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Prognostic Implications of Sialosyl-Tn Antigen Expression in Sinonasal Intestinal-type Adenocarcinoma

A. Franchi[1] and O. Gallo[2]

[1]Institute of Anatomic Pathology; and [2]Otolaryngology Clinic, University of Florence, Florence, Italy

The expression of the mucin antigen sialosyl-Tn (S-Tn) was evaluated immunohistochemically in a series of 30 intestinal type adenocarcinomas of the nasal cavities and paranasal sinuses to assess the relationship between the histological features of the lesions and their clinical behaviour. In grades 1 and 2 adenocarcinomas, the staining localised at the apical pole or within the cytoplasm of neoplastic cells, and in the content of glandular structures. Grade 3 adenocarcinomas had a very scanty expression of the antigen. Mucinous adenocarcinomas showed an intense immunoreaction within the cell cytoplasm and in the extracellular mucous pools. Conversely, non-neoplastic sinonasal mucosa had a very focal distribution of the antigen. Immunostaining was scored according to the percentage of low power microscopic fields showing positivity. Twenty-one adenocarcinomas (70%) were considered positive. No significant relationship was found between S-Tn positivity and the histological degree of differentiation of the lesion. The 5-year survival rate and disease-free interval of patients with S-Tn positive adenocarcinomas were significantly lower than those with negative adenocarcinomas (17.8% versus 72%, $P=0.0001$; 16.6% versus 40%, $P=0.0001$, respectively). These results indicate that S-Tn immunostaining appears to be a significant prognostic factor in patients with sinonasal intestinal type adenocarcinoma. Copyright © 1996 Elsevier Science Ltd

Keywords: nasal cavity, paranasal sinus, adenocarcinoma, sialosyl-Tn, mucin antigen

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INTRODUCTION

Intestinal-type adenocarcinomas (ITACs) of the nasal cavities and paranasal sinuses are an uncommon but well recognised group of tumours, which closely resemble colonic neoplasms and normal small intestine mucosa in histological, histochemical, immunohistochemical, and ultrastructural profile [1–3]. A peculiar feature of these adenocarcinomas is their association with work exposure to wood and leather dust which has been recognised in several studies from different countries [4–7]. They present with a variety of histological aspects, and several morphological classifications have been proposed, having some prognostic implications [8–10]. In particular, well differentiated forms, such as the "papillary type" seem to have a better prognosis than "alveolar" and "solid" forms [8–10]. However, the heterogeneity of the proposed classification schemes may limit their applicability and reproducibility. Therefore, the identification of novel molecular markers indicative of aggressive tumour behaviour, which may be better determined and quantified, would be extremely useful in assessing the prognosis and managing the treatment of patients with ITACs.

In searching for putative markers with prognostic significance in ITACs, we concentrated our attention on mucin glycoprotein expression by neoplastic cells. Mucin glycoproteins are complex molecules consisting of carbohydrates linked to proteins through an oxygen bond, which are commonly produced by glandular epithelia [11]. Important quantitative and qualitative changes in mucin production occur during neoplastic transformation. In particular, incomplete aberrant glycosylation may result in the excessive formation of mucin antigens, such as sialosyl-Tn, by neoplastic cells. Immunohistochemical studies have confirmed that sialosyl-Tn antigen has a very limited distribution in normal tissues, but is highly expressed by a variety of carcinomas having mucin producing ability [12]. In a recent immunohistochemical study of intestinal adenocarcinoma, sialosyl-Tn antigen expression was demonstrated to be an independent predictor of prognosis [13]. In particular, patients with sialosyl-Tn positive tumours had a significantly worse survival rate and a shorter disease-free interval [13]. Conversely, the expression of other simple mucin carbohydrate antigens, such as T and Tn, had no prognostic significance in the same series [13]. Mucin production has been identified in ITACs using histochemical

Correspondence to A. Franchi at the Istituto di Anatomia e Istologia Patologica, Viale G.B. Morgagni 85, 50134 Firenze, Italy.
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methods [9], but mucin antigen components expressed by these tumours have not been studied in depth. In consideration of the histomorphological similarities existing between intestinal adenocarcinomas and ITACs of the sinonasal mucosa, it is reasonable to expect that sialosyl-Tn antigen is expressed by these latter neoplasms. To verify this hypothesis, we analysed S-Tn antigen distribution in a series of ITACs, focusing on the relationship with the histological features of the tumour and on its possible prognostic implications.

