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Circulating Markers in Squamous Cell Carcinoma of the Head and Neck: A Review

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Biological markers of disease enhance the ability to diagnose, treat and evaluate results of therapy and are especially intriguing for their potential use in the management of malignant tumours. The serum levels of various biochemical substances have been shown to be abnormal for many cancers and are utilised in the management of affected patients. Several markers have been thoroughly investigated for potential clinical utility in head and neck carcinoma. Although no single marker has been found to be adequately sensitive and specific, combinations of markers may improve the utility for some aspects of patient management. This review highlights the literature to date in the realm of circulating markers for head and neck carcinoma. A discussion of the potential usefulness and limitations of such markers follows.
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INTRODUCTION

AN ESTIMATED 42 400 new cases of cancer of the upper aerodigestive tract will be reported in the U.S.A. in 1993. The majority of these cases are squamous cell carcinoma (SCC) of the larynx, with oral cavity carcinoma a close second. Despite improvements in therapeutic and reconstructive modalities, head and neck carcinoma represents an important cause of cancer morbidity and mortality. The 5-year survival rates for malignancy of the oral cavity and pharynx remains lower than 55% and the head and neck is the only anatomical region in which 5-year survival rates have not improved significantly in the past two decades. It is estimated that over 2000 people will die from oral cancer alone in 1993 [1].

Detection of these malignancies is often delayed until advanced stage disease is present, since early lesions often do not produce symptoms. This is true for recurrent or second primary tumours as well as primary tumours. Monitoring response to therapy for carcinoma in this region is limited to techniques such as radiographic investigation and close clinical observation. The development of a reliable circulating tumour marker could assist in the evaluation of patients, by potentially expediating the detection of occult malignancy and could be used to monitor therapy. In addition, such a marker might be used to develop new forms of treatment and might predict the biological behaviour of a tumour.

The ideal tumour marker would be a sensitive indicator of disease such that a high percentage of patients would have serum levels above the established normal. It would also be specific in that normal controls would not have levels above the accepted standard. Serum concentrations would correlate with tumour burden and clinical outcome and would return to normal with successful therapy and reappear prior to clinical recurrence. The ideal marker would also be stable enough to be assayed in low concentrations at a cost which is not prohibitive [2].

Unfortunately, no such "ideal" marker has been identified, however, there are several markers which have been found to be worthy of study in head and neck carcinoma. These include oncofetal proteins and other proteins, enzymes, hormones, metabolic byproducts such as erythrocyte polyamines, immune parameters, lipids, oncolipids and gangliosides, viral markers, tumour-associated antigens, prostaglandins and prostacyclins, and base elements (Table 1).

The following is a review of the most important markers that have been investigated in head and neck carcinoma.

CARCINOEMBRYONIC ANTIGEN

Carcinoembryonic antigen (CEA) was first described by Gold and Freeman [3] as a tumour-specific antigen for colorectal cancer. The subsequent reports indicating that CEA is a marker for gastrointestinal malignancies [4] were followed by the finding that elevated levels of CEA were present in a multitude of malignancies and levels of CEA returned to normal after successful therapy [4, 5]. In addition, elevation of circulating CEA has been associated with various non-malignant conditions including inflammatory diseases of the bowel, pancreas and liver and benign lung disease, as well as uraemia and chronic cigarette smoking [6]. Silverman *et al.* studied CEA levels of the 276 patients with head and neck

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Table 1. Tumour markers investigated in head and neck carcinoma

