to the conflicting results that have been reported in numerous studies regarding the role of ploidy in OSCC. By far the majority of studies on DNA content in OSCC were performed using flow cytometers not dedicated for DNA analysis. This has resulted in relative high cv's with resultant false diploid classification in many cases. The flow cytometer in the Department of Oral Pathology an Oral Biology is the only dedicated DNA unit in South Africa.

The p53 mutation profile of OSCC from a Black population sample in South Africa was determined in study (18). Exons 5-9 of the p53 gene were amplified. Mutations were identified in 23.6% (13/55) of the tumours. The majority were single base pair substitutions and 2 were deletions. Two novel mutations were identified. The hot spot region at codons 238-248 for p53 mutations was not prominent in this study, but rather the region between codons 272 to 292. There also appeared to be a geographical

distribution in the exons affected. The importance of the p53 gene in oral carcinogenesis in a local population sample was established.

Study (19) is a follow up on the previous study. The p53 mutation profile was compared with p53 protein and PCNA expression. No association between p53 protein expression and p53 gene mutation could be established. Overexpressed p53 cannot be described as mutated protein and no conclusions can be made on the presence of p53 gene mutations based on the immunohistochemical evaluation of the p53 protein alone. A possible difference between PCNA and p53 expression was suggested but the difference was not statistically significant.

Special circumstances occasionally necessitate the utilisation of a rapid processing technique. These circumstances usually involve malignancies. Study (20) was undertaken to evaluate the rapid acetone processing technique used in the department pertaining to histotechnical quality and reliability of histochemical and immunohistochemical staining techniques. The staining intensity of the rapidly processed sections was similar or superior to that of routine processed sections showing that it can be used with confidence in histopathology laboratories. It is unfortunately a labour intensive method and therefore not suitable as a routine procedure.

The expression of the fragile histidine triad (Fhit) protein in OSCC and adjacent dysplastic and normal epithelium was evaluated in study (21). This was the first study to evaluate Fhit expression using

immunohistochemistry. Normal epithelium showed a strong expression of Fhit protein while a reduction or loss of expression was found in 66% of the OSCC. Loss of expression was also present in the atypical cells in epithelial dysplasia. This study suggested that *FHIT* gene inactivation plays a role in oral carcinogenesis.

Study (22) was done to correlate the pattern of Fhit protein expression in OSCC with detectable abnormalities of the *FHIT* gene. RT-PCR was used because the *FHIT* genomic locus is very large while the coding region is small. Immunohistochemistry for the Fhit protein was negative in all cases of abnormal RT-PCR results. The *FHIT* gene is inactivated by deletion rather than mutation. The complexity of these alterations in studies has suggested that protein detection through immunohistochemistry may be the best method to assess the involvement of Fhit in malignancies.

Study (23) was a follow-up to compare the different antisera against Fhit protein and to evaluate the staining pattern in different epithelial disease states. Two of the antisera used were commercially available. This study showed that all three antisera could be used for evaluation of Fhit expression in OSCC although different staining characteristics were observed in non-neoplastic epithelial states.

STUDIES ON DENTAL HARD TISSUES AND TUMOURS AND CYSTS OF THE JAWS

Declaration

Nineteen articles are submitted in this section. Study (1) was initiated by Weber. I was involved with the management of the patient and participated in preparation of the manuscript. I initiated studies (2 and 3), participated in the clinical and histological evaluation of the material and was responsible for preparation of the manuscripts. Study (4) was initiated by Pretorius. I took part in the study design, did the histological, morphometrical and statistical analyses and participated in preparation of the manuscript. Studies (5 and 6) were initiated by Raubenheimer. I took part in the manuscript preparation of the manuscripts. I initiated study (7), participated in the clinico-pathological evaluation of the cases and was responsible for preparation of the manuscript. Study (8) was initiated by Raubenheimer. I participated in the histological evaluation and in preparation of the manuscript. Study (9) was initiated by Weber. Our department supplied two cases. I participated in the histological interpretation of the cases and preparation of the manuscript. Studies (10 and 11) were initiated by Raubenheimer. I took part in reviewing the histopathology of all cases and in the preparation of both manuscripts. I initiated studies (12 and 13), took part in the histological evaluation of the cases and were responsible for preparation of the manuscripts. Studies (14-16) were initiated by Raubenheimer. I was involved in evaluating and

reviewing of the cystic lesions and participated in preparation of the manuscripts. I initiated studies (17 and 18), participated in the histological interpretation of the cases and was responsible for preparation of the manuscripts. I initiated study (19), participated in the study design and preparation of the manuscript.