PATIENTS AND METHODS

Cases studied

A group of 30 consecutive cases of ITAC treated at the Otolaryngology Clinic, University of Florence, was selected for this study. The series included 28 males and 2 females (male/female ratio 14:1), with age at the time of diagnosis ranging between 41 and 79 years (mean 59). In 18 cases (60%) there was evidence of occupational exposure to leather dust (9 patients) or wood dust (9 patients). In addition, 12 patients were heavy smokers. No patient had previous, concurrent or subsequent gastrointestinal adenocarcinoma. The most common presenting symptoms were unilateral nasal obstruction (16 patients) and epistaxis (6 patients). Both nasal obstruction and epistaxis were present in 9 cases, and in 4 cases epistaxis was associated with rhinorrea. 2 patients with ethmoidal tumour presented with exophthalmus, while in a case of maxillary sinus adenocarcinoma, swelling of the cheek and anaesthesia of the infraorbitary branch of the third nerve were present at the onset. The mean duration of symptoms before diagnosis was 6 months. On rhinoscopy, the lesions had polypoid or nodular shape, were pink-reddish or white-grey, and frequently presented areas of haemorrhage or ulceration. Owing to the advanced stage of the tumours at the time of diagnosis, it was difficult to determine the site of origin of most of the lesions. Eighteen tumours (60%) involved the ethmoid sinus, the maxillary sinus and the nasal fossa. Bilateral extension to the ethmoid sinus and nasal fossa was present in 4 cases. Extension to the orbit was observed in 5 patients, and intracranial extension in one. In 8 cases (26%), the tumour had a more limited growth, involving the nasal fossa, the ethmoid and/or the rhynopharynx. Laterocervical metastases were detected at the time of diagnosis in 2 cases, one with bilateral involvement, and in one patient there was evidence of cerebral metastases. 11 patients (36%) received radiation therapy, with doses ranging between 56 and 70 cGy. 11 patients underwent surgical resection of the tumour, consisting of maxillectomy and/or ethmoidectomy, associated in 2 cases with exenteratio orbitae, followed by radiotherapy in 9 cases and by radio- and chemotherapy in 3 cases. One patient underwent elective neck dissection with superficial parotidectomy. Follow-up information ranging from 3 to 108 months (mean 29.7 months) was obtained for 29 patients. Local recurrence occurred in 15 patients (51.7%), and distant metastases (lymph node and pulmonary) were detected in 7 (24.1%). 18 patients (62%) died of their tumour, 3 (10.3%) died of unrelated causes without evidence of disease, 3 (10.3%) were alive with disease, and 5 (17.1%) were alive with no evidence of disease at last follow-up.

Immunohistochemistry

Paraffin blocks of all cases were retrieved from the files of the Institute of Anatomic Pathology, University of Florence. Haematoxylin-eosin and periodic acid Schiff stained sections were employed for preliminary histological examination, and to classify the lesions in grade 1 (well differentiated), grade 2 (moderately differentiated), grade 3 (poorly differentiated) adenocarcinomas and mucinous adenocarcinomas, using the criteria described previously [14]. According to this classification, there were 3 grade 1, 16 grade 2, 2 grade 3, and 9 mucinous adenocarcinomas in this series.

