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# Sialic acids in head and neck squamous cell carcinoma

## Kwasy sjalowe w raku płaskonabłonkowym głowy i szyi

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## Summary

Aberrant glycosylation is a universal trait of cancer cells, and various types of glycan structures are well-known markers of tumour progression and invasion. This article discusses the respective role of sialic acids, biosynthesis of sialylglycoconjugates and the genetic basis of such phenomenon as well as effects of sialisation, different from normal, and their correlation with clinical prognosis in the head and neck squamous cell carcinoma (HNSCC).
So far, only few studies of the level of sialic acid in head and neck tumours have been carried out. Conclusions of the published reports confirm the elevated levels of total sialic acid in these tumours. Not all the authors, however, agree about correlation between the level of free or associated forms of sialic acid with the tumour size and severity or condition of lymph nodes. Comparing the progress in diagnosis and treatment of other types of cancer that has been made thanks to extensive work on the role of sialic acids, we came to conclusion that only further, detailed studies of this subject, related to HNSCC would be able to define the role of the glycoforms of sialic acid as a tumour marker or the target of immunotherapy.

**Key words:** **sialic acids, head and neck squamous cell carcinoma, markers in head and neck cancer, N-acetylo-5-neuraminic acid**

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## Introduction

During embryogenesis, the cells are activated, start growing and proliferate rapidly. In vertebrates, the development of the embryo as well as cell activation are accompanied by changes in glycosylation. Therefore, it is not surprising that such changes are a permanent feature of cancerous transformation. Further stages of carcinogenesis, i.e. invasion of tissues through ability of adhesion to cells of other types and cellular matrix, as well as colonization of places distant from the primary tumour and angiogenesis, are also underlain by mechanisms observed in normal cells, e.g. leukocytes, dependent on changes in glycosylation. A part of them influences the behaviour of malignant cells in a confirmed manner; therefore knowledge may be used to predict aggressiveness and invasiveness of a tumour and also upon monitoring and planning the regimen. However, vast majority of disorders in glycosylation has not been examined yet; we do not know their role or significance in biology of malignant cells.

One of the key forms of changes in glycosylation of malignant cells is elevation of the amount of sialic acid on their surface. Populations of those cells are known to be promoted during cancerous transformation which enhance proliferation, invasiveness, angiogenesis, ability to metastasis, and which are resistant to immunological reactions and apoptotic signals. The increase in surface sialylation of glycans of malignant cells seems to meet such criteria, affecting both, intercellular reactions and those between the cell and the matrix.

## Structure and role of sialic acids

Sialic acids (SA) comprise a family of aceto-derivative neuraminic acid (Neu) - 9- carbonic 5-amino-3,5-dideoxy-2-nonuloson acid. There are about 40 derivatives of neuraminic acid, which play a significant role in cell functioning. SA attach to glycans in the terminal, non-reducing position, which charges the particles of glycoconjugates negatively, thus enabling them to perform a significant function in intercellular and intermolecular interactions. Increased surface sialylation of glycoforms causes the cells repel each other, therefore decreasing their adhesion, elevating motility and enabling the malignant cell to reach blood circulation. About 70% of SA build cell membranes. The remaining part is distributed among endoplasmic reticulum, mitochondria and lysosomes. Sialic acids that bound to cell membranes are called binding sialic acids – BSA. Those attached to proteins and lipids are called protein-associated sialic acids – PSA or PASA and lipid-associated sialic acids – LSA or LASA respectively. Free form of SA can be identified only in trace amounts (free sialic acids - FSA) [8,20,57]. In blood plasma, the presence of sialic acids is associated with their occurrence as a component of acute phase proteins: alpha - 1- acid glycoprotein (AAG), haptoglobin, plasminogen, ceruloplasmin or transferrin. This is probably why elevated total amount of sialic acids (total sialic acids - TSA) and free sialic acids - FSA, correlate with elevated ESR and CRP, as well as with the named acute phase proteins. Such relation can be observed not only in cancer but also in inflammatory conditions, coronary disease, pregnancy, alcohol abuse or microalbuminuria [22,26]. Because of the negative charge of SA, they influence significantly the solubility, viscosity, stability and the charge of glycoproteins to which they attach. While occluding the polypeptide part (e.g. antigenic or receptor), the sialylated glycoconjugates protect it against the effect of proteolytic enzymes and immunological reactions. The enzymes called sialidases (neuraminidases) hydrolize the glycosidic bond between sialic acid acceptor oligosaccharide, influencing the receptor-ligand interactions. It was shown that desialylated glycoproteins are more rapidly removed from circulation by specific receptors for sialylglycoproteins, localized on hepatocites, Kupffer's cells or peritoneal macrophagocytes. The cells expressing desialylated glycoproteins are often recognized by receptors called galectins. After identification by particular galectins, erythrocytes, lymphocytes, blood platelets and malignant cells with little or no sialic acids, are removed from the organism, as desialylation uncovers galactose residues in glycoconjungates which are ligands for galectins. Such mechanism, underlying removal of old and damaged cells is also sometimes used by some pathogens. For example, in sepsis caused by *Streptococcus pneumoniae*, the crucial role is taken by sialidases removing the remaining sialic acid from thrombocytes, which leads then to lethal coagulopathy [36].

SA determine also multiple pathomechanisms of infections and colonization by microbes. Identification of sialic acid attached in a proper configuration to a glycoprotein or a glycolipid of the cellular membrane of the host, through lectins (haemagglutinins) on the pathogen's surface, initiates adhesion or endocytosis. On the other hand, sialidases of microbes uncover cellular antigens of the host, triggering autoaggressive reaction, despite subsidence of the primary infection. As a component of sialyl-Lewis structures (membrane antigen determinants), they take part in reactions of those antigens with cytokines (IL-1, TNF) and selectines E, L and P. These processes regulate, among other, colonization of lymph nodes by circulating leukocytes, their permeation through the endothelium in inflammatory conditions, and on the same basis, they also have a role in the processes of cancer metastasis.

Most significant in humans is N-acetyl Neu derivative, i.e. N-acetyl-5-neuraminic acid (Neu5Ac, previously NANA). Other derivatives, e.g. O-acetyl were found in the tissues of fetus and in patients suffering from melanoma. N-glycol derivatives were identified on cellular surfaces of most mammals, including chimpanzees and other anthropoid apes [19]. However, N-glycolyl derivatives are not present on the surface of human cells, because of the lack of CMP-Neu5Ac hydroxylase (CMAH), transforming Neu5Ac into N-glycolylneuraminic acid. (Fig.1) Regarding the fact that humans have contact