Abstract

Study (1) reported an unusual case of injury to a permanent incisor tooth following trauma to the deciduous predecessor. The patient presented with a soft tissue tag extending from the gingiva, entering the pulp chamber in the middle third of the crown of a maxillary incisor. The possible chain of events leading to this unique presentation was discussed.

Study (2) presented the clinicopathological features of two patients with rough hypoplastic amelogenesis imperfecta presenting with multiple impacted teeth. The impactions were associated with pericoronal odontogenic fibromas of the WHO type. These fibromas were considered to be the main reason for the impactions in both cases and were regarded as hamartomatous growths from follicular origin.

Study (3) reported a case of regional odontodysplasia associated with a soft tissue tumour. Histological examination of the teeth showed hypoplastic dentine with a prominent predentine layer and numerous interglobular masses. The associated tumour had a hamartomatous

appearance consisting of odontogenic epithelial islands associated with amorphous calcifications in a cellular fibrous stroma.

Study (4) was performed to evaluate the sealing ability of a tricure glass ionomer material (Vitremer) as an apical sealant after apicectomy and to compare it with amalgam. Micro leakage was determined according to the extent of dye penetration measured by an image analyses system on ground sections of prepared teeth. The extent of dye penetration was significantly less in the glass ionomer group compared to the amalgam group. These results were obtained with "bulk" placement of the glass ionomer to make the study more clinically relevant. It was concluded that Vitremer could be recommended for routine clinical use as an apical sealant.

The amino acid composition of dentine in permanent human teeth was determined using ion-exchange chromatography in study (5) in order to compare the results with previously published data obtained from different analytical methods. Nineteen amino acids were detected, including small quantities of asparagine and 1-methylhistidine which have not previously been documented in human dentine. This finding and other quantifiable differences with previous reports might have been the result of different analytical methods, but might also reflect dietary and other regional factors that might influence dentinogenesis.

Study (6) is a follow-up of the previous study to compare the inorganic contents of opaque and translucent dentine. A significant difference in the fluoride, magnesium and zinc contents were found.

The clinicopathological features of eight cases of giant ossifying fibromas were reported in study (7). All cases had a prominent fibrous component. The resorption of mineralised tissue is indicative of altered cellular differentiation and proliferative activities in large ossifying fibromas. Focal areas of aneurysmal bone cyst formation were identified in the majority of cases.

Study (8) reported two cases of adenomatoid odontogenic tumour, both measuring in excess of 7 cm. The progressive growth and cortical perforation in these two cases substantiated the view that it is a benign neoplasm rather than a hamartoma.

Three cases of diffuse peripheral odontogenic fibromas were described in study (9). The diffuse involvement of the gingiva in all three cases supported the likelihood that the peripheral odontogenic fibromas have a hamartomatous origin rather than being a true neoplastic lesion. The one case, which was associated with ocular and skin lesions, could be part of a yet undescribed syndrome.

A rare case of a peripheral dentinogenic ghost cell tumour was reported in study (10). The lesion was present on the mandibular right alveolar ridge

and presented as a broad based polypoid lesion. The lesion was excised and no recurrence was detected after a six-year follow-up period.

The clinicopathologic diversity of ameloblastomas was described in a study (11) of 108 such tumours. An association with ameloblastic fibroma, adenomatoid odontogenic tumour and aneurysmal bone cyst formation in some cases was illustrated. Ameloblastomas with melanocytes and mucous metaplasia were also found. These changes emphasized the differentiation potential of neoplastic odontogenic epithelium.

A peculiar presentation of an intrabony ameloblastoma was reported in study (12). Histological examination of a multicystic mandibular ameloblastoma revealed a papillomatous lesion resembling a verruca vulgaris in one cyst. HPV type 18 DNA was identified in this lesion using radiolabelled *in situ* hybridisation. The absence of HPV DNA in other epithelial areas of the ameloblastoma was suggestive of a secondary infection although the mode of infection could not be established.

The clinicopathological features of unicystic ameloblastomas were reported in study (13). Most lesions were located in the mandible and were frequently associated with impacted teeth, root resorption and tooth displacement. The value of thorough histological evaluation of the cyst wall to determine the appropriate sub-classification was emphasised.

An extensive review of cystic jaw lesions in a German population sample was reported in study (14). Although a large percentage of the patients were military personnel with a bias towards young males, unicystic ameloblastomas presented one and a half decades later than is generally reported. Radicular and residual cysts were as expected the most common cystic lesion.

Recent new developments in the classification of odontogenic cysts of the jaws were described in study (15). The significance of a correct diagnosis as well as the importance of communication between the clinician and histopathologist in the diagnostic process was emphasised.

In study (16), the clinical and radiographic features of a series of unilocular lesions resembling dentigerous cysts were correlated with the microscopic diagnosis. A significant number was found to be unicystic ameloblastomas or odontogenic keratocysts. The importance of histological evaluation of all pericoronal cystic lesions was underlined.

The clinicopathological features of two cases of glandular odontogenic cysts were reported in study (17). The electron microscopy characteristics were the first to be reported in these types of cysts. It demonstrated a process comparable to apoptosis in the superficial eosinophilic cuboidal cells of the epithelial lining.

Study (18) reported the largest pigmented neuroectodermal tumour of infancy yet. These tumours are usually 1-3 cm in size but the excised tumour in this case measured 18 cm in diameter. The tumour had a normal histological appearance and no malignant change could be detected. No recurrence was found.

The prevalence of Epstein-Barr virus in Burkitt's lymphoma in a South African population sample was investigated in study (19). This was the first study involving a South African sample of its kind. *In situ* hybridisation for EBERs found EBV DNA to be present in 50% of the BLs. No difference was observed in the positivity and proliferation index between the oral-maxillofacial and the non-facial group of BL supporting the view that EBV is no longer thought to be the sole cause of BL, but is still accepted as a co-factor in the pathogenesis of this neoplasm.

STUDIES ON SALIVA AND SALIVARY GLAND NEOPLASMS

Declaration

Ten publications are submitted in this section. Study (1) was initiated by Raubenheimer. I was involved in the fieldwork obtaining some of the material and participated in preparation of the manuscript. Study (2) was initiated by Raubenheimer while I performed the statistical analysis and participated in preparation of the manuscript. I initiated study (3), took part in reviewing the material and participated in preparation of the manuscript. I initiated studies (4 and 5), was responsible for the study design and participated in the interpretation of data and manuscript preparation. Raubenheimer initiated study (6) while I participated in preparation of the manuscript. Study (7) was initiated by Hemmer while I took part in preparation of the manuscript. I initiated study (8), was responsible for the study design, interpretation of the histological slides and manuscript preparation. I initiated study (9), participated in the case selections and reviews and was responsible for the manuscript preparation. Study (10) was initiated by Raubenheimer; I participated in the manuscript preparation.

Abstract

The function of saliva and the role of salivary glands in a variety of vertebrates were discussed in study (1). The adaptation of salivary glands to suit the range of environments of the different vertebrates was emphasised.

Multiple myeloma (MM) is typed according to the circulating monoclonal immunoglobulin and or light chain type produced by the neoplastic plasma cells. Immune suppression due to a decrease of circulating normal immunoglobulins is a serious complication of MM. Study (2) demonstrated that the patients with MM had significantly increased concentrations of the specific immunoglobulin related to the type of MM in their saliva compared to the control group. It further showed that the salivary IgA concentration in non-IgA MM and salivary IgG concentration in non-IgG MM patients were within normal range despite a significant decrease in circulating normal immunoglobulins in these patients. This lack of suppression of normal salivary immunoglobulin concentrations in patients suffering MM was supported by the lack of clinical evidence of an opportunistic infection in the oral cavities in any of the MM patients.

The purpose of study (3) was to determine the relative frequency and distribution of intraoral salivary gland neoplasms in a black African population taking newly described entities into account. The majority of tumours (52%) were malignant. Polymorphous low-grade adenocarcinoma was found to be the most common intraoral malignancy in contrast to mucoepidermoid carcinoma reported in the majority of other studies. It was

suggested that these differences were probably related to criteria used for diagnosis of polymorphous low-grade adenocarcinoma. The malignant tumours were also found to occur at a significantly older age than benign tumours.

The diagnosis of salivary gland neoplasms can be problematic, especially when small biopsy specimens are submitted for histopathologic interpretation. The evaluation of the nucleolar organizer region associated proteins (AgNOR) staining technique as an additional microscopic criterion to benefit the diagnostic process was evaluated in study (4). Although the difference between the mean AgNOR count per nucleus between benign and malignant tumours and between polymorphous low-grade adenocarcinoma and adenoid cystic adenocarcinoma were highly significant, the presence overlapping AgNOR count between various tumours prohibited the use of this technique as an absolute criterion in establishing a final diagnosis. It could however be used as a diagnostic aid in differentiating between salivary gland neoplasms.

Study (5) was a follow-up on the previous study to correlate the AgNOR counts in salivary gland tumours with the proliferation index and DNA ploidy status as determined by a standard flow cytometer. Although a positive correlation between the AgNOR count and proliferation index was found, it was not statistically significant. Only three tumours (3/33) showed aneuploid DNA content. The low number of aneuploidy tumours was most

likely the result of paraffin-embedded blocks for obtaining a cell suspension to use on a non-dedicated DNA flow cytometer.

The role of DNA ploidy analyses, histochemistry and immunohistochemistry in the diagnosis of epithelial salivary gland neoplasms was reviewed in study (6). The utilisation of fine needle aspiration and frozen sections for the establishment of a diagnosis were discouraged. It was concluded that although special investigations may contribute, the diagnosis still relied mainly on the growth pattern and cytological features of a tumour.

Cytogenetic studies have proposed that three groups of epithelial cells with different karyotypic patterns exist in Warthin's tumour of salivary glands, suggesting the existence of etiologically different subsets of this tumour. Study (7) was undertaken to evaluate the role of high-resolution DNA flow cytometry to the cytogenetic analysis of Warthin's tumour. All 28 cases of Warthin's tumour consisted of flow cytometrically diploid cells. The mean cv was 1.31% indicating the sensitivity of the DNA analyses. This study did not support the hypothesis of existence of cytogenetically distinctive subgroups of Warthin's tumour.

The histogenesis of Warthin's tumour is controversial. The heterotopic theory suggests entrapped salivary gland epithelium in associated lymph nodes while the immune theory postulates a lymphocytic response to epithelial changes. Study (8) was a follow-up on the previous study to

evaluate a proposed role of EBV in the pathogenesis of Warthin's tumour. Using *in situ* hybridisation, no signals using EBER1/2 probes could be detected in the epithelium of 20 cases of Warthin's tumour or adjacent normal salivary gland tissue. Individual positive lymphocytes were present in 7 cases. Although this study did not prove any theory regarding the histogenesis of Warthin's tumour to be correct, it demonstrated that EBV was not involved in the pathogenesis of Warthin's tumour as had been suggested by other studies.

Study (9) reported 5 cases of intraoral salivary duct carcinoma (SDC), a high-grade malignancy usually encountered in the parotid gland. This was the largest series of these tumours reported to date. The histological features were similar to those SDC originating from the major salivary glands. The immunohistochemical profile confirmed a ductal origin while four tumours displayed DNA aneuploidy suggesting aggressive behaviour and poor prognosis. Due to the rarity of intraoral SDC, the diagnostic criteria and differential diagnoses were discussed in detail.

Several publications had regarded the presence of tyrosine-rich crystalloids as a unique microscopic feature of pleomorphic adenomas. Study (10) reported the presence of tyrosine-rich crystalloids in a well-documented case of polymorphous low-grade adenocarcinoma with obvious important diagnostic implications. It was speculated that the presence of tyrosine-rich crystalloids in polymorphous low-grade adenocarcinomas might imply a level of differentiation closer to that of

pleomorphic adenomas than more malignant tumours of salivary gland origin.