For immunohistochemical studies, tissue sections were stained using a standard streptavidin–biotin technique (LSAB kit, Dako Co., Carpinteria, California, U.S.A.). The primary monoclonal mouse antibody against sialosyl-Tn antigen (HB-STn-1, Dako Co., Carpinteria, California, U.S.A.) was applied at 1:50 dilution. The specificity of this antibody is analogous to that of TKH2 antibody. Paraffin sections of colorectal carcinomas were employed as positive controls; negative controls were obtained by substituting the primary antibody with non-immune mouse serum. Scoring of the immunohistochemical staining was performed without information on the clinical follow-up data, according to the criteria estabilished by Itzkowitz *et al.* [13]. The percentage of low-power (× 10 objective) fields showing sialosyl-Tn antigen expression was determined, and cases with less than 5% of positive fields were considered negative.

Statistical methods

Considering the day of initial biopsy as the starting day of the observation, the disease-free and overall survival curves were calculated according to the Kaplan–Meier method [15]. Patients who died of other causes without evidence of disease or who were unavailable for follow-up were censored either at the time of death or last follow-up. The disease-free interval was calculated considering the time to first recurrence. Differences in survival and locoregional control between sialosyl-Tn positive and negative cases were assessed using the log rank test [16]. The pattern of associations between S-Tn antigen expression and clinico-pathological parameters was determined using Fisher's exact test. Relative risk and corresponding 95% confidence interval were calculated using the odds ratio method.

RESULTS

Sialosyl-Tn antigen positive expression was detected in 21 cases (70%). In grades 1 and 2 adenocarcinomas, the immunostaining localised at the cell membrane of the apical pole of neoplastic cells, as well as in the cytoplasm, and in the content of glandular structures (Fig. 1). In grade 3 adenocarcinomas, there was a very limited expression of the antigen, and staining was seen only in relation to the membrane of a few neoplastic cells. In mucinous adenocarcinomas, there was a diffuse and intense staining of the extracellular mucous pools, while neoplastic cells showed a prominent diffuse cytoplasmic or peripheral positivity (Fig. 2). In seven biopsy specimens, fragments of non-neoplastic nasal mucosa were present. Sialosyl-Tn antigen was expressed focally in the superficial layers of squamous epithelium, in transitional epithelium and in mucosal glands (Fig. 3). The distribution of positive cases according to the histological type of adenocarcinoma is shown in Table 1: no statistically significant association was found between these variables, although all grade 1 and all mucinous adenocarcinomas were scored as positive. The percentage of

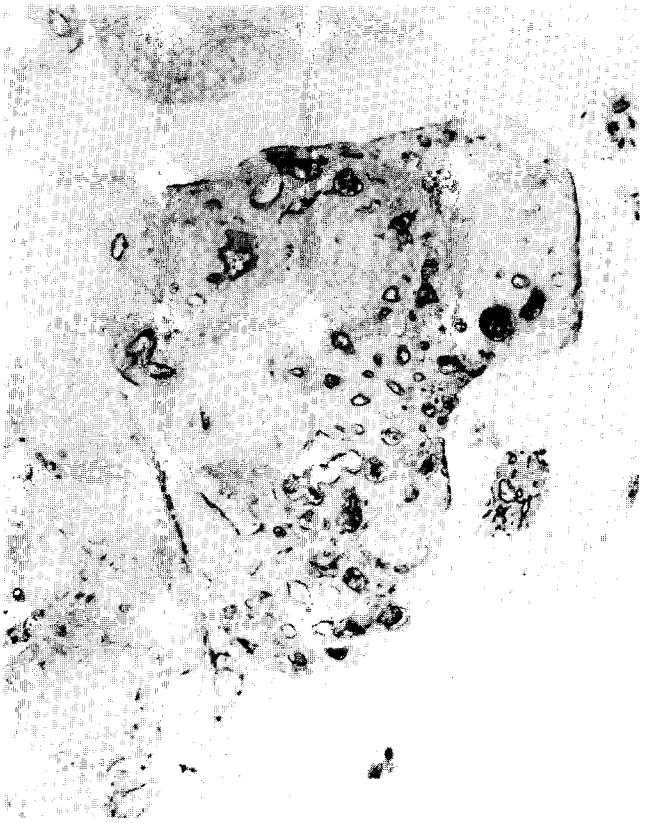

Fig. 1. Grade 2 intestinal type adenocarcinoma showing intense and diffuse staining for sialosyl-Tn antigen in the content of glandular structures (original magnification x 120).

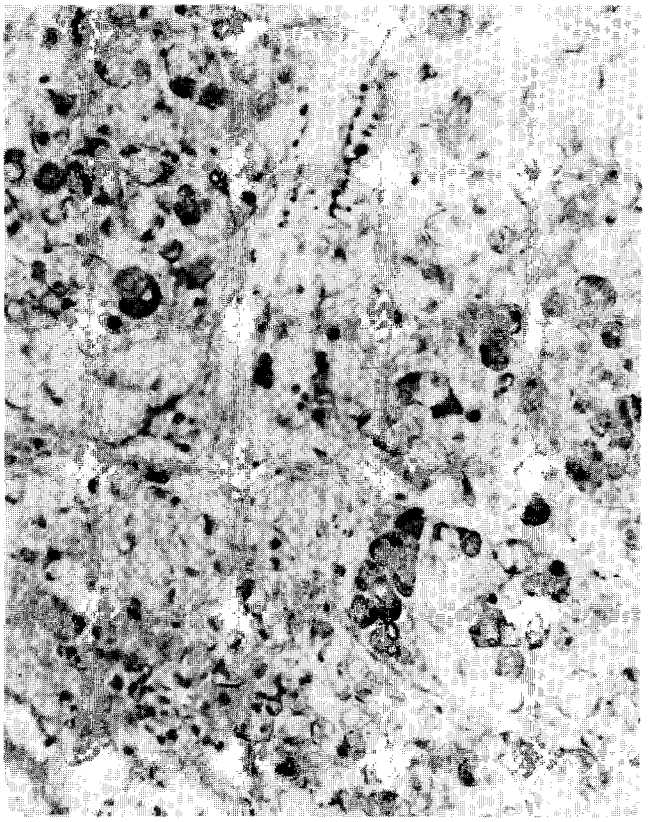

Fig. 2. Mucinous adenocarcinoma showing positive reaction for sialosyl-Tn antigen in the cytoplasm of neoplastic cells and in extracellular mucus (original magnification x 240).

S-Tn positive adenocarcinomas in the group of patients with occupational exposure to wood and leather dust was comparable to that of the group without occupational exposure (72 and 70%, respectively). Moreover, there was no difference in the treatment received between S-Tn positive and S-Tn negative adenocarcinomas.

The clinical outcome of patients according to S-Tn tumour expression is summarised in Table 2. Patients with sialosyl-Tn antigen positive tumours tended to have a less favourable clinical course. 11 out of 21 (52.3%) developed local recurrence, while 4 of the 8 (50%) negative tumours relapsed. All patients developing distant metastases had S-Tn positive tumours, while none of the S-Tn negative adenocarcinomas metastasised. Of the 18 patients who died of their disease, 16 had a S-Tn positive tumour and only 2 had a S-Tn negative tumour ($P=0.02$). Overall 5 year survival rate of patients according to S-Tn antigen expression was 17.8% for positive tumours and 72% for negative tumours ($P=0.0001$) (Fig. 4). The disease-free interval rate for patients with positive tumours was 16.6%, and that of patients with negative tumours was 40% ($P=0.0001$).

DISCUSSION

Our results show that ITACs express S-Tn antigen, in accordance with adenocarcinomas of other districts [12]. As already observed in colorectal cancer, we found no association between S-Tn expression and the histological degree of differentiation of the adenocarcinomas [13]. Conversely, non-

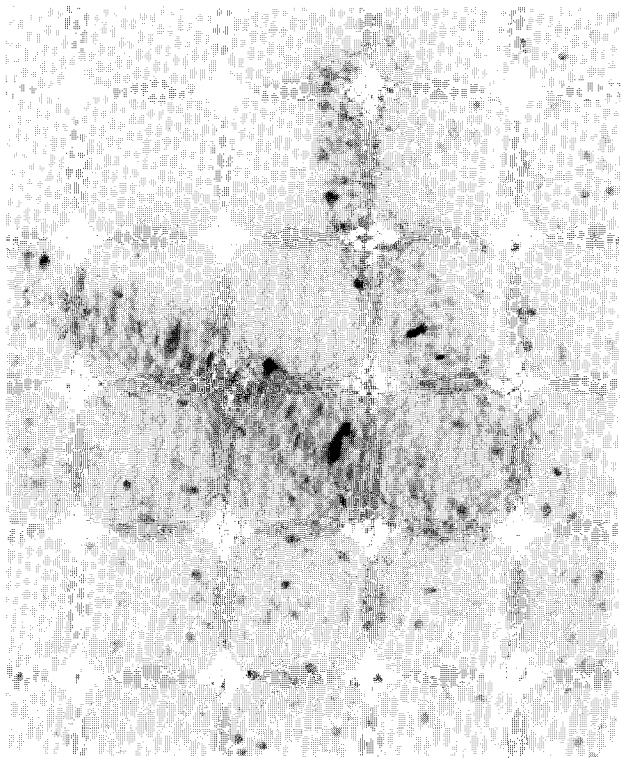

Fig. 3. In non-neoplastic sinonasal mucosa focal immunoreactivity for sialosyl-Tn antigen is detectable (original magnification x 500).

Table 1. Sialosyl-Tn antigen expression in different histological subtypes of sinonasal ITAC

Histological type	Sialosyl-Tn antigen +	−	Total
Grade 1	3	0	3
Grade 2	9	7	16
Grade 3	0	2	2
Mucinous	9	0	9
Total	21	9	30

Table 2. Clinical outcome of patients according to S-Tn antigen expression in sinonasal ITAC

	S-Tn+	S-Tn−	*P*	RR (95% CI)*
Local recurrences	11/21	4/8	0.6	1.03 (0.65; 1.61)
Distant metastases	7/21	0/8	0.1	1.57 (1.15; 2.16)
Deaths for disease	16/21	2/8	0.02	1.96 (1; 3.81)

*Relative risk and corresponding 95% confidence interval.

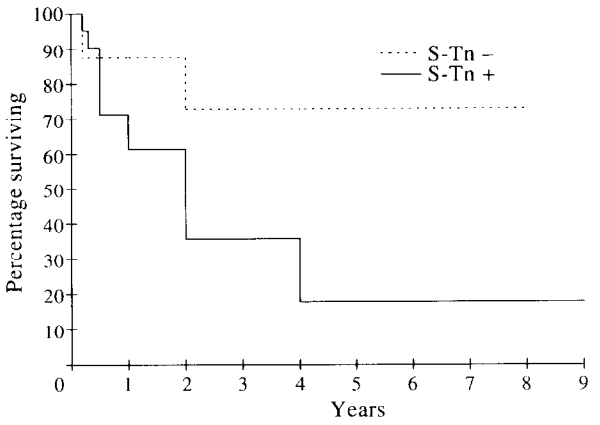

Fig. 4. Actuarial survival in patients with intestinal type sinonasal adenocarcinoma as a function of sialosyl-Tn antigen expression.

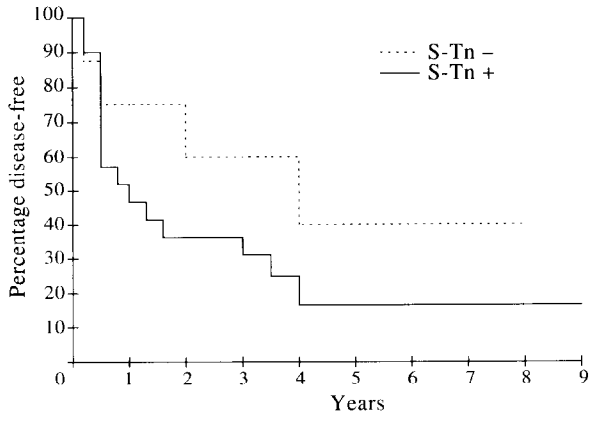

Fig. 5. Disease-free interval in patients with intestinal type sinonasal adenocarcinoma as a function of sialosyl-Tn antigen expression.

neoplastic sinonasal mucosa showed a very limited distribution of the antigen, thus confirming the association of S-Tn immunohistochemical expression with cancer [12]. Differences in S-Tn expression between normal and neoplastic tissues may result from modifications along the biosynthetic pathways of the molecule during carcinogenesis. The initial step of *O*-linked oligosaccharide synthesis is the glycosylation of serine and threonine amino acid residues of protein to form the Tn antigen. This structure may be further glycosylated by the addition of sialic acid, resulting in the S-Tn antigen, or alternatively by galactose to form the T antigen. In normal tissue S-Tn antigen is not detectable, as it is further glycosylated. Aberrant incomplete glycosylation may occur in cancer tissue, thus leading to the accumulation of S-Tn antigen, which becomes immunohistochemically detectable [11]. Another possible mechanism to explain the tumour-associated expression of S-Tn antigen in the colon has been recently suggested by Ogata *et al.* [17]. These authors obtained a positive immunostaining for S-Tn in normal colonic mucosa using a saponification method to remove *O*-acetyl esters from sialic acid residues. They concluded that S-Tn antigen is expressed in an *O*-acetylated form in the normal colonic epithelium which is not recognised by the antibody used in the immunohistochemical reaction [17].

The analysis of this series confirms that ITACs of the sinonasal mucosa are highly aggressive tumours with a poor prognosis, which often occur in association with occupational exposure to wood and leather dust. Moreover, we provide evidence that S-Tn expressing ITACs are more likely to follow an aggressive clinical course, as patients with positive tumours had a significantly lower overall 5 year survival rate and disease-free interval in comparison with patients with antigen-negative tumours. This seems to be of particular importance as, until now, no biological prognostic marker has been available for these neoplasms. Previous reports have suggested that specific histological subtypes of ITAC of the sinonasal mucosa, such as the alveolar-goblet cell and the signet ring cell types, may have a more aggressive clinical course [8]. Interestingly, in our series all mucinous adenocarcinomas, which correspond to the above-mentioned subtypes in other classifications, expressed S-Tn antigen. If our findings are confirmed on a larger series, S-Tn antigen immunohistochemical determination could be of considerable importance for deciding which patients affected by ITAC of the sinonasal mucosa might benefit from aggressive therapeutic protocols and a strict follow-up.

The mechanism by which S-Tn expression may influence tumour behaviour remains to be determined. Some experimental evidence indicates that sialoglycoprotein expression may affect the invasive and metastatic potential of tumour cells [18]. In particular, sialylated mucin-type glycoproteins have been associated with increased metastatic potential in a murine colon cancer cell line [19], and could, therefore, play a key role in the spread of tumour growth. Other lines of evidence support a role for mucin antigens in interfering with the immune system. Recently Ogata and coworkers have shown that S-Tn antigen is able to inhibit natural killer cytotoxicity, while mucins lacking the S-Tn antigen and non-mucin glycoproteins are not [20]. Therefore, it is conceivable that a reduced immunosurveillance could favour tumour aggressiveness in patients with S-Tn expressing ITACs.

Our findings indicating S-Tn production and secretion by neoplastic cells of ITACs may have other important clinical

implications. It has been suggested that S-Tn antigen serum determination may be useful in monitoring patients with gynaecological malignancies and in evaluating therapy [21]. Similarly, S-Tn determination in serum and nasal mucus could be useful in monitoring individuals at risk of developing ITAC, such as wood workers and leather workers, or in following the clinical course of ITAC patients after therapy. Further studies comparing S-Tn levels in blood serum or nasal mucus in patients with ITAC and normal controls are presently underway in our laboratories.

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