Markers	References
Oncofetal proteins	
CEA	[2, 6, 7, 17, 49, 50, 52–54]
Alpha fetoprotein	[2]
Proteins	
Ferritin	[2, 8, 11, 25, 52, 54]
β-protein	[2]
Tissue polypeptide antigen	[2, 52–54]
β-2-microglobulin	[2, 12, 25]
Ceruloplasmin	[56]
Transforming growth factor-α	[47]
Glycoproteins	
α-1-antitrypsin	[2, 14, 56]
α-1-acid glycoprotein	[2, 14]
α-2-HS glyocoprotein	[2, 14]
Pre-albumin	[2, 14]
Albumin	[2, 14]
Haptoglobin	[14, 56]
TAG-72	[60]
CA 19-9	[17, 52, 60, 61]
CA-50	[17, 54]
CA-125	[61]
CA 15-3	[61]
Enzymes	
Alkaline phosphatase	[2, 20–22]
Placental alkaline phosphatase	[2]
Cathepsin D	[63]
Lactate dehydrogenase	[2, 50]
Aliesterase	[2, 23]
Leukocyte elastase	[28]
Phosphohexose isomerase	[2, 24, 25]
Adenosine deaminase	[2]
Neuron-specific enolase	[2]
Alkaline deoxyribonuclease	[17, 27]
Thymidine kinase	[26]
Hormones	
Calcitonin	[2]
Prostaglandins and prostacyclins	[2]
Metabolic byproducts	
Erythrocyte polyamines	[2, 29]
Immune parameters	
Immunoglobulins	[2, 25, 30, 31]
Immune complexes	[31, 32]
Cytokines and cytokine receptors (TNF, IL-2 receptor)	[33]
Immunosuppressive substance	[34, 35]
Immunosuppressive acidic protein	[35]
Lipids	
Lipid sialic acid (also protein-bound and free forms)	[2, 25, 45–47, 49, 52, 55, 56]
Oncolipids	[2, 37]
Gangliosides	[57]
Viral markers	
Epstein–Barr virus	[2]
Herpes simplex virus	[2]
Tumour-associated antigens	
Squamous cell carcinoma antigen	[2, 38–43, 49, 55, 62]
Gastrointestinal carcinoma-associated antigen (same as CA 19-9)	[17, 60, 61]
Tumour-associated trypsin inhibitor	[59]
Other cancer-associated antigens (CA-50, CA-125, CA 15-3, TAG-72)	
Base element	
Zinc	[62]
Copper	[62]
Multiple markers	
CEA, LSA, SCC-Ag and CA-125	[49]
CEA, LSA, SCC-Ag and LDH	[50]
CEA, LSA, SCC-Ag, ferritin, TPA and CA 19-9	[52]
CEA, SCC-Ag and TPA	[53]
CEA, SCC-AG, ferritin, TPA and CA-50	[54]
LSA and SCC-Ag	[2, 55]
LSA, ferritin, β-2-microglobulin, IgE and PHI	[2, 25]
PSA and SCC-Ag	[51]
CEA and α-1-acid glycoprotein	[2]
LSA, α-1-antitrypsin, haptoglobin and ceruloplasmin	[56]

carcinoma of whom 154 were smokers and 122 were non-smokers. Both the incidence and magnitude of CEA elevations correlated with the clinical stage of disease; however, if patients with advanced disease were excluded similar levels of the antigen were found in tumour-bearing patients, tumour-free previously treated patients, and healthy controls. If the definition of abnormal CEA levels is 5 ng/ml, then 36% of tumour-bearing patients had abnormal levels whereas only 5% of the non-smoking control patient population had an abnormal level. If the definition of abnormal is a CEA greater than 7 ng/ml, 17% of the tumour-bearing patients and 5% of smoking control patients had abnormal levels. Schneider *et al.* [7] found that 47% of 85 patients with head and neck carcinoma had levels greater than 5 ng/ml but did not find a correlation with site or stage of disease and the level of the CEA. Currently, CEA is not felt to be a good marker for head and neck carcinoma.

PROTEINS AND GLYCOPROTEINS

Ferritin is a storage and major iron binding protein that is normally found in nanogram quantities in human serum. Elevated serum ferritin has been demonstrated in patients with lymphoma, leukaemia, multiple myeloma, breast cancer, lung cancer, colorectal cancer, liver cancer, malignant melanoma, neuroblastoma, tumours of the testes and ovaries and non-malignant conditions [8–10]. The potential for ferritin as a serum marker in head and neck cancer was investigated by Maxim and Veltri in 1986 [8]. These investigators found that there was a significant difference in serum levels between non-smoking control patients and smoking control patients when compared to patients with head and neck cancer. The prognostic significance of persistently elevated ferritin levels during treatment was postulated. More recent studies suggest that early laryngeal cancers are not readily diagnosed due to a lack of specificity [11]. Thus, ferritin is not currently a useful marker for head and neck cancer.

Other proteins such as β-2-microglobulin and β-protein have been found to be elevated in serum of patients with head and neck carcinoma, however, the usefulness of these substances is unknown [2, 12]. Glycoproteins have been studied in various types of malignancies including those of the liver [13, 14]. Szymendera [15] showed that a variety of malignant tumours produced elevated levels of glycoproteins CA 19-9, CA-50 and CA-125. Wolf *et al.* [16] studied six glycoproteins: haptoglobins, α-1-antitrypsin, α-1-acid glycoprotein, α-2-HS glycoprotein, pre-albumin and albumin. They demonstrated a correlation between levels of α-1-antitrypsin and α-1-acid glycoprotein and tumour burden. Haptoglobin was elevated significantly in a group of tumour-bearing patients but no correlation between levels and tumour burden were demonstrated. The levels of α-2-HS glycoprotein decreased with increased tumour burden. More recent studies of glycoproteins have suggested that they are not adequate alone in head and neck tumour evaluation [17].

Fazekas-May [18] studied 14 patients with SCC of the head and neck and found that urinary transforming growth factor alpha levels did correlate, however, with the course of disease in 43% of the patients studied. This marker appears to warrant further study in head and neck carcinoma. However, at this time, measurement of this transforming growth factor (TGF-α) in head and neck cancer is not clinically useful.

SERUM ENZYMES

The level of various enzymes in serum has been correlated with different types of cancer. The best example of this is alkaline phosphatase which has been found to be present in elevated levels in hepatic carcinoma [19]. A study has been performed correlating response to chemotherapy for carcinoma of the head and neck with the pretreatment levels of alkaline phosphatase, [20] however, others presented data which did not agree with this finding [21, 22]. Aliesterase is an enzyme involved in fat metabolism and in contrast to enzymes required for glycolysis, aliesterase levels have been shown to decrease in patients with malignancy. One study in head and neck tumours revealed that serum levels of aliesterase varied inversely with tumour stage and that levels rose during radiotherapy but did not reach control levels [23]. Phosphohexose isomerase (PHI) is an enzyme that transforms glucose-6-phosphate and elevated levels have been found in patients with advanced tumours including head and neck cancers, [24] however, the marker is not sensitive enough to be useful in carcinoma of the head and neck [25]. Thymidine kinase was found to be elevated in the sera of oral carcinoma patients, but with a sensitivity of only 47% [26]. Alkaline deoxyribonuclease levels appear to correlate with successful treatment of head and neck cancer [27]. Another enzyme that was studied by Harbans Lal was serum adenosine deaminase which correlated with tumour burden and response to tumour therapy, however, these changes were not sufficiently specific to warrant their use as a diagnostic tumour marker [19]. Most recently, leukocyte elastase has been found to be elevated in a variety of pharyngeal cancers [28]. The usefulness of various enzyme markers in head and neck cancer requires further study. There is no apparent clinical utility of these markers currently for head and neck cancer.

ERYTHROCYTE POLYAMINES

Erythrocyte polyamines were studied in patients with head and neck cancer in 29 previously untreated patients of whom 31% had increased levels which correlated with tumour burden. The levels also decreased after surgery or irradiation [29]. These data suggest that this marker is also not sensitive enough for diagnostic purposes.

IMMUNE PARAMETERS

Abnormalities of the immune system are well documented in patients with head and neck carcinoma. Immunoglobulins, particularly IgE, and circulating immune complexes have been investigated, however, these are also not sensitive or specific enough to be valuable tumour markers [25, 30–32]. Recently, investigations of interleukin-2 (IL-2) receptor, tumour necrosis factor (TNF), immunosuppressive substance (IS) and immunosuppressive acidic protein (IAP) have begun which will probably change our understanding of these potential markers [33–35]. Hsu *et al.* found that soluble IL-2 receptor levels were elevated in patients with nasopharyngeal carcinoma and correlated with clinical staging, whereas levels of TNF were elevated, but did not correlate with tumour stage. IL-2 receptor is a blocking factor causing decreased mitogenic response, but its use as a marker requires further study [33].

Yamanaka *et al.* used a single radial immunodiffusion in 108 patients with head and neck cancer and found serum immuno

suppressive substance (IS) positive in 46%. They also found that elevation correlated with extent of disease and predicted recurrence [34]. Kubota *et al.* found 58% of patients with oral cancer had positive IS levels and found IS to be a useful parameter for monitoring disease stage and therapy [35]. These newer makers still lack adequate sensitivity to be useful as diagnostic aids.

ONCOLIPIDS

In 1986, Fossel *et al.* [36] described a new non-specific cancer marker involving the use of water suppressed proton nuclear magnetic resonance spectroscopy of plasma. Lipoprotein particles found in the plasma of cancer patients have been termed "oncolipids" and preliminary tests have suggested that this assay may provide a universal cancer screening test. One study [37] of 46 patients who had biopsy-proven head and neck carcinoma and 32 controls revealed significant differences between patients with disease and controls. However, the conclusion of this study was there was no clinical utility due to the tremendous overlap between disease and non-disease groups.

SQUAMOUS CELL CARCINOMA (SCC) ANTIGENS

A squamous cell-derived antigen TA-4 was initially isolated and purified from squamous cell carcinoma from the uterine cervix by Kato and Turagoe [38] in 1977 using a radioimmunoassay (RIA). Kato *et al.* demonstrated that serum levels of this antigen correlated with extent of disease as well as success of therapy [39]. Maruo *et al.* [40] demonstrated that elevated serum TA-4 levels fell very rapidly after complete tumour excision or following definitive radiation therapy. Studies of this TA-4 indicate that it is not a single substance but a series of at least 14 proteins with a common antigen and a molecular weight of approximately 48 000 daltons [40]. Initial studies demonstrated elevated SCC antigen in 13 out of 25 patients with head and neck carcinoma [41]. Johnson *et al.* [42] demonstrated elevated pretreatment SCC antigen levels in 45% of 60 patients with head and neck SCC. Eibling *et al.* found elevated pretreatment levels in 44% of 89 patients with head and neck carcinoma [43]. Although the mean SCC antigen level increased with increasing T or N stage of the disease, the percentage of patients with elevated levels did not vary significantly with increasing tumour burden. There was a strong correlation between serum antigen levels and clinical course in patients who developed recurrence. Until recently, this marker seemed to have the highest sensitivity of the markers studied in head and neck cancer. Although the sensitivity is still less than 50%, in those patients who present with elevated levels, post-therapy serum levels may provide clinically useful data. Routine clinical use of SCC-RIA is not recommended.

LIPIDS AND FORMS OF SIALIC ACID

Serum lipid-bound sialic acid (LSA) have been found to be useful in assessing disease progression in identifying patients resistant to therapy with breast and colorectal carcinoma although it is neither sensitive nor specific enough for cancer screening [25, 44, 45]. Fisher *et al.* [46] found that sialic acid levels in head and neck cancer patients were elevated in proportion to the extent of tumour and that 71% of patients with recurrence of tumour had elevated levels. A previous study found the protein-bound form to be a better indicator, than LSA or free sialic acid of poor prognosis in head and neck malignancies [47]. The significance of these and other studies is discussed in the section on multiple markers. Sialic acid seems to be one of the more useful markers which should be investigated further, but still cannot be currently advocated as a diagnostic aid.

MULTIPLE MARKERS

The lack of a single tumour marker has led investigators to search for combinations of multiple markers. Gail *et al.* [48] found that the combination of CEA and total sialic acid were found to be useful in advanced lung cancer. Based on this model, Straka *et al.* [49] reviewed the use of a tumour marker panel and studied the utility of the combination of SCC antigen, CEA, LSA and CA-125. They confirmed that SCC antigen RIA was the most sensitive marker, however, various combinations of the markers did not significantly improve either specificity or sensitivity of the markers for diagnostic purposes. Dreyfus *et al.* [50] evaluated LSA, SCC antigen, CEA and lactate dehydrogenase (LDH) levels in squamous cell carcinoma of the head and neck in 52 patients. 42 of these patients had active measurable disease and 10 had no evidence of clinical disease. In patients with active disease, LSA, SCC antigen, CEA and LDH were elevated in 71, 33, 27 and 18%, respectively. None of the markers were elevated in the group with no evidence of disease. The incidence and magnitude of LSA and SCC antigen elevations correlated with the extent of disease and the authors concluded that LSA appeared to be the most promising and sensitive marker of SCC of the head and neck followed in decreasing order of sensitivity by SCC antigen, CEA and LDH. The difference between these two studies may be related to using a different assay method for LSA. Similarly, Ropka *et al.* found that the LSA test sensitivity was 63.4 vs. 27.6% for SCC-associated antigen with a specificity of 77.9% for LSA and 85% for SCC-associated antigen. When the combination of both tests were positive, sensitivity was 18.7% and specificity was 95%. If either was positive in parallel combinations, sensitivity was 72.4% and specificity was 68%. They concluded that further evaluation is required that applies different definitions of normal and determines longitudinal changes with disease status [51]. Vinzenz found elevated levels of β-2 microglobulin, IgE, PHI, LSA and ferritin in 50%, 55–62%, 62%, 60% and 50%, respectively, and concluded that LSA, β-2 microglobulin and ferritin merit further study for early detection [25]. Mevio *et al.* found LSA and ferritin to be more useful than CEA, TPA, SCC-Ag, CA19-9 in 50 patients with laryngeal cancer [52]. Palermo *et al.* found benefit from using the combination of SCC-Ag, CEA and TPA with an increased sensitivity of up to 71% [53].

Screm *et al.* studied CEA, ferritin, CA-50, TPA and SCC-Ag in 54 "ENT" cancers with a 72% combined sensitivity, and concluded that they were not useful since objective data is available even in early tumours [54]. Bhatavdekar also found PSA to be a more reliable marker than SCC antigen [55]. It would appear from all the data from these combined studies that sialic acid is the most sensitive marker for SCC, followed by SCC antigen and possibly ferritin. It would seem to follow that further investigations of these markers, and particularly the various forms of sialic acid is indicated. Combinations

including newer immune markers like IS should also be investigated further.

CONCLUSIONS

The identification of a circulating tumour marker has great potential for clinical application. Unfortunately, efforts to date to identify a single tumour marker or even a combination of markers for head and neck carcinoma are limited by an inadequate sensitivity and specificity. Therefore, serum markers are not widely used clinically and are primarily used in research. Despite this lack of a useful marker for diagnostic screening and recognition of occult malignancy, markers may be useful for monitoring therapy. The development of new multiple marker batteries may provide the closest approximation to a perfect marker that is feasible. Additional studies into the nature and function of oncogenes may provide clues to the variability of these tumours and the resultant heterogenieity of their marker expression.

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