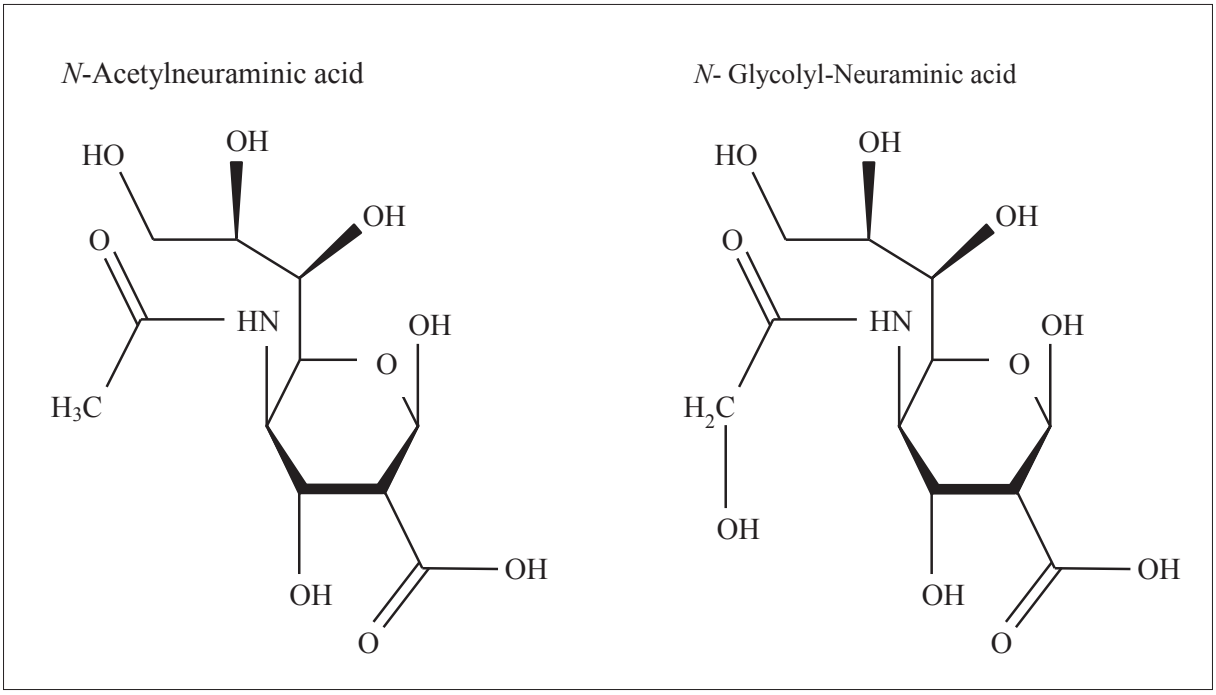


**Fig. 1.** Structures of N-Acetylneuraminic acid and N-Glycolylneuraminic acid.

with this acid - consumed with food, there are sometimes anti-Neu5Gc antibodies found in healthy individuals serum – the so called Hanganutziu-Deicher antibodies. N-glycolylneuraminic acid was also found in tissues of some cancers, usually as a component of gangliosides (G3), less often as a component of other glycolipids or glycoproteins. The mechanism of this phenomenon is not explained. It may be caused by an alternative course of transformations in cells or, more likely, by consumption of Neu5Gc in food [49]. Independently of the source, the sialic acid - absent in normal tissues - stimulates the immune response in a form of Hanganutziu-Deicher antibodies, which may explain some instances of spontaneous cancer remissions. It may also constitute the target of specific cancer immunotherapy [30].

## Biosynthesis of glycoconjugates containing sialic acid

Enzymes that synthesize sialic acid-containing glycoconjugates are called sialyltransferases; they reside in Golgi apparatus and transfer sialic acid residue from its CMP-activated form to the appropriate acceptor. Until now, twenty genes belonging to the ST family have been identified. They have been divided into four families: ST3GAL, ST6GAL, ST6GALNAC i ST8SIA. Enzymes from the family of ST3GAL catalyze adhesion of the sialic residue to terminal galactose in glycoproteins and glycolipids with the α2-3 linkage. Changes in expression of ST3GAL 1, 3 and 6 were found in breast, ovarian, stomach and cervical cancer [33]. ST6GAL1 and 2 add SA to galactose with the α2-6 binding. It is generally believed that increased degree of sialylations enhances tumour aggression. Some of the known substrates of that enzyme are: E-selectin, β1 integrin, ICAM-1 and VCAM. Elevation of the ST6GAL1 gene expression in numerous cancers depends on activity of ras oncogene. That is why, in cancers where its mutation is identified, including laryngeal cancer, elevated level of sialylation α2-6 should be expected. In addition cytokines such as TNF-α, IL-1, or IL-6, may induce elevated expression of ST6Gal-1. Disturbed STGAL1 modulates significantly the cell interactions with other cells. Its overexpression was confirmed in head and neck cancer, colorectal carcinoma, cervical cancer, breast or liver cancer [55]. Liu et al. investigated the macrophages' apoptosis controlled with the TNFR1 receptor, demonstrating that it depended on sialylation of α2-6 receptor, caused by ST6GAL1. The cells with elevated sialylation were significantly less sensitive to apoptosis stimulated by TNF than other cells. The authors concluded that removal of α2-6 sialylation of macrophages during differentiation stage should decrease their survival by sensitizing them to TNF-stimulated apoptosis [27]. Cancers with elevated expression of ST6GAL1 are characterized by lower degree of cells differentiation, higher motility and invasiveness. The studies performed indicated that many of those effects were caused by excessive sialylation of β1 integrin [12]. It was hypothesized that decrease in ST6GAL 1 activity reduced invasiveness of malignant cells, but only those with increased level of integrin [39]. Depending on ST6GAL1, excessive sialylation of integrin causes its increased binding with collagen I, fibronectin and laminin, which effects in higher motility of cells, and therefore correlates with increased invasiveness and predisposition to metastasize. On the one hand, a cell with highly sialylated β1 integrin

shows better adhesion to the collagen I, IV and fibronectin, improving its migration and making it easier for the cell to detach from the tumour. However, the same mechanism is responsible for enhanced, selective adhesion to endothelial cells and metastasis [39]. Such increase is often caused by elevated levels of the sialic acid attached with a binding to external lactosamine in units of Galβ1-4GlcNAc, or in internal units of O-glycans GalNAc-O-Ser/Thr. The last epitope, the so-called sialyl--Tn (Siaα-2-6GalNAcα1-0 Ser / Thr), is currently the aim for immunotherapeutic attempts in some cancers [44]. What is more, radiation-stimulated overexpression of ST6GAL1 improves survival of radiated cells, i.e. causes resistance to radiation [25]. The evaluation of ST6GAL overexpression, or indirectly of the amount of α2-6 sialylation, e.g. in laryngeal cancer cells, may become an indicator of cancer tissue radiosensitivity. Thus, it may facilitate selection of therapies when surgery and radiotherapy seem to be equivalent regimens.

## Sialic acid in known tumour markers

Multiple carbohydrate antigens containing sialic acids have been reported to have a role in neoplastic transformation. They change significantly during carcinogenesis, where the changes may be: aberrant sialylation, increased or decreased antigen expression as well as appearance in an uncommon place. Tumour-associated carbohydrate antigens resulting from improper glycosylation accompanying cancer transformation include mucin related Tn, Sialyl Tn, and Thomsen–Friedenreich antigens. Furthermore, there are antigens connected with Lewis blood group: $Lewis^y$, $Lewis^a$, sialyl-$Lewis^a$, sialyl-$Lewis^x$ and $Lewis^x$ (SSEA-1, stage-specific embryonic antigen-1). Finally, glycosphingolipids Globo H and SSEA-3, and gangliosides GD2, GD3, GM2, Neu5GcGM3 as well as polysialic acids [13].

None of the above antigens has been studied thoroughly in the context of head and neck cancer. The research has focused rather on the role of sialic acid in other cancers; especially in colorectal, breast, lung, pancreatic cancer and melanoma.

## Sialic acids in glycoproteins

### 1. Structure of sialylglycoproteins

Neu5Ac is attached in the terminal position of oligosaccharide structures of numerous glycoproteins. Generally, two types of covalent bond between a sugar particle and a peptide fragment are distinguished. N-glycoside bonds of an amino group of the asparganine residue (Asn) and O-glycoside bonds of a hydroxyl group of residue of serine (Ser) or threonine (Thr) [36,17].

### 2. Sialic acids in blood group antigens

O- glycosylation is a common covalent modification of serine and threonine residues of mammalian glycoproteins. This glycoproteins that are often termed mucins. The simplest mucin O- glycan is a single N- acetylgalactosamine residue linked to serine or threonine named the Tn antigen. Biosynthesis of mucin-like O-glycosides depends on activity of several different glycosyltransferases. After adding sialic acid to the Tn antigen (GalNAc-O-Ser/Thr) in α2-6 position sialyl-Tn antigen is produced, and if instead of Neu5Ac, galactose is added through ß1-3 linkage, T antigen is obtained. Further modifications include addition of Neu5Ac with α2-3 binding (sialyl-T antigen) or α2-6, or in both positions simultaneously. These antigens are usually masked with sialic acid residues in normal cells, so such epitopes are invisible for the immune system (in sialyl-Tn antigen, O-acetylation of Neu5Ac residue causes that it is undetectable by monoclonal antibodies). They appear after desialylation in glycoproteins on cells aiming at tumorigenesis [8].

Masked T and Tn antigens are also present on glycophorin A. It is richly glycosylated and sialylated protein found on red blood cells, which carries blood group antigens M and N. Glycophorin A conteins one N-linked and 15 O-linked oligosaccharides, which after desialylation became the antigens T, and after degalactolylation Tn antigens. All oligosaccharides constitute a glycocalyx, which is important factor protecting cells from pathogens; many of such oligosaccharides are blood group antigens [7].

Increased expression of T antigens (Thomsen-Friedenreich antigen (TF)), Tn and sialyl-Tn is observed in 90% of cancers and in some leukaemias [42]. The levels of T/Tn antigens are directly proportional to progression of cancers and possibility of metastasis. Tn antigen is present in more invasive cancers with high metastatic potential, while the dominance of T antigen allows for better prognoses. Neu5Ac has probably double role. On the one hand, increased sialylation of glycoproteins on the surface of malignant cells lowers their one to another adhesion and towards the intercellular matrix, which supports detachment of cells from the tumour, while on the other, sialylation α2-6 is a signal of ligand activation for several galectins, and required e.g. for siglecs, which in turn causes higher adhesion of malignant cells to the endothelial cells which produce galectin 3, and as a consequence causes metastasis. Sialyl-Tn synthesized by ST6GalNAc-1 with the α2-6 binding is a significant antigen of numerous epithelial carcinomas, e.g. stomach, pancreatic, colorectal, ovarian or breast cancer and usually correlates with bad prognosis. Its expression is a reliable marker for example in colorectal cancer, while in breast cancer it correlates with decreased differentiation of cells [54]. In turn, the presence of sialyl-Tn in metaplasia of gastric mucosa is a valuable marker of high risk of gastric carcinoma [50].

Other alteration of glicosylation during carcinogenesis are changes in the expressions of Lewis histo-blood group antigens. Increased or decreased expression of

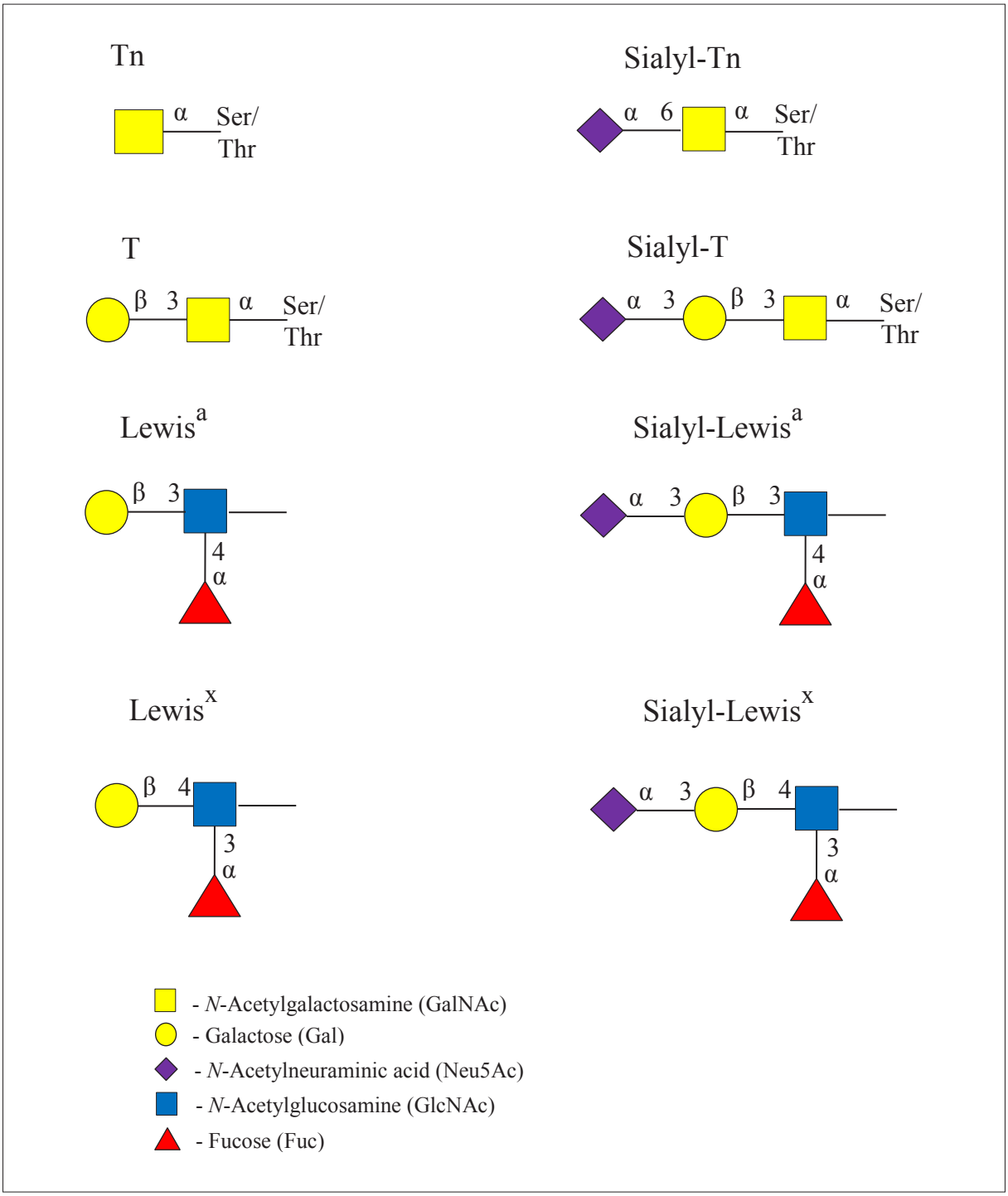


**Fig. 2.** Structures of T, Tn, SiaT, SiaTn and Lewis-type antigens.

Lewis type antigens often accompanies cancer transformations, and the type of pathological biosynthesis depends on the tissue, which triggered carcinogenesis. Regarding the role of the sialic acid, sialyl-Lewis$^x$ and sialyl-Lewis$^a$ antigens are interesting [51]. (Fig. 2) Both antigens are produced through α2-3 sialylation, depending on α 2-3 - sialyltranferase, and their presence on the surface of malignant cells enhances metastasis and reduces the patients' survival rate. Such relation was observed in pancreatic, colorectal, lung, bladder, stomach, breast, kidney and liver cancer [18]. Sialyl-Lewis$^a$, better known as CA 19-9 antigen, is a commonly applied marker, particularly in pancreatic cancer. Both antigens are ligands for E and P selectins, which physi-

ologically are particles responsible for adhesion of leukocytes for vascular endothelium. Selectins are one of the families of intercellular adhesion particles (CAMs). It includes transmembranous leukocyte glycoproteins-L, endothelial-E glycoproteins and platelet-P. Selectins are present in small amounts on the surface of normal cells [24,31]. Affected by inflammatory mediators, such as TNF, IL-1 or thrombin, they are transferred onto the cell surface, binding then to specific ligands on the surface of leukocytes, platelets and endothelial cells. In tumour cells, similar role is played by Lewis type antigens. They enable the tumour cells present in the circulation to bind with endothelium, which is the first stage of metastasis.

## Sialic acids in glycolipids

Glycolipids are lipids with a carbohydrate attached by glycosidic bond. Lipids are non-polar molecules, which make them capable of interacting with the lipid-bilayer of the cell membrane and anchoring the glycolipid to the cell surface. Neu5Ac has an important role in construction of gangliosides. These are glycosides of ceramides, containing oligosaccharide residuum attached with the O-glycoside binding, composed mainly of four types of sugar: glucose, galactose, galactosamine and N- acetyl neuraminic acid. Characteristic of gangliosides is the presence of at least one residue of sialic acid, which is reflected in their nomenclature. The letters M, D and T refer to mono-, di- and tri- sialylgangliosides respectively. These lipids are mainly present in the membranes of ganglion cells of the nervous system, but also in other tissues [34]. Linear polymers of the sialic acid with α2-8 bonds, may also appear as component of gangliosides or glycoproteins. Increased expression of gangliosides and polysialic acids may be correlated with expansion of cancer invasiveness and progression of melanoma, microcellular lung cancer, Wilms' cancer, astrocytoma, neuroblastoma and glioma. These studies have now reached the stage of highly advanced preclinical and clinical investigations of immunotherapy making use of monoclonal antibodies against gangliosides [13].

## Sialic acids in head and neck squamous cell carcinoma

In the past scarce studies focused on the correlation of sialic acids levels with the stage of head and neck cancer, and only some individual investigations were carried out in the area of laryngeal cancer.

The latest estimates regarding laryngeal cancer were published in 2005. Only a few studies on the role of SA in nasopharyngeal and oral cancer have been published recently. Reports on positive relation between the levels of sialic acids, both in a free form (FSA) and one associated with proteins (PASA) or lipids (LASA), with the tumour size, stage and condition of the lymph nodes, are more congruent in most of the publications. As for sialic acid in its free form (FSA), the results differ remarkably. Most authors report that the measurement of serum concentration of sialic acid in patients with head and neck cancer is a high sensitivity method. However, the usefulness of sialic acids as a marker is significantly limited by the fact that their level increases also in inflammatory conditions, especially the chronic ones, and in patients with alcohol abuse. Consequently, the method may be characterized by low specificity. Most authors suggest the use of high sensitivity of increased sialic acid concentrations along with another marker of high specificity, e.g. SCCA or CYFRA 21-1 [23, 24, 37].

Uslu et al. 2003 found positive correlation of serum levels of bound sialic acid with the laryngeal cancer stage with simultaneous lack of correlation in the levels of free sialic acid. BSA levels were significantly higher in patients with laryngeal cancer and increased with the severity of the disease [46]. Akay et al. 2001, identified significantly increased levels of TSA in patients with laryngeal cancer compared to a control group and a positive correlation between the levels of ICAM-1 and TSA and the stage of cancer of the larynx. The highest levels of TSA were observed in patients with stage IV [1]. Kiura et al. 2000 examined the level of antigen SCCA, CEA, ferritin, IAP and sialic acid in HNSCC. They found, inter alia, a positive correlation between the level of sialic acid in plasma with tumour size, metastasis in the lymph nodes, clinical stage and the survival rate [20]. Ayude et al. 2003 assessed total serum concentration of sialic acid of patients with head and neck cancer, corrected for the total protein content (TSA / TP). After determining the cutoff level of 12.0 ng / mg, TSA / TP method showed the level of sensitivity (63%) in the diagnosis of HNSCC specificity level 94%, 50% and 90%, relating to a group of healthy patients, smokers and drinkers and patients with benign diseases. Sensitivity of the method in the diagnosis of HNSCC (in the absence of the spread) may be improved using a combination of TSA / TP + CYFRA 21-1up to 71% of the TNM I, 80% of TNM II and 60% of the TNM III [2]. Subsequent authors, Fisher Egg 2003, compared serum concentrations of sialic acid in patients with newly diagnosed cancer, relapsed and patients clinically treated previously without the recurrence of a disease, with a group of healthy controls. They recognized measurements of sialic acid serum level as a useful parameter for early detection of a recurrent disease. In this study, the highest levels correlated positively with tumour size were observed in patients with active cancer. 71% of patients with recurrent cancer were above the level of SA established by the authors on the basis of the control group, the upper limit of normal (82 mg / dl) [10]. In 2015, two teams of Chinese scientists published simultaneously the results of their studies, confirming significantly increased serum level of sialic acids in patients with nasopharyngeal cancer compared to patients with rhinitis and control groups [45,56].These studies showed statistically increased sialic acids levels in HNSCC. However, such knowledge is not enough to use SA as a marker or as a target of immunotherapy. Much more detailed information on glycoforms specific for HNSCC is needed. Direction of future investigations

may be determined by earlier studies which indirectly provided information on improper sialylation of cancer tissues in the head and neck region.

The presence of mucins with changed glycosylation was found in head and neck squamous cell carcinoma, also correlated with tumour progression and ability to produce metastasis [44]. Studies of laryngeal cancer confirmed elevated expression of mucins, positively correlated with T feature and poor prognosis. However, they rarely evaluated the epitope of glycosylation disorders [6,16]. What is more, the results by various authors show significant discrepancies, probably because of different test methods. It is hard to decide then whether any antigen of MUC1, MUC2, Lewis, T/Tn group may have a role of a marker in HNSCC [41]. Bearing in mind the role of mucins in the development of cancer depending on their increased sialylation, often correlating with enhanced activity of sialyltransferases, and the fact that desialylation stimulates identification of MUC1 in cells [6], it is possible that focusing the studies on determination of sialic acids levels of in tumour tissue, preferably with differentiation of the binding type, type of glycoconjugate or identification of a glycoform, may provide more homogeneous results and allow for administration of targeted therapies [41].

Investigating oral squamous cell carcinoma, Shimizu used a monoclonal antibody directed against MUC1, obtaining 22% response, with 57,9% of malignant cells with confirmed presence of MUC1 antigen. This response is too weak, however, to be accepted as basis for a therapy. To improve effectiveness, the author suggests an adjuvant therapy or the use two antibodies at a time [40]. An alternative solution could be identification of a specific MUC1 glycoform. In this respect, studies on adenocarcinomas appear interesting, especially those of the breast cancer, where an immunodominating O-glycopeptide epitope (Tn/STn-MUC1) was identified, resulting from improper glycosylation and probably enhancing production of a specific antibody, regarding the lack of immunological tolerance for this structure [53, 43]. Bhatavdekar and al. 1988 and 1991, evaluated serum levels of protein-associated sialic acid (PSA) in patients with head and neck cancer, comparing them to the control group. The results presented point to PSA as a good predictor. The elevated levels were found in 57% of patients with benign tumours, 52% of primary head and neck cancer, and 75% of metastatic HNSCC. PSA levels decreased in patients during effective treatment and increased with poorer prognosis [4,3]. The literature includes no publications on the levels of gangliosides related to HNSCC, however, their role may be confirmed indirectly by studies of the level of sialic acid associated with lipids. Thirty years ago, Galioto et al. 1987 examined the level of LASA in patients with laryngeal cancer qualified for total or partial laryngectomy with surgical junction. It pointed to significantly higher concentrations of LASA in patients with lymph node metastasis as compared to those without metastasis, it was estimated, however that the study showed a large margin of error [11]. Throughout the following years, the results of LASA were more promising. In his studies of the amount of SA connected with lipids, Mevio 1991 found high specificity and sensitivity of 98-99% for this method in laryngeal cancer [32]. Straka et al. 1992 evaluated usefulness of radioimmunoassays for detection of squamous cell carcinoma, the level of sialic acid bound to lipids (LSA), carcinoembryonic antigen (CEA) and CA-125 in a population of cancer patients and the control group. The radioimmunoassay was rated as the most sensitive method - 47.5%, the level of CEA was elevated in 40.6% of patients with cancer, LSA only in 16,8%, and CA-125 in 7.9%. False positive results were observed in 18.2% of patients in the radioimmunoassay of CEA and 10.2% in LSA and 15.9% of CA-125. Different combinations of markers did not bring any statistical changes in specificity or sensitivity [41]. According to López Sáez et al., a medium level of sialic acid associated with fats (LSA) is significantly lower in healthy patients, and increases substantially along with progression of the disease. However, no significant difference was identified between patients with chronic diseases and those with breast cancer, head and neck cancer, lung cancer and tumours of the gastrointestinal tract [35]. High sensitivity of this method was also confirmed by Kaplan et al. 1993 with sensitivity of 94,4 % [21], and Dreyfuss et al. 1992 showing sensitivity of 71% [9]. According to Inal et al. 2004, sensitivity was 89,3% at specificity of 100% [15]. In all the studies, the levels of LSA increased along with progression of the disease, especially in relation to T characteristics and metastasis to the lymph nodes.

Recent publications do not report on any studies focused on sialic acids associated with lipids in head and neck squamous cancer. However, similar research referring to e.g. non-small cell lung cancer, neuroblastoma or melanoma, is already at the preclinical stage and clinical immunotherapy with use of monoclonal antibodies against gangliosides has been investigated [14,52]. It is likely that the same antibodies will prove effective in the laryngeal cancer therapies as well as other head and neck tumours. Hence, it is worthwhile to take a closer look at the causes of elevated LSA in HNSCC, and pay attention to particular sialylconjugates, with potentially higher usability, e.g. NeuGcGM3. The presence of N-glycoliloneuraminic acid NeuGc as a component of gangliosides (G3) was identified in tissues of some cancers, even though a human body does not produce this compound. The cause may be an alternative course of transformations in muted cells or, what is more probable, its consumption together with food. The presence of Neu5Gc in laryngeal cancer tissues has not been evaluated yet. Most of the studies in this area focus on malignant melanoma and breast cancer; some authors observed its presence also in tumour cells of gastric, colorectal, nasopharyngeal and uterine cancer, and in germ cell tumours as well as leukaemias or lymphomas [29]. Of special interest is the presence of Neu5Gc in epipharyngeal cancer, taking into account the viral etiology of this carcinoma. Earlier studies deny any

possibility of emergence of CMAH in human cells. Other enzymes, which could take a role of this enzyme in malignant cells, are searched for. It would be recommended to carry out more detailed evaluation of the levels of Neu5Gc in head and neck tumours, especially the nasopharyngeal cancer and such mutation within its cells which could enhance Neu5Gc expression. If proved, significant presence of Neu5Gc in cells of e.g. cancers of the larynx or the epipharynx, would make a way towards specific immunotherapy, aimed at a relation which does not occur in healthy cells, therefore is potentially highly effective and safe. Studies of an antibody against NeuGcGM3 have already reached the stage of first clinical tests, bringing some encouraging results in advanced malignant melanoma. Similar studies have also been designed for breast and lung cancer [14].

Possible use of α2-6 sialylation as a marker in head and neck tumours requires further detailed research. However, studies confirming e.g. elevated activity of integrin β1 or TSA in laryngeal cancer have already been performed [1]. The main, recurring conclusion from this research is the correlation of the level of integrins with activity of malignant cells as well as a possibility to influence the course of the disease through regulation of expression of genes responsible for creation of particular subtypes of integrins. Until now, the authors have not dealt with the question of biochemical forms or reasons for increased activity of specific subtypes of integrins in HNSCC, neither with molecular implications of such changes. Multiple investigations however, were carried out in other types of tumours.

Some reports on HNSCC suggest a significant role of α2-6 sialylation in cancerous transformation. Studies of the cervical cancer proved that along with the increase of malignancy, expression of α2-6 sialylation elevates in the basal layer and of α2-3 sialylation in the interlayer and superficial layer [28]. Holíková et al. 2002 mapped the profiles of glycoconjugate epitope expression depending on the type of sialylation, for epiderm and epithelium of the larynx, cornea as well as, oral and pharyngeal mucosa, using for that purpose plant lectins and comparing them with endogenous galectins. They found α2-6 sialylation in basal and spinous layers of the epidermal and epithelial cells of the mouth and vocal folds; and α2-3 sialylation, strictly suprabasally in the epithelium of the mouth and vocal folds. What is more, in studies of cancerous tissues from these regions, it was found that the number of bindings α2-3 decreases and the number of bindings α2-6 increases along with decreased cell differentiation [14]. In the study of Shah et al. 2007, TSA and TSA/TP were significantly higher in untreated oral cancer patients compared to oral precancerous conditions (OPC) and the controls. Also, the increase of serum TSA and TSA/TP was more significant in patients with OPC than in the controls. Elevated levels of α2-6- and α2-3-linked sialic acid as well as ST6GAL and ST3GAL activity in the tumour tissue and serum of cancer patients were also found [38].

According to Vajaria et al. 2013, serum and salivary TSA/TP ratios are significantly higher in OPC and oral cancer patients, compared to the controls. Salivary TSA/TP ratios were elevated at greater intensity than the serum levels. Both, salivary and serum TSA/TP levels were higher in smokers of the study group and in the controls.

The same team published a 2014 study of glycosylation changes in saliva of oral cancer patients, oral precancerous conditions and patients following treatment, compared to the control group. The authors found significantly higher values of the TSA / TP, α2-6- and α2-3-linked sialic acid and the activity of neuraminidase and ST-s activity in patients with oral cancer as compared to the precancerous lesion and compared OPC to the control group. The levels of studied factors also changed during treatment, demonstrating positive correlation [47, 48].

## Summary

The presence of sialic acids in human cells is common, therefore finding relations between changes in sialylation and tumour biology demands not only determination of correlations between the amount of Neu5Ac and the tumour stage but also a detailed research is necessary to comprise e.g. type of chemical binding, kind of glycoconjungate or changes in its function, depending on sialylation. Potential use of sialic acids as markers of head and neck cancers depend mainly on defining which specific glycoforms of this compound accompany cancerous transformation in a particular organ, as well as relation between their amount and the tumour malignancy, invasiveness and histological profile.

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