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Sialic acid as a potential biomarker for cardiovascular disease, diabetes and cancer

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Abstract

Cardiovascular disease (CVD), diabetes and cancer pose increasing global healthcare burdens. New biomarkers could enable earlier diagnosis of these diseases, leading to more effective treatment and lower associated healthcare burden. Elevated total sialic acid (TSA) concentration in plasma and serum has been positively correlated with cardiovascular conditions diabetes andthe development of malignant tumours. This article reviews the use of TSA as a potential biomarker in these disease and makes a comparison with existing markers. Elevated TSA has been shown to be indicative of the pathogenesis of CVD, diabetes and malignant tumours. While not a specific marker for one disease there is promise in utilising TSA as a method for monitoring disease progression and effectiveness of treatment programs.

1. Introduction

Cardiovascular disease (CVD), diabetes and cancer represent a global burden on healthcare systems with risk factors such as obesity rising in recent years. This has resulted in increased healthcare costs and rising mortality rates, with a combined 20.1 million deaths worldwide in 2019 resulting from CVD and diabetes, representing 34% of all deaths. Cancer was the cause of 10.8 million (17%) deaths in 2019.[1] These healthcare challenges affect both economically developed and developing nations and can be attributed to an increase in access to unhealthy foods, tobacco products and increasingly sedentary lifestyles.[2] To combat this problem and reduce mortality rates and global healthcare burden, early diagnosis is of paramount importance to allow for earlier intervention and treatment. Biomarkers play a key role in this, as they provide measurable characteristics that can be indicative of the presence or progression of a disease. Biomarkers for many diseases have been identified already: troponin for myocardial infarction,[3] serum cholesterol[4] and blood pressure for cardiovascular disease,[5] and carcinoembryonic antigen (CEA) for certain cancers.[6] These markers, when combined with other predictive measures such as risk calculation algorithms, for example QRISK3, and frequent physical examinations, can provide robust methods for the diagnosis of diseases and health conditions. This review aims to critically appraise the value of sialic acid as a biomarker, reviewing research into different CVDs, diabetes, diabetic complications and cancers in different biological fluids offering insight into the potential for sialic acid to act as a marker in different situations and offer potential new perspectives on future research into this developing area.

Sialic acid has long been shown to be a marker for the presence and pathogensis of cardiovascular disease. It is of interest due to the relative unreliability of current cardiovascular biomarkers. For example, cholesterol and blood pressure, and other markers for CVD, can be affected by external factors: food and alcohol consumption, tobacco usage and stress. Identifying and validating more accurate biomarkers that are less affected by external factors is crucial in aiding early diagnosis and prevention of these diseases. Further to this, diabetes, which is closely linked to CVD acts as a main risk factor for the development of CVD. The increased inflammation that is caused by diabetes could also benefit from usage of sialic acid as a marker for better early detection of diabetes and greater

prevention of related complications that can have a great impact on those suffering from diabetes.

New markers for cancer would also be extremely useful. General markers for cancer indicate the presence of malignancies, however there is greater scope to utilise biomarkers for the differentiation of different stages of cancer, and to track the progress and success of clinical cancer therapies. This could in turn offer significant positive impact on patients, as most markers are currently used to detect and confirm suspected cases of cancer. This means that replacing these, or combining them with a marker adequate for screening for cancers before symptoms are present, could allow for much earlier detection and treatment, leading to increased survival rates. There is also an incentive to remove the need for more invasive diagnostic tests such as biopsies, and replace these with minimally invasive tests such as blood tests (via finger prick to minimise invasiveness as much as possible), or in the case of oral cancer, collection of a saliva sample which takes only minutes and is non-invasive.

1.1 Glycosylation of cells and proteins

Glycosylation is integral to the function of healthy cells and proteins, aiding in immune and inflammatory response, and regulating apoptosis and cell interactions.[7] It is the process whereby glycans (chains of sugars) are attached to the surfaces of cells and excreted proteins. Glycosylation changes, whether this is changes in whole glycans or just some sugar units, have long been observed in the pathogenesis of many health conditions such as inflammatory bowel disease and diabetes. *N*-Glycans decorate the surfaces of cells and proteins and are generally found as one of three main types: oligomannose, complex and hybrid (Figure 1).[8] *N*-Glycans exhibit a common ‘core’ consisting of *N*-acetylglucosamine (one of which can be fucosylated) and mannose. Oligomannose glycans exhibit only mannose attached to the common core. Complex glycans have ‘antennae’ attached to the core. Hybrid glycans contain both a mannose ‘antennae’ and complex glycan ‘antennae’. The antennae can be capped with *N*-acetylneuraminic acid (Figure 2), also known as sialic acid.

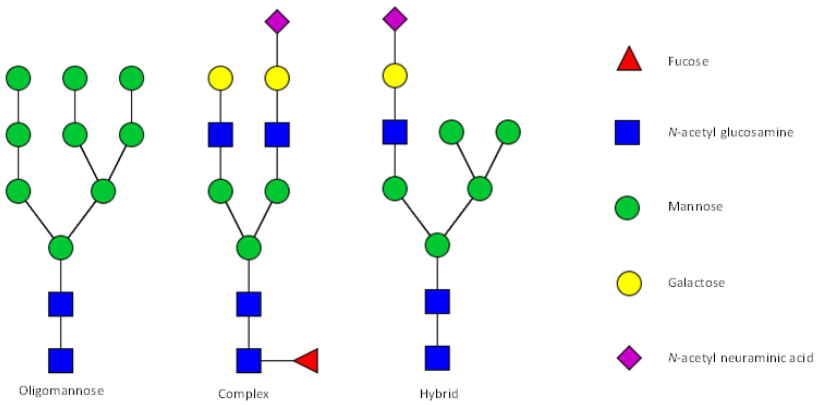

Figure 1: Three types of *N*-glycan: oligomannose, complex and hybrid

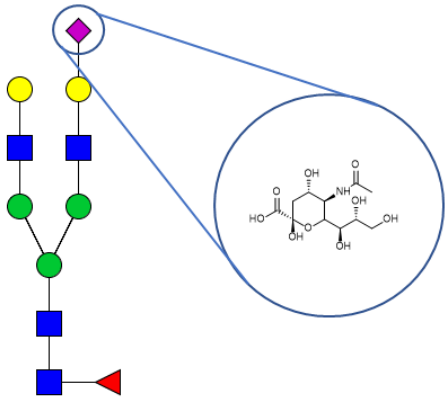

Figure 2: *N*-acetylneuraminic acid (sialic acid) and its position as part of a complex glycan

1.2 Sialic acid

Sialic acid (*N*-acetylneuraminic acid) is a nine-carbon backbone monosaccharide containing a carboxylic acid functional group and is generally found as the terminal unit on glycans which form parts of glycoconjugates such as glycoproteins. Sialic acid can also be found elsewhere: as internal residues in glycans, as part of polysialic acid chains, as part of gangliosides (more commonly referred to as lipid-bound sialic acid (LSA) (Figure 3) and a small quantity can be found as free (unbound) sialic acid in bodily fluids such as plasma, serum, urine and saliva. It plays many important biological roles: mediating cell interactions, modulating immune response and preventing aggregation of cells in blood vessels by providing an overall negative charge to the endothelium.[9] Importantly, sialic acid and some acetylated derivatives have been shown to be overexpressed in the endothelium under the influence of inflammation, although it is not fully understood why this is the case. Cancers overexpress sialic acid on the cell surface, which masks cell surface tumour antigens minimising attack by the immune system, hence allowing for further cancer cell proliferation.[9]

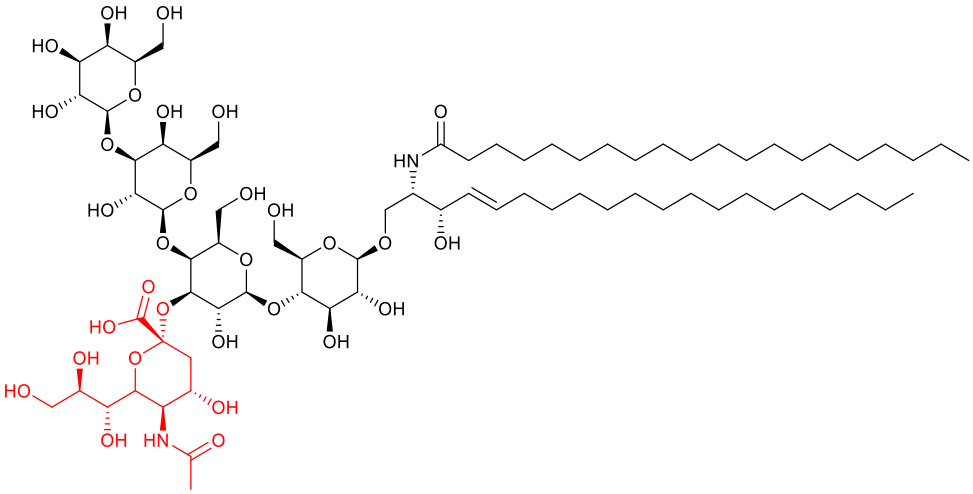

Figure 3: GM1 ganglioside, *N*-acetylneuraminic acid highlighted in red

1.2 Quantification of sialic acid

Measuring sialic acid concentration in biological fluids to determine its effectiveness as a biomarker requires utilising assays that allow for quantification of sialic acid in the sample to be analysed. This can be performed using a variety of assays, falling into four main categories: colorimetric, fluorometric, enzymatic and chromatographic. [10] Some of the most common assays are: thiobarbituric acid assay[11], orcinol/resorcinol assays [12,13] (colorimetric), 3,5-diaminobenzoic acid [14] (fluorometric), pyruvate release *via* sialic acid aldolase [15] (enzymatic) and labelling of sialic acid with 1,2-Diamino-4,5-methyleneoxybenzene [16] (DMB) followed by HPLC/UPLC analysis (chromatographic).

Each assay has its advantages and disadvantages. Colorimetric and fluorometric assays are cheap and quick to perform but suffer from many issues with interference and high limits of detection. This is where compounds other than the target compound (in this case sialic acid) are affected by the assay and can have a negative effect on the quantitation. On the other hand, enzymatic and chromatographic assays are more cumbersome and expensive to perform requiring specialist instruments which not every laboratory will have access to. These assays avoid the issues present with colorimetric/fluorometric methods and have much lower limits of detection. With such differences present between assays and such a variety of assays available there can be a lot of variation in the values for sialic acid obtained between studies, this is especially obvious when comparing values for different cohorts of healthy controls who should have similar sialic acid concentrations (Tables 2-13). Some of these variations may also be down to differences in sialic acid between different geographic populations or ethnicities due to environmental or genetic factors. One such study highlights the difference

in sialic acid concentration between a Unites States based population cohort and a Japanese based population cohort. [17]

2. Sialic acid concentration in serum and plasma and its association with cardiovascular disease (CVD) and markers of CVD

Total sialic acid (TSA) concentration in plasma has been associated with general mortality from all CVD causes,[18] and specifically from coronary heart disease (CHD) and stroke (Table 1).[19] These long term observational studies took data from 54, 385 volunteers over a period of 21 years, and followed mortality from all causes with a focus on investigating the relationship between plasma sialic acid concentrations and those that were CVD related. Participants were assigned to one of four quartiles based on sialic acid concentration. The study showed a relative risk of 2.62 in women and 2.38 in men for those with elevated sialic acid in the highest quartile compared with the lowest, potentially indicative that sialic acid concentration correlates with CVD risk. Further analysis showed the relative risk of mortality from CHD in populations with the highest sialic acid levels compared to the lowest was 1.76 in men and 1.98 in women, much less than for general CVD risk. For stroke the relative risks were 1.62 and 1.68 for men and women respectively. This indicates that sialic acid is a possible predictor of mortality from CVD, CHD and stroke given that the relative risk for mortality from these conditions was significantly higher in volunteers with elevated sialic acid (lowest versus highest quartile).

		Relative Risk
Overall CVD	Women	2.62 (1.93; 3.57)
	Men	2.38 (2.01; 2.83)
CHD	Women	1.94 (1.61; 2.34)
	Men	1.76 (1.58; 1.96)
Stroke	Women	1.68 (1.28; 2.21)
	Men	1.62 (1.26; 2.09)

Table 1: Age-adjusted relative risk for CVD, CHD and stroke in (27065 men and 28037) (comparing lowest and highest quartiles of sialic acid concentration in plasma). Data is presented as 95% confidence interval (95% CI)

2.1 Atherosclerosis

Atherosclerosis is the build-up of plaques on the arterial wall. These plaques are made up of deposits of fat, cholesterol, calcium and other blood constituents. These form after damage to the interior arterial wall. Serum sialic acid levels have been shown to be elevated in those

with atherosclerosis versus those without.[20] TSA has also been correlated with risk factors of CVD, one such study in patients on haemodialysis indicated that sialic acid was positively correlated with high sensitivity C-reactive protein (hs-CRP), lipoprotein (a) and carotid intima media wall thickness (CIMT).[21] A further study also suggested a link between sialic acid and CIMT, as well as other factors: low density lipoprotein (LDL), hs-CRP and uric acid.[22]

Other studies probing sialic acid as a marker for CVD have shown a good correlation between controls and disease cohorts with different levels of vessel disease, indicating greater severity of atherosclerosis.[23,24] Patients with 1, 2 or 3 vessel disease (VD) had higher levels of serum TSA than controls. The patients with 2 or 3 VD showed significantly raised TSA levels over those with 1 VD (Table 2). This gives good grounds to claim that TSA is an indicator of the progression of coronary artery disease (CAD) and atherosclerosis. These findings are backed up by data that appears to indicate changes in sialic acid concentration in controls who have CAD and are not undergoing treatment versus those who are undergoing treatment. TSA concentration is significantly lower and negatively correlates between those in treatment versus those who are not, showing that TSA is a good indicator of disease progression.[25] One group of authors report that there is no discernable difference in TSA levels between healthy controls and those with VD. [26] The authors called into question values provided by other studies based on the analytical methods for quantifying sialic acid. Colorimetric methods are relatively inaccurate for sialic acid quantitation [10], where the authors make the correct assertion that their enzymatic method is most likely more accurate. The papers discussed above also utilise enzymatic methods for the quantitfication of sialic acid and all agree that sialic acid correlates with presence of CAD and atherosclerosis.

Number of participants (N)	Control (mg/dL)	1VD (mg/dL)	2VD (mg/dL)	3VD (mg/dL)	p-value	Ref
90	65.4 (61.7; 69.1)	72.5 (68.0; 77.0)	81.0 (77.7; 84.3)	N/A	Control vs 1VD: 0.05 Control vs 2VD: 0.001	[23]
180	51.0 (49.2; 52.8)	106 (102.7; 109.3)	138.3 (133.9; 142.7)	169.9 (165.5; 174.3)	All groups vs control: 0.001	[24]

Table 2: Serum sialic acid concentration comparison between controls and patients with different severity of coronary artery disease. Data is presented as 95% confidence interval (95% CI)

2.2 Coronary Heart Disease (CHD)

Coronary Heart Disease is a condition where blood flow to the heart is decreased due to narrowing of the arteries from conditions such as atherosclerosis. Studies have focussed on CHD and sialic acid as a marker for this. One such study by Knuiman et al. showed that in patients with CHD versus controls without CHD there were elevated levels of sialic acid in serum.[27] The study also investigated CVD mortality and found that a 25 mg/dL increase in serum sialic acid correlated with an overall relative risk of CHD mortality of 1.40 in women and 1.06 in men.

Lifestyle and biological risk markers have also been correlated with sialic acid and CHD in a study performed by Lindberg et al. in 1993. [28] Tobacco consumption and alipoprotein B were positively correlated with sialic acid concentration. Leisure time physical activity was also negatively correlated. However, no correlation was found between sialic acid concentration and alcohol, alipoprotein A1, lipoprotein(a) and diastolic blood pressure. The non-significant correlation between alcohol consumption and sialic acid has been contradicted by many other studies.[29–31] These studies indicate that alcohol consumption was associated with raised levels of serum TSA. It was also observed that the quantity of alcohol consumed affected sialic acid concentrations. When more alcohol was consumed this resulted in greater increases in TSA concentration. This was further proven by showing that after no consumption of alcohol for a period of one to two weeks sialic acid levels returned to normal levels.

2.3 Other Cardiovascular Conditions

Chronic heart failure (CHF) is a condition which limits the amount of blood that the heart can pump, resulting in symptoms such as breathlessness, fatigue and fluid retention. This condition has an extremely high morbidity rate. One of the major causes is CAD, it can also be caused by other conditions however, such as hypertension. Three studies undertaken in recent years have outlined how sialic acid is significantly elevated in serum in patients with CHF as compared to a control group (Table 3). [27,32,33] giving a good indication that serum TSA could be utilised as a marker for the presence of this condition.

Number of participants (N)	Control (mg/dL)	Disease (mg/dL)	p-value	Ref
78	72.0 (56.55; 87.45)	173.0 (107.5; 238.6)	0.001	[32]
69	80.34 (75.1; 85.6)	95.2 (89.9; 100.5)	0.001	[33]

Table 3: Serum sialic acid concentration for CHF, patients vs healthy controls. Data is presented as 95% confidence interval (95% CI)

Myocardial infarction, more commonly known as heart attack, is a serious medical condition where blood flow to the heart is suddenly stopped. This is usually because of a blood clot or in some cases a piece of atherosclerotic plaque that has broken off from the arterial wall. Elevated serum TSA has been shown to correspond with heart attacks and elevations in levels of acute-phase proteins (antitypsin) in serum. TSA and lipid bound sialic acid were found to be elevated in patients 24 hours post-heart attack.[34] Findings from a second study also showed an increase in TSA in those suffering from myocardial infarction as well as a correlation between sialic acid and acute phase proteins commonly associated with infarction.[35] These studies show that TSA is related to acute phase proteins, both in myocardial infarction patients and healthy controls. Further to this, TSA levels have been shown to increase up to 3 days after a heart attack (Table 4).[36] Levels increase day on day for 3 days post infarction and then drop slightly, but do not return to pre-infarction levels after this time point.

Number of participants (N)	Control (mg/dL)	24 hr PI (mg/dL)	48 hr PI (mg/dL)	72 hr PI (mg/dL)	p-value	Ref
70	54.3 (48.5; 60.0)	67.0 (57.7; 76.4)	N/A	N/A	N/A	[34]
66	51.7 (45.4; 57.9)	63.4 (48.9; 57.9)	74.6 (59.8; 89.3)	86.1 (79.9; 92.4)	0.001	[36]

Table 4: Serum sialic acid concentration comparison between controls and patients at different time points post infarction. Data is presented as 95% confidence interval (95% CI)

Hypertension is a condition where arterial blood pressure is elevated. Hypertension has been associated with elevated serum TSA, as has prehypertension[37] (where blood pressure is

above normal, but not enough to diagnose high blood pressure). Both hypertension and prehypertension groups showed significantly higher TSA than the control group. An elevation in TSA was also seen between prehypertension and hypertension groups. This indicates that elevated serum TSA is a marker for the presence of hypertension and high blood pressure in general, possibly acting as a good diagnostic marker to differentiate between the two conditions.

Other studies have been carried out to assess the general CVD risk relationship to TSA in various conditions such as periodontitis[38] and menopause.[39] Periodontitis is a risk factor of CVD and menopause has previously been shown to increase the risk of developing CVD. More research is required into these areas as there has only been one study into each of these, with SA being linked to CVD in periodontitis but not being linked to menopause (no increase in post-menopausal women versus pre-menopausal women).

3. Sialic acid concentration and its association with type 1 and type 2 diabetes and diabetes complications

Diabetes is a disease associated with a lack of ability by the body to modulate blood glucose levels. Diabetes can be categorised as type 1 or type 2. Type 1 is an autoimmune condition where the immune system attacks the beta cells of the pancreatic islets, causing reduction in the ability to produce insulin. Type 2 is caused by either the lack of ability to produce insulin, or the insulin that is produced being ineffective at modulating glucose.

3.1 Type 1 and 2 diabetes

Type 1 and 2 diabetes have been extensively studied to determine if sialic acid is a biomarker for these diseases. Good correlation was shown between type 2 diabetes and sialic acid with multiple authors reporting raised levels of serum sialic acid in type 2 diabetes patients as compared to healthy controls (Table 6) (Figure 4).[40–48] However, this was not found to be the case for type 1 diabetes, where no significant correlation was found between diabetic patients and healthy controls (Table 7) (Figure 4).[40,49–51] It is not currently well understood as to why this is observed. More research is required in this area to determine the source of this discrepancy between type 1 and 2 diabetes in relation to elevated sialic acid levels.

Number of participants (N)	Control (mg/dL)	Type 2 (mg/dL)	p-value	Ref
40	61.2 (52.1; 70.3)	71.70 (66.1; 77.3)	0.001	[40]
43	60.0	69.0	0.05	[41]
50	53.2 (51.9; 54.5)	64.4 (62.9; 65.9)	N/A	[42]
100	56.6 (56.0; 57.2)	55.0 (54.2; 55.8)	0.001	[43]
160	61.0 (59.0; 63.0)	70.7 (68.9; 72.5)	N/A	[44]
40	60.0 (50.4; 69.6)	74.0 (69.2; 78.8)	0.02	[45]
420	52.2 (50.9; 53.5)	68.0 (66.4; 69.6)	N/A	[47]
90	44.5 (40.6; 48.4)	62.4 (60.1; 64.7)	N/A	[48]

Table 6: Serum sialic acid concentration in type 2 diabetes patients and controls. Data is presented as 95% confidence interval (95% CI)

Number of participants (N)	Control (mg/dL)	Type 1 (mg/dL)	p-value	Ref
40	61.2 (52.1; 70.3)	61.8 (56.8; 66.8)	NS	[40]
300	66.4	67.0	NS	[49]
44	106.2 (95.4; 117)	119.5 (108.1; 130.9)	NS	[50]
42	67.0 (62.4; 71.6)	68.9 (64.8; 73.0)	NS	[51]

Table 7: Sialic acid concentration in type 1 diabetes patients and controls. Data is presented as 95% confidence interval (95% CI)

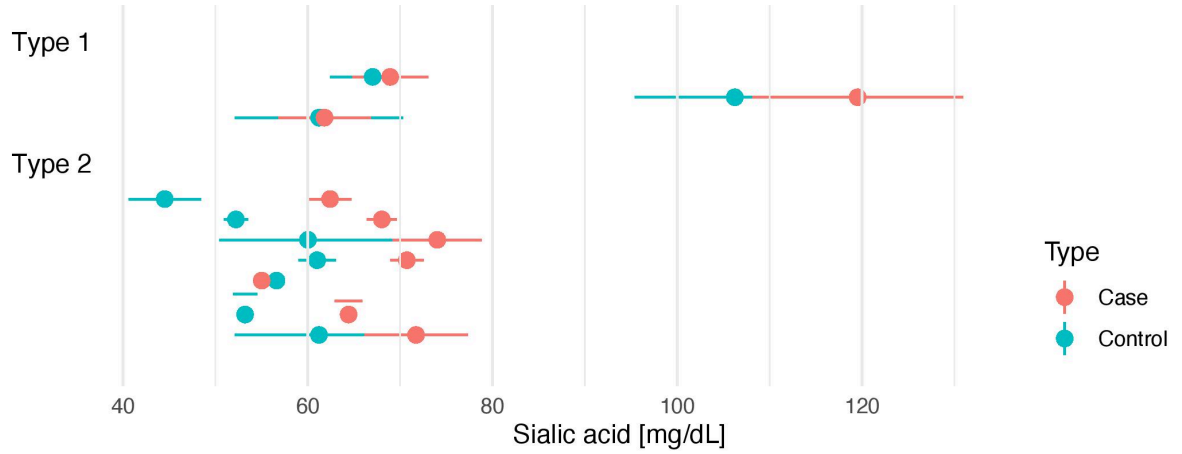

Figure 4: Forest Plot highlighting the difference in sialic acid concentration (mg/dL) in serum between healthy controls versus diabetic patients (type 1 and type 2).

3.2 **Type 1 and 2 diabetes complications: Neuropathy, nephropathy and retinopathy**

Poorly controlled blood sugar levels can lead to capillary damage which can cause further damage to nerves which results in neuropathy in the extremities leading to degeneration of the nerves leading to pain, tingling and burning symptoms. It also leads to nephropathy from damage to capillaries in the kidneys affecting filtration of blood, eventually culminating in albuminuria. Finally, it can cause retinopathy where the eyes become damaged due to this capillary damage eventually leading to macular degeneration and possible blindness.

Neuropathy, nephropathy and retinopathy have all been associated with elevated serum sialic acid levels. Patients suffering from these diabetic complications and type 2 diabetes are shown to have raised sialic acid levels over those with just type 2 diabetes and even further over healthy controls (Tables 8-10).[42,43,48,52] This reinforces the hypothesis that type 2 diabetes is associated with serum sialic acid concentration and further to this, it indicates that these diabetic complications are also associated with a further rise in sialic acid levels.

Number of participants (N)	Control (mg/dL)	Neuropathy (mg/dL)	Disease + Complications (mg/dL)	p-value	Ref
75	53.2 (51.9; 54.5)	64.4 (62.9; 65.9)	73.9 (72.2; 75.6)	N/A	[42]
363		53.3 (50.4; 56.2)	56.7 (50.1; 63.3)	0.92	[52]

Table 8: Serum sialic acid concentrations in patients with diabetes, and those with diabetic neuropathy versus healthy controls. Data is presented as 95% confidence interval (95% CI)

Number of participants (N)	Control (mg/dL)	Nephropathy (mg/dL)	Disease + Complications (mg/dL)	p-value	Ref
200	56.6 (56.0; 57.2)	55.1 (54.3; 55.9)	85.1 (84.4; 85.8)	0.001	[43]
363		53.3 (47.6; 59.0)	62.5 (59.0; 66.0)	0.006	[52]

Table 9: Serum sialic acid concentrations in patients with diabetes, and those with diabetic nephropathy versus healthy controls. Data is presented as 95% confidence interval (95% CI)

Number of participants (N)	Control (mg/dL)	Retinopathy (mg/dL)	Disease + Complications (mg/dL)	p-value	Ref
200	56.6 56.0; 57.2)	55.1 (54.3; 55.9)	75.1 (74.4; 75.8)	0.001	[43]
90	44.5 (40.6; 48.4)	62.4 (59.2; 65.36)	80.0 (74.8; 85.2)	N/A	[48]
363		46.8 (41.1; 52.2)	57.8 (54.6; 61.2)	0.002	[52]

Table 10: Serum sialic acid concentrations in patients with diabetes, and those with diabetic retinopathy versus healthy controls. Data is presented as 95% confidence interval (95% CI)

Albuminuria is a condition that follows damage to the kidneys and is indicated by the excretion of excess albumin into the urine via the kidneys. It is a complication of diabetes, caused by nephropathy and other such damage to the kidneys. The disease contains three stages: normoalbuminuria, microalbuminuria and clinical proteinuria which are characterised by the increasing levels of excretion of albumin. This condition has been studied to determine whether sialic acid concentration is an indicator of its presence in diabetic patients. Serum sialic acid concentration has been measured in patients with diabetes and in those suffering from various degrees of albuminuria (Table 11).[53,54] On average, type 2 diabetes patients without complications showed reduced serum sialic acid levels over those with complications. Measurements were also compared between different severities of albuminuria with increasing severity showing increased serum sialic acid concentrations indicating that this serves as a potential marker for the presence of these complications and their severity.

Number of participants (N)	Control (mg/dL)	Complications (mg/dL)	p-value	Ref
106	54.4 (51.7; 57.1)	Microalbuminuria: 59.6 (57.1; 62.1) Clinical Proteinuria 72.3 (69.6; 75.0)	0.01 0.001	[53]
69	49.4 (48.2; 50.6)	Normoalbuminaria: 58.7 (52.9; 63.3) Microalbuminuria: 61.8 (56.6; 67.0)	N/A	[54]

Table 11: Serum sialic acid concentrations in patients with diabetes, and those with varying stages of albumninuria. Data is presented as 95% confidence interval (95% CI)

The onset of these complications has also been shown to be predicted by elevated sialic acid levels. Elevated levels of sialic acid were shown to occur 3 years before diagnosis in patients with type 2 diabetes.[55]

4. Sialic acid concentration and its relation to various cancers, cancer stages and classifications

Cancer encompasses a number of diseases involving abnormal cell growth which can invade organs and spread to other parts of the body. Cancerous cells can destroy surrounding healthy tissues leading to an array of symptoms depending on the type of cancer, with some cancers presenting with more easily identifiable symptoms than others. Cancer has a high mortality rate, with 18.1 million new cases in 2018 and 9.6 million deaths[56], some cancers

are more fatal than others however. For example, brain cancer has a five-year survival rate of 12.2%, oral cancer has a five-year survival rate is 50% whereas testicular cancer has a five-year surivival rate of 95.3%.[57] This is due to a number of factors including the ability to detect the disease sufficiently early, and the ability to treat the disease effectively. It has been shown in general studies covering different types of malignancies that sialic acid could serve as a good indicator and marker for the presence of these malignancies.[58] Sialic acid has been identified as a potential maker for different types of cancer with multiple studies across different disciplines showing increased sialic acid levels in patients when compared to healthy controls (Table 12) (Figure 5). Changes in sialylation levels have also been shown in siglecs [59] and other receptors such as epidermal growth factor receptor (EGFR) [60–62] in lung and colorectal cancers.

Number of participants (N)	Cancer type	Control (mg/dL)	Disease (mg/dL)	p-value	Ref
107	Lung	LSA: 17.2 (12.7; 21.7)	LSA: 32.4 (16.0; 48.8)	N/A	[66]
58	Bladder	LSA: 12.7 (12.2; 13.2)	LSA: 21.3 (21.2; 21.4)	0.05	[70]
118	Bladder	LSA: 20.3 (14.5; 26.1)	LSA: 28.7 (22.4; 38.9)	0.001	[71]
		PSA: 5.6 (4.4; 6.7)	PSA: 7.2 (5.9; 8.5)		
91	Cervix	TSA: 60.0 (56.9; 63.1)	TSA: 116 (114.8; 117.2)	< 0.001	[75]
67	Breast	TSA: 83.6	TSA: 108	< 0.01	[78]
540	Prostate	TSA: 54.1 (45.1; 63.1)	TSA: 56.8 (45.3; 68.3)	0.013	[82]
56	Prostate	TSA: 74.6 (61.8; 87.4)	TSA: 80.5 (56.1; 104.9)	0.0036	[83]
107	Colorectal	TSA: 49.0 (41.1; 56.9)	TSA: 71.3 (54.4; 88.2)	<0.0001	[84]
111	Colorectal	TSA: 66.8 (53.1; 80.5)	TSA: 114.1 (77.3; 150.9)	<0.0001	[85]
		BSA: 65.9 (52.3; 79.5)	BSA: 112.9 (76.1; 149.7)	<0.0001	
242	Colorectal	TSA: 69.2 (59.9; 76.5)	TSA: 76.1 (60.2 ; 92.0)	< 0.001	[86]
60	Oral	TSA: 29.0 (28.2; 29.8)	TSA: 45.3 (43.8; 46.8)	0.05	[93]
		LSA: 16.7 (16.1; 17.4)	LSA: 23.0 (22.1; 23.9)		

Table 12: Comparison of sialic acid levels between cancer patients and healthy controls in different types of cancer. Data is presented as 95% confidence interval (95% CI)

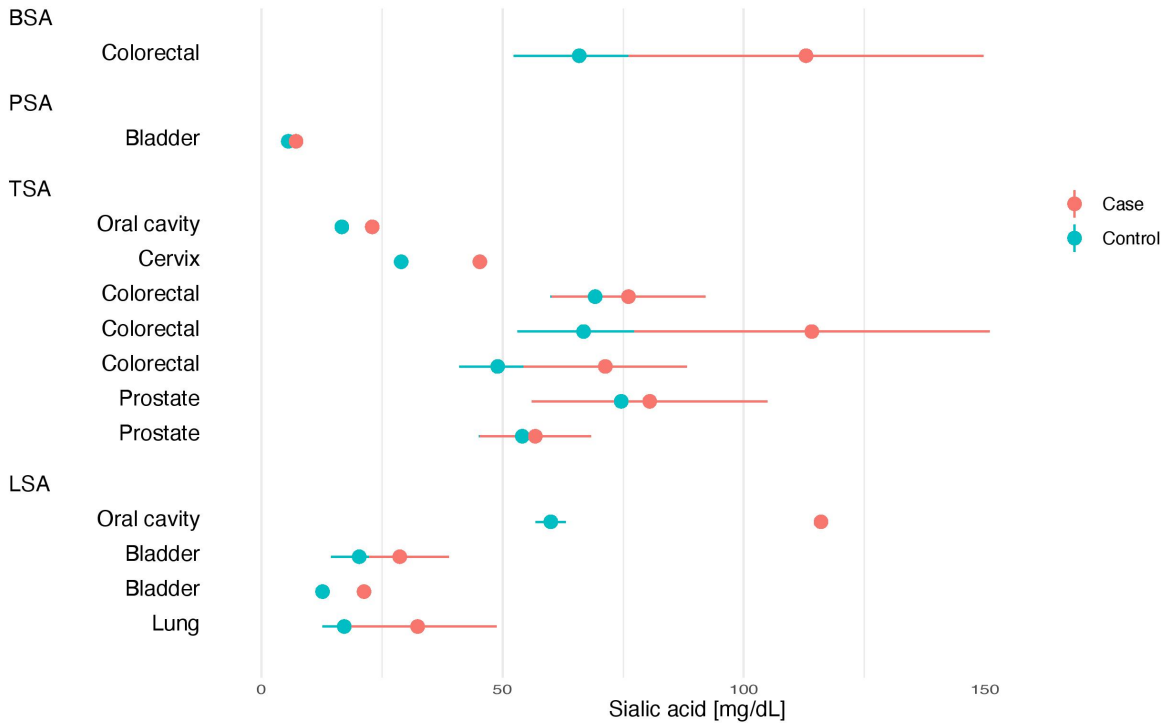

Figure 5: Forest plot highlighting the difference in sialic acid concentration (mg/dL) in healthy controls versus disease patients.

4.1 Lung cancer

Multiple studies have confirmed that there is a significant increase in serum TSA[63,64] and serum lipid bound sialic acid (LSA)[65,66] in patients with lung cancer when compared to healthy controls and patients with benign pulmonary disease (BPD). It was also noted that when patients with lung cancer underwent successful treatment for the disease there was a significant decrease in serum TSA[67] and LSA.[66,68] The decrease could be tracked as the course of treatment progressed allowing for monitoring of whether the treatment for lung cancer was successful. Sialic acid has been posited as a more sensitive marker than CEA, but it is not necessarily more specific in all cases. Combination of these markers may be pertinent as they are both good indicators of disease and when used together could give an excellent picture of whether or not lung cancer is present in a suspected case. More recent research into lung cancer and its associated with sialic acid has revealed an interesting link between sialylation and EGFR. [61] Increased sialylation of EGFR inhibited cancer cell growth indicating that downregulation of enzymes that sialylate these receptors plays an important

role in cancer cell proliferation. Research into inhibiting the routes by which these enzymes are downregulated could provide new treatments to slow the spread of cancer cells.

4.2 Bladder cancer

The current methods for diagnosis of bladder cancer are urine cytology, or the measurement of markers such as chromosomal changes, bladder tumour-associated antigen, CEA and nuclear matrix protein 22.[69] However, these current markers are quite unreliable, sometimes being unable to identify certain cancers or being abnormal in cases where no cancer is present leading to a possible false positive result. It is therefore important to expand this area of research to identify potential markers for the identification and diagnosis of this type of cancer. An early study performed in 1992[70] investigated serum LSA as a marker for bladder cancer. Although it was found that serum LSA was elevated in cancer patients as opposed to healthy controls, the authors questioned the usefulness of LSA as a marker for bladder cancer diagnosis, due to the relative inaccuracy of the method used for the measurement of LSA. This perhaps highlights the need for easier and more accurate assays for sialic acid analysis.[10] The study did however offer some interesting insights that sialic acid may be useful for tracking tumour staging and grading, with statistically significant ($p < 0.05$) elevations in serum LSA levels found between healthy controls and patients with tumours stage I-IV. These findings were built upon by a more recent study, that included a larger sample size.[71] The authors of this study also indicated an increase in LSA levels in cancer patients versus healthy controls. These authors also determined that LSA could be a marker for bladder cancer diagnosis, stating good sensitivity, specificity and accuary as a marker for the disease. The authors did comment briefly on the relation between serum sialic acid concentration and tumour staging but did not offer any statistical analysis so it cannot be determine if the elevation was statistically significant. Both of these studies agree on a futher point however, that LSA could serve as a biomarker for the monitoring of treatments and their effectiveness during the clinical course of treatment, with a marked decrease in LSA observed in patients who respond well to a course of treatment for the disease. This is compared to the observation of a smaller or no decrease in LSA in patients who did not respond, or only partially responded, to the treatment course. Further to the study of TSA and LSA, urinary sialic acid has also been investigated as a marker for bladder cancer showing elevated urinary sialic acid in patients with bladder cancer compared to healthy controls.[72] The authors of this paper also noted the levels of urinary sialic acid in patients grouped by tumour stage. It is interesting to note that the authors report numbers that indicate lower

levels of urinary sialic acid in patients with stage T1 bladder cancer which is contradictory to the evidence provided by other authors. No statistical analysis was offered on this data by the authors so it is not clear if this decrease is statistically significant. However, the authors of this paper do report an interesting find however, in that when comparing pre- and post-treatment levels of urinary sialic acid in stage T1 there was a significant decrease in sialic acid levels ($p < 0.05$) This is in contrast to observations in patients who did not respond to treatment who exhibited unchanged, or in some cases, elevated sialic acid levels post-treatment.

4.3 Cervical, breast and prostate cancer

Cervical, breast and prostate cancer are amongst a small percentage of cases diagnosed once symptoms present in an obvious manner. Earlier diagnosis is achievable, however, with regular physical screenings. These screenings can be invasive, potentially uncomfortable for the person undergoing the procedure and are normally only performed on patients over certain ages due to other risk concerns such as radiation in the case of breast cancer screening. This aspect can dissuade some people from voluntarily undergoing monitoring at regular intervals and can allow early stage cancers in younger patients to be overlooked or not detected until the cancer further develops. Replacing these screenings, or complementing them with a less invasive screening process in the form of a test for a marker such as sialic acid that can be performed with patients of all age groups would be very beneficial for earlier detection of these diseases.

Serum sialic acid levels were found to be significantly elevated in patients with carcninoma of the cervix when compared to patients with benign gynecological disease and healthy controls. TSA, LSA and free sialic acid (FSA) were shown to be biomarkers for the disease, each under different circumstances.[73] TSA was found to only be significantly elevated ($0.01 < P < 0.05$) in stage II A carcinoma, LSA was only significantly elevated ($0.01 < P < 0.1$) in stage I B carcinoma, meanwhile, FSA was significant elevated for three stages of carcinoma of the cevix (IA: $0.001 < P < 0.01$, IIA: $P < 0.001$, IB: $0.001 < P < 0.01$). The study concluded that while TSA and LSA could potentially be viable markers, they showed limited usage and value, only being able to possibly aid in a diagnosis in limited circumstances. In contrast, FSA was shown to be significantly elevated for mutliple types of cervix cancer studied versus healthy controls, with good sensitivity and specificity. These findings are confirmed by a second study[74] which found that TSA was found to correlate

with late stages of the disease only (IIB, IIIB and IV: $P < 0.05$), while LSA increased in stage IV carcinoma ($P < 0.01$). The authors concluded that TSA and LSA were limited in their usefulness as a marker for cervix cancer, showing poor ability for use as a diagnostic tool or as a complement to clinical staging of the tumours post diagnosis. These findings were backed up by a later study which showed that serum sialic acid was elevated in cancer patients versus healthy controls, with a good possibility for tracking treatment of the disease with TSA falling in step with the treatment progress.[75] A significantly higher fall in TSA levels was observed in patients with a complete clinical response to treatment as opposed to a partial or non-response. It was also observed that there was a statistically significanty larger drop in sialic acid concentration in early stage versus late stage cancers post-treatment ($P < 0.05$). More research is required in this field as the information available is limited, it seems as though sialic acid shows promise as a marker for cervical cancer but requires follow-up in further studies to determine its true effectiveness especially given the limited scope most studies fall into with regards to the tumour stages studied at the same time

Patients with breast cancer have been shown to exhibit elevated serum TSA[76–78] and LSA [77,78] when compared to healthy controls. TSA was shown to positively correlate not only with the presence of malignancies but also the stage of the disease with sialic acid levels increasing further as the stage of the disease increased. However, the authors offered no statistical analysis which is a limitation of this study. [76] The authors indicate that there was less of an increase of sialic acid concentration in serum in early stage breast cancer patients versus late stage breast cancer patients. The issue of the lack of statistical analysis also hampers this claim, however it could show that TSA and LSA as a marker for breast cancer could be better as a marker for the detection of late stage disease. The authors also noted a drawback, that TSA and LSA were specific but not particularly sensitive. Adding to this, while there was no difficulty differentiating between healthy controls and those with tumours, there was an issue in differentiating between those with malignancies and those with benign breast disease further limiting the usefulness of this marker. More recent studies have indicated that the measurement of salivary sialic acid could be used as an indicator for the presence of malignancies given that there was a significant difference in sialic acid concentration in samples collected from patients with malignancies versus those without observed in saliva samples. Further to this, clear differences in sialic acid concentration was shown between cancer stages, this method appears to show promise as an marker for early stage breast cancer. [79] This is further backed up by another study by the same authors

whereby samples were collected from female patients before they underwent a biopsy for suspected breast cancer. Of the 164 patients, 35 were diagnosed with breast cancer. This group showed clear elevations in salivary SA. [80] Saliva offers the same advantages as serum for the diagnosis of breast cancer in that a determination can be made of the presence of malignancies based on SA concentrations. Saliva may offer an advantage in being able to more easily differentiate late stage from early stage cancers.

Patients with prostate cancer (PCa) have shown significantly elevated serum TSA and PSA when compared to healthy controls and benign prostatic hyperplasia (BPH) patients.[81–83] PSA was also elevated in those with BPH when compared to healthy controls, but not to the same levels as with PCa. Sialic acid was found to be both highly sensitive and specific, making it an ideal candidate, with the added ability to differentiate well between BPH and PCa. It was also found that prostate cancers with bone metasteses showed significantly higher sialic acid levels than cancers without ($P < 0.005$), allowing for the possible use of the marker for determining how far a cancer has progressed at the time of diagnosis. With regards to sialic acid specificity, one group of authors commented that it is not specific for prostate tumours, i.e. it can be associated with many different tumour types and as such it was not necessarily useful for diagnosis on its own and would be better combined with other markers and screening processes. Sialic acid does however show promise in this case for the monitoring of tumour progression and staging.

4.4 Colorectal cancer

Colorectal cancer patients have shown elevated serum TSA, LSA and BSA when compared to healthy controls.[84–86] There was also good indication that there was a significant correlation between late stage disease and TSA. TSA became more elevated as the staging of the cancer increased (Table 13), CEA was shown to not be affected by tumour stage indicating that sialic acid has an advantage here over an existing marker for the disease. This shows that the marker could be good for monitoring the progress of malignant colorectal diseases and possibly the reaction of the disease to therapeutic approaches as shown for other types of malignancies. One study compared TSA with CEA with regards to the finding outlined above, indicating that CEA was perhaps still a better marker for cancer being present, but combination of CEA and TSA could provide a more sensitive approach to the detection of colorectal cancer. Further to this, as with lung cancer, a link between colorectal cancer proliferation and sialylation of receptors such as EGFR has been found. [60,62] Decreased

sialylation of the receptor leads to increased cancer cell growth. One of the groups of authors indicated that this appears to be due to downregulation of the ST6Gal-I enzyme which carries out sialylation of EGFR. Treatments targeting this downregulation however may be tricky because the authors also report that this enzyme is responsible for N-glycan sialylation of cancer cells which aids proliferation and metastatis.

Healthy Control (mg/dL)	Duke's A (mg/dL)	Duke's B (mg/dL)	Duke's C (mg/dL)	Duke's D (mg/dL)
49.0 (41.1; 56.9)	N/A	57.9 (47.9; 67.8) (P < 0.001)	72.15 (57.5; 86.8) (P < 0.0001)	80.8 (63.6; 98.0) (P < 0.0001)
66.8 (53.2; 80.4)	85.4 (65.9; 104.9) (P < 0.0001)	117.2 (81.0; 153.4) (P < 0.0001)	117.2 (77.3; 157.1) (P < 0.0001)	N/A
63.0 (54.1; 71.9)	73.0 (59.0; 87.0) (P < 0.05)	78.0 (60.0; 87.0) (P < 0.05)	77.0 (60.0; 94.0) (P < 0.05)	83.0 (60.0; 106) (P < 0.05)

Table 13: Comparison of sialic acid concentrations in serum in different stages of colorectal cancer. Data is presented as 95% confidence interval (95% CI)

4.5 Oral Cancer

Depending on the measure, oral cancer is the sixth most common cancer in the world representing something in the region of 2-4% of all cancers (3-400,000 cases per year), with most cases classified as Oral Squamous cell carcinoma (OSCC).[56,87] However, despite many years of searching for early diagnosis biomarkers it's mortality rate has remained stubbornly high with 5-year survival rates still around 50% and recurrence rates similarly high.[88] The gold-standard for diagnosis is still specialised histology, a factor contributing to late diagnosis in many cases.[89] Therefore the potential impact for salivary or serum based diagnostics is very high. Unsurprisingly maybe, given the origin of the word sialic (sialon- from saliva), a relationship between elevated plasma TSA/ LSA and indeed salivary TSA has been established over the years Several studies from groups in India, where OSCC is particularly prevalent, have established that OSCC patients have raised salivary TSA when healthy and confirmed cancer cases are compared. A TSA/ Total Protein (TP) ratio is often used as a measure of disease with up to 2-fold higher TSA/TP ratio being evident in advanced

cancer over healthy patients, and 1.5-fold higher TSA/TP ratio being evident in early cancer, indicating potential prognostic value but with variation depending on the study. [90] In addition, there is also a correlation with cancer grading in several studies, with raised TSA and TSA/TP in saliva and serum dating back well over 20 years [91,92] as well as a presentation of oral pre-cancer known as Oral Leukoplakia in which TSA was 1.5x higher at 45.3 (43.8; 46.8) than healthy controls at 29.0 (28.2; 29.8) ($P < 0.05$, n=30) but less so for LSA at 23.0 (22.1; 23.9) versus 16.7 (16.1; 17.4).[93]. More recently, the levels of enzymes in saliva and serum that are associated with sialic acid based surface glycosylation modulation, such as sialyltransferases (STs) and sialidases, have been shown to be slightly raised in oral cancer patients.[90,94] Others have also begun to analyse levels of 2,3-linked sialic acids versus 2,6 levels using lectins, although this work is hindered by a lack of easily accessible tools.[90]

Overall, it is clear from over 20 years of work that TSA might be a reliable measure of Oral Cancer prognosis-precancer observation, as well as more advanced cancers; and with the accessibility of saliva sampling it seems an excellent candidate for increased screening and study.

5. Conclusion

Sialic acid is correlated with the presence and pathogensis of different diseases but cannot be used to differentiate between different diseases. However, sialic acid, is a potential biomarker for monitoring the effectiveness of treatment and disease progression or remission. More specifically: atherosclerosis, CHF, CHD and hypertension have been associated with an increase in serum sialic acid concentration between control and disease cohorts. Sialic acid serum concentration has also been shown to be useful in differentiating the severity of CHD with differences in sialic acid levels between patients with 1 VD and 2 or 3VD, making it a useful marker for the progression of the disease. Interestingly there is some debate on this topic, with not all studies agreeing that there is a correlation between elevated sialic acid concentration and the presence of CHD, more studies in this area may be of use to confirm whether sialic acid can be a marker for CHD severity. Alongside this, myocardial infarction was associated with elevated SA post infarction, as well as with acute phase proteins, further confirming the use of SA as a marker for myocardial infarction. Sialic acