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# Circulating blood group related carbohydrate antigens as tumour markers

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The various blood group related carbohydrate structures which are in clinical use as circulating tumour makers are reviewed. Their location on carbohydrate chains and their structural characteristics are shown, and their clinical performance in various malignant diseases is reviewed. The available data on their sensitivity, specificity and predictive value are shown; and carcinomas of the pancreas, ventricle, colon–rectum and ovary are identified as diseases in which these markers can be of good benefit for follow-up. Future research should be devoted to studies of the function of these structures, and to studies of their gene-transcription.

*Keywords:* blood groups, cancer, carbohydrate antigens

## Introduction

The ideal tumour marker should only be present, or increased, in patients with malignant tumours, should correlate with tumour burden, should be easy to analyse, have a short biological half-time, and should be present in all tumours of a specific organ, no matter what their grade of atypiais.

A few tumour markers have these characteristics. These are hormones which are actively produced by the tumour cells such as chorionic gonadotropin from choriocarcinoma cells, or gastrin from a subset of gastric carcinomas. In haematology, malignant transformation of plasma cells leads to immunoglobulin producing tumours – the well known M-components of myelomatosis. These products are the normal physiological components of cells from which the tumour arises.

Unfortunately, however, the most frequently found carcinomas are of epithelial origin, colon, lung, breast, bladder etc. The normal counterpart of these tumour cells do not produce any known specific substance that can be used to monitor them. Other markers for common epithelial cancers have been investigated, and almost all of these are identified by the use of monoclonal antibodies. Accordingly, the tumour specificity depends on the specificity of the epitope. The epitope can be a peptide, a carbohydrate structure on a glycoprotein, a glycolipid, or a combination of these.

This review focuses on carbohydrate epitopes as tumour markers. It excludes markers like CA125, CEA (carcino-

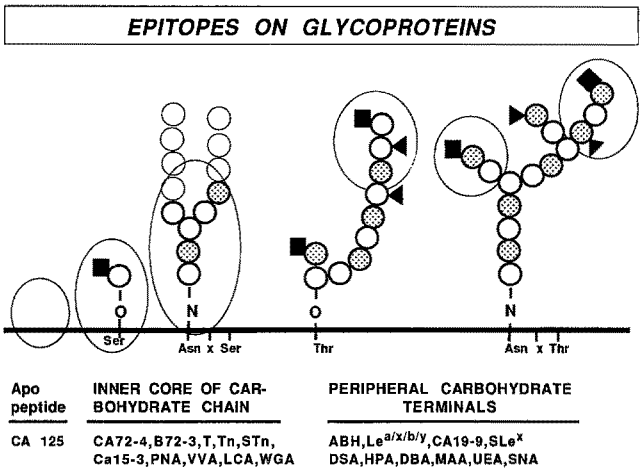


**Figure 1.** Circles indicate epitopes on glycoproteins. The epitope to the left is the naked peptide, which is the target for antibodies like anti-CA125. The inner core epitopes can be either O- or N-linked, and are targets for the antibodies and lectins summarized in the Figure, most of which react with the O-linked structures. The peripheral epitopes can be identical on O- and N-linked structures. Among these are the common blood group antigens.
DSA, datura stramonium agglutinin; DBA, dolichos biflorus agglutinin; HPA, helix pomathia agglutinin; LCA, lens culinaris agglutinin; MAA maackia amurensis agglutinin; PNA, arachis hypogaea agglutinin; UEA ulex europaeus agglutinin; VVA, vicia villosa agglutinin; WGA triticum vulgaris agglutinin; SNA, sambucus nigra agglutinin. See text or Table 1 for explanation of other abbreviations.

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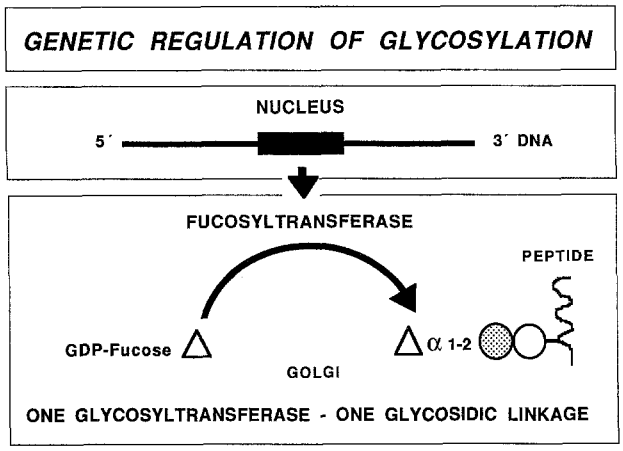


**Figure 2.** Schematic presentation of the genetic regulation of glycosylation. The gene encodes a fucosyltransferase that transfers a fucose residue to an alpha 1-2 position in the acceptor structure.

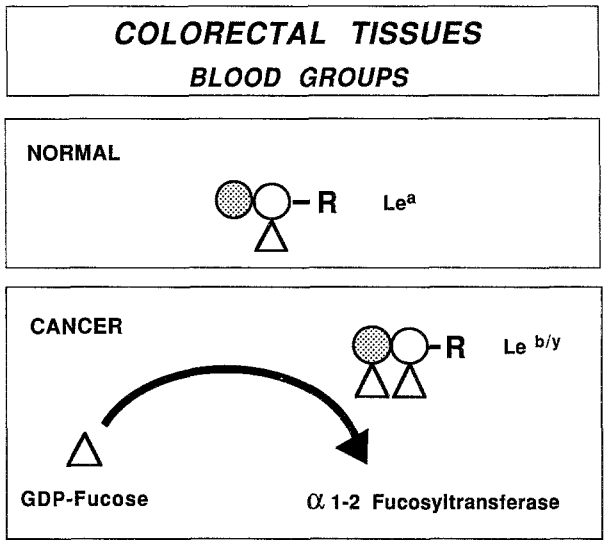


**Figure 3.** In colorectal tumours, an alpha 1-2 fucosyltransferase is activated and leads to formation of a range of structures like $Le^{b/y}$ and ABH.

embryonic antigen), and PSA (prostate-specific antigen), which are all detected by protein specific antibodies.

## Carbohydrate tumour markers

The carbohydrate epitopes of tumour markers are shown schematically in Fig. 1. The apo-protein part of a glycoprotein is that which is devoid of carbohydrates (CA125 is given as an example in Fig. 1). The carbohydrate epitopes can be either on the inner-core structures as shown by the O-linked mucin-type structures, or they can be terminal structures as shown on the branched N-linked structures. Well known antibodies and lectins that react with these structures are indicated on the Figure.

To understand how carbohydrates function as tumour markers, a little knowledge is needed about their biosynthesis. As shown in Fig. 2, carbohydrate structures are *not* the primary gene products. This is the glycosyl transferase which transfers a sugar residue from a donor to an acceptor (glycoprotein/glycolipid); in this way the carbohydrate chain is successively built up. Several families of transferases are known, for example, at least five different genes, located on two different chromosomes, are known to encode fucosyltransferases that add a fucose to a α1-3 position on a carbohydrate chain [1]. It is assumed that these transferases recognize a carbohydrate chain as the acceptor structure, no matter what protein it is located on. In this way many different glycoproteins can have identical carbohydrate side chains, or at least, have identical terminal glycosylation. Even a single glycoprotein can have several carbohydrate chains that are identical.

The molecules we measure as carbohydrate tumour markers in immunological techniques are based on using an immobilized anti-carbohydrate capture antibody, and an anti-carbohydrate detecting antibody. From an antibody point of view the carbohydrate epitope has to fit the antigen binding site, whereas the protein linking the carbohydrates is present as a simple physical back-bone, no matter what its nature. A tumour cell can produce a variety of glycoproteins, having highly different physiological function, however, if they have two carbohydrate chains that fit the capture and detecting antibodies, they will be measured by a tumour marker assay.

## What happens to glycosylation in carcinomas?

It is well known that dramatic changes in glycosylation occur in almost every carcinoma. This has been reviewed elsewhere [2]. In brief, the main alterations can be summed up as either: (i) a block in biosynthesis of one of the pathways that glycosylation follows in normal cells – this block directs the biosynthesis in another direction, leading to overproduction of structures that are only produced in small amounts in normal cells; or (ii) opening up of new glycosylation pathways that lead to formation of new tumour-associated structures – structures that are not present in normal cells.

This regulation of pathways is directed by up- or down-regulation of glycosyltransferase activities. At a molecular level , several mechanisms can alter the activity of glyco syltransferases: gene-mutation/deletion; altered transcription-translation; inappropriate Golgi location; lack of nucleotide sugars, etc.

Examples of the tumour-associated glycosylation are plentiful. In colon carcinomas the relatively immature O-linked mucin type structures Tn and sialosyl-Tn are absent from normal mucosa but produced in large amounts in carcinomas [3]. The nature of this change is probably due to a reduced elongation of the carbohydrate chain. In the rectum, blood groups A and B are produced only in tumours, as an α1-2 fucosyltransferase (FT) is upregulated, and forms the necessary acceptor structure, see Fig. 3 and reference [4].

In bladder tumours, blood groups A and B antigens are lost due to a reduced activity of the ABO transferase [5].

### Why are carbohydrate structures good tumour markers for serological detection?

In summary, the following properties of carbohydrate structures makes them good candidates for serological detection:

(a) They are often highly polar due to the presence of sialic acid (NeuNAc) and readily enter the water-phase of the circulation. Most of the structures that are clinically useful are sialylated, like sialyl-Lewis a and x ($sLe^a$ and $SLe^x$) sialyl-Tn etc.

(b) They are produced in an increased amount in most malignant cells due to upregulation of glycosyltransferases. There is a steady production of structures, often with some back-up, as several glycosyltransferases seem to be able to synthesize similar structures although with different efficiency.

(c) They can, in most cases, be detected on several different proteins that originate from the tumour cell. This is of importance, as high grade tumours tend to acquire many genetic abnormalities, and a tumour marker based on a specific peptide sequence/structure could easily be lost in high grade tumours. Examples of this situation in this issue of *Glycoconjugate Journal* are α-1-acid glycoprotein and transferrin that alter their glycosylation in hepatic carcinomas.

(d) The structure does not have to be tumour specific. Although there is a change in glycosylation of a given cell compared to its normal counterparts, the structures formed are found in other cells as normal structures. Both $sLe^a$ and $sLe^x$ are present in normal epithelia, and leucocytes etc, however they occur in large concentrations in serum from many cancer patients. Other structures like sialyl-Tn antigens are not present in most normal tissues, but do have a widespread distribution in tissue like endothelium of small capillaries, in the gut wall [4].

(e) They are resistant to degradation. Most tumours contain necrotic areas with dead cells. Cells that are malnourished or dying often start to leak proteins into the surroundings. These proteins can be degraded by proteases, whereas the carbohydrates are more likely to be conserved for longer.

### The carbohydrate structures used for serological tumour detection in commercially available kits

Blood group related carbohydrates were originally discovered on red blood cells, however, they are present on the surface of most cells, and are, therefore, also termed histo-bloodgroup antigens [6]. The lacto-series structures can traditionally be divided into Type 1 and Type 2 chain structures on basis of their galactose to *N*-acetyl-glucosamine linkage, see Table 1. The mucin-type structures form a large group characterized by short O-linked structures. The reagents available to measure carbohydrate antigens are listed on the left in Table 1. It is obvious that several antibodies detect $sLe^a$ structures, fewer antibodies detecting $sLe^x$ structures. This is not logical, as the Lewis a ($Le^a$) structure is partly dependent on the genetic status of the individual. Five to twenty percent of the population, depending on race, are Le(a–b–) individuals, with mutations of the *Le* gene, whereas such mutations seem to be rare in the $Le^x$ gene-complex. Based on this fact, one would expect $Le^x$ to be a better, more generalized tumour marker than $Le^a$. However, tumours, even in Le(a–b–) individuals, seem to be able to synthesize $Le^a$ structures to some extent [7], which supports its use as a general tumour marker.

**Table 1.** Commercially available antibodies and their specificity.

| *Monoclonal antibody* | *Carbohydrate specificity* | *Name of structure* |
|---|---|---|
| *Type 1 chain* | | |
| CA 50 | Galβ1-3GlcNAc<br>$\alpha 2/3$<br>NeuNAc | Sialyl-Lacto-*N*-biose 1 |
| CA 50<br>CA 19-9<br>CA 242 | Galβ1-3GlcNAc<br>$\alpha 2/3$ $\alpha 1/4$<br>NeuNAc Fuc | Sialyl-$Le^a$ |
| *Type 2 chain* | | |
| CSLEX | Galβ1-4GlcNAc<br>$\alpha 2/3$ $\alpha 1/3$<br>NeuNAc Fuc | Sialyl-$Le^x$ |
| FH6 | Galβ1-4GlcNAcβ1-3-Galβ1-4GlcNAc<br>$\alpha 2/3$ $\alpha 1/3$ $\alpha/3$<br>NeuNAc Fuc Fuc | Sialyl-dimeric $Le^x$ |
| *Mucin-type* | | |
| TKH2<br>B72.3<br>CA72-4<br>JT10e | GalNacα1-*O*-Ser/Thr<br>$\alpha 2/6$<br>NeuNAc | Sialyl-Tn |
| PNA | Galβ1-3GalNAcα1-*O*-Ser/Thr | T antigen |
| CA549<br>CA15-3 | Peptide specific | |

Gal, galactose; GlcNAc, N acetylglucosamine; Fuc, fucose; GalNAc, *N* acetylgalactosamine; Ser, serine; Thr, threonine. For other abbreviations see text.

### Which carbohydrate tumour marker for which tumour?

Carbohydrate tumour markers have been investigated as serological tumour markers for a large range of malignant diseases. Some of these are summarized in Table 2. It is evident that both a high sensitivity and specificity are obtained only in pancreatic, gastric, colorectal, and ovarian carcinomas. Among these diseases we find reasonable positive and nega-

**Table 2.** Malignant diseases in which circulating carbohydrate antigens serve as tumour markers.

| *Malignant disease* | *Antibody/ Carbohydrate* | *Sensitivity %* | *Specificity %* | *+ Pred. value* | *– Pred. value* | *Correlations* | *Reference* |
|---|---|---|---|---|---|---|---|
| Bladder | CA 50/sLe$^a$, sLac | | | | | Invasion, stage, | [27, 28] |
| Brain | MA126/$G_{D2}$ | 91 | 91 | | | | [29] |
| Breast | CA-50 | 20% | 64% | | | | [30] |
| | CA-19.9/sLe$^a$ | 17% | 78% | | | | [30] |
| Colorectal | CA 242/sLe$^a$ | 100% | 64% | 67% | 100% | Mortality 1st | [8] |
| | CA 50 | | | | | year stage | [9] |
| | CA 72.3/sTn | 41% | 98% | | | | [11] |
| Head and neck | CA-50/sLe$^a$ | 31–36% | 64–95% | | | | [31] |
| | CA 19.9/sLe$^a$ | 31% | 78% | | | | [31] |
| Gastric | CA 72-4/sTn | 94% | 95% | | | Stage, serosa infiltration | [32] |
| | CA 19-9/sLe$^a$ | 41% | 95% | | | | |
| | CA 50/sLe$^a$ | 71% | 46% | | | | [30] |
| Leukaemia | FH6/sLe$^x$ | | | | | Acute leukaemia, not in remission | [33] |
| Lung | | | | | | | |
| Small cell | 1D7/FucGM1 | | | | | Stage, progression CEA, therapy resistance | [34] |
| Non-small cell | CA 242/sLe$^a$ | 29% | 96% | 71% | 78% | | |
| | CA-50sLe$^a$, sLac | 44% | 67% | | | | |
| Mix | CA-19.9sLe$^a$ | 41% | 87% | | | | [35] |
| Ovary | TKH2/sTn | 50% | 93.5% | 72.2% | 84.7% | Present in all | [36] |
| | CA 19-9/sLe$^a$ | | | | | Mucinous adenocar- | [37] |
| | CA 72-4/sTn | 63% | 91% | | | cinoma | [38] |
| Pancreas | CA 50/sLe$^a$, sLac | 70–96% | 34–90% | 26–94% | 86–99% | Cholestasis in | [12, 17, 39, 40] |
| | CA 242/sLe$^a$ | 62–94% | 65–95% | 40–76% | 61–96% | benign dis. | |
| | CA 19-9/sLe$^a$ | 81–84% | 79–98% | 99% | 77% | | [40, 41] |
| | CA 195/Le$^a$, sLe$^a$ | 86% | 92% | 94% | 76% | | [40] |
| Prostate | CA-50/sLe$^a$ | 13% | 64% | | | | [30] |
| | CA-19.9/sLe$^a$ | 50% | 78% | | | | |
| Uterus | CA-50/sLe$^a$ | 30% | 64% | | | | [30] |
| | CA-19.9/sLe$^a$ | 30% | 77.5% | | | | [30] |

tive predictive values. In other diseases, the sensitivity is low, whereas the specificity is high. This is due to the selection of the cut off-point, above which a sample is regarded as indicative of the presence of a tumour. This requires construction of ROC (receiver operating characteristics) curves, which, unfortunately, has only been done for a limited number of markers. In most cases, the cut off-point is selected based on the average amount of marker present in serum of a large group of normal individuals, plus twice the SD.

When a marker has a high specificity it is reasonable to use the marker as an indicator of a malignant disease. This has a clinical value in the follow-up of patients after primary therapy, in which case a positive lead time can be obtained from the marker compared to the clinical symptoms, and appropriate further investigations (X-ray, scanning, endoscopy, etc.) can be initiated. It should be stressed that these markers are not specific for a certain tumour location, and hence must be considered as generalized tumour markers.

The use of these markers for screening purposes, in populations where the prevalence of disease is low, should be avoided, due to the large number of false positives which can be obtained.

### Markers for colorectal carcinomas

Both the $sLe^a$ and $sLe^x$, as well as the mucin type sialyl-Tn, seem to be good markers for colorectal carcinoma. In one prospective study [8] of 33 patients, it was found that the specificity for CA 242 ($sLe^a$) was 100%, the positive predictive value was 100%, and the negative predictive value was 60%. It was stated that a tumour response was never seen in the absence of falling marker levels, and similarly, rising marker levels invariably indicated tumour progression within 16 weeks [8]. In another paper [9], the preoperative level of CA-50 ($sLe^a$ and sialyl Lac) strongly predicted the cancer-specific mortality within the first year after surgery, and less strongly during the second year. Similar results have been obtained by others [10], who found that CA-50 provided a preoperative predictive value, that was not given by the Duke's staging system.

The sialyl-Tn epitope (CA 72.3) was found to supplement CEA, as 49% of CEA negative patients scored positive with TAG-72 [11]. In 60% of patients in which significant changes of CEA levels could not be detected, TAG-72 showed rising positive levels prior to clinical evidence of recurrent disease [11].

### Markers for pancreatic carcinomas

In benign pancreatic diseases there exists a relationship between the amount of CA 50 or CA 242 ($sLe^a$) in serum and cholestasis e.g. as measured by serum bilirubin or alkaline phosphatase (12, 13). This is supposed to be due to the fact that these glycoconjugates, like CEA, are produced in the hepatic bile ducts, pancreatic ducts and pancreatic centroacinar cells [14–16].

Obstruction of the bile outflow could, at least theoretically, lead to the passage of the glycoconjugates from bile to the blood stream together with bilirubin and alkaline phosphatase. This is supported by the fact that decompression of the bile duct in benign diseases leads to normalized levels of CA 50 and CA 19-9 in serum [12].

The combination of serum carbohydrate tumour markers with other serological markers like CEA and tissue polypeptide antigen; and with other procedures like ultrasound, computed tomography and endoscopic retrograde cholangiopancreatography has improved the specificity of CA-50 for the detection of pancreatic cancer. However, the sensitivity is reduced if the requirement is that both markers should be positive. If just one of a panel of markers should be positive the sensitivity is increased, with a small loss of specificity. An efficiency of more than 80% is possible on the basis of serum carbohydrate assays, and an efficiency of more than 92% is found when serum markers and imaging procedures are combined [17, 18].

The serum level of carbohydrates with $\alpha$1-4 fucosylation (Le structures) seems to be related to the Lewis genotype in normal individuals. Individuals with the Le(a–b–) genotype have lower levels of $Le^a$ based structures (CA19-9, CA50, CA242, Span-1) in several publications [19–21]. It is likely, however, that these structures are present in those individuals, probably due to overlapping specificity of the many different FTs. The Le structures may be primarily dependent upon the Le transferase activity in a particular tissue (e.g. endothelium), and, therefore, knowledge of these structures on red blood cells does not necessarily provide information on the activity of FTs in other tissues. Furthermore, the DNA mutations observed in Le(a–b–) individuals vary, and lead to enzymes with different characteristics, some with a low, but readily detectable activity. Several publications have focused on the tissue-specific expression of FTs [7, 22, 23], and the very high levels of $Le^a$ based structures which can be found in the serum of some Le(a–b–) patients indicate that (i) the Le transferase can be upregulated; or, that (ii) other FTs with some $\alpha$1-4 activity are switched on in carcinomas.

In patients with pancreatic cancer and no increase in tumour markers (false negative), the Le negative phenotype is approximately three times more frequent than in the background population [9].

The $Le^x$ structures are not dependent on the Le status, and the $Le^x$ negative phenotype is very rare. Based on this it might be more logical to measure $sLe^x$ in serum from pancreatic patients, however, this remains unproven.

### What is the molecular mechanism behind the shedding of carbohydrate structures from tumours?

It is interesting to note, that in one study [24] of the correlation between concentrations of $sLe^x$ and $sLe^a$ in tissue extracts, and the serum levels of these antigens, no correlation could be found. This indicates that it is not the total amount present in a tumour that determines the serum level, but other mechanisms like location in tumour cells, shedding from tumour cells, the number of invasive cells and tumour vascularization. Further studies are needed to define these mechanisms.

### Do the carbohydrate structures shed by tumour cells serve a function?

When a normal cell is forced through the steps that lead to malignant transformation, be it activation of protooncogenes, and/or deletions or mutations in tumour suppressor genes, several cellular properties are altered. These alterations vary from an increased rate of cell division to the ability to invade. We have information about a few of these alterations at the molecular level, like the presence of an increased number of proteins that stimulate cell division, ranging from membrane receptors to nuclear peptides. However, many of the properties of malignant cells are not well understood: why for example

do they escape the immune system? How do they adapt to the different microenvironments that exist in the primary tumour e.g. in the colon, or to the environment in a lymph node or liver sinusoid? Are some of these unresolved problems related to the glycosylation of the cellular proteins; highly dynamic and diversified carbohydrate structures that can add or remove recognition sites, adherence sites, alter intracellular trafficking pathways, change receptor function etc? Our basic knowledge is mainly structural, a large number of glycoforms of various proteins and lipids have been structurally characterized, but these still await the assignment of biological functions. This is similar to the situation in which many proteins have been sequenced and cloned, but still have an unknown cellular function.

## Future perspectives in the clinical use of tumour-associated glycosylation

Several carbohydrate antigens that are present on tumour cells have been used as targets for scanning procedures. Labelled antibodies against Sialyl-Tn have been injected intraperitoneally for the detection of ovarian carcinoma, or carcinoma of the peritoneum [25]. The same antibodies have been injected intravenously, for intra-operative scanning of tissues and lymphnodes, in patients with primary and metastatic large bowel cancer [26]. These are just a few examples of potential procedures for the staging and localization of tumours and their metastases.

The new molecular biological techniques for the examination of glycosyltransferase genes and their mRNA seem very promising. With these techniques, basic information regarding the level of gene transcription can be obtained, and an accurate identification of mutations or loss of heterozygocity of glycosyltransferase genes can be obtained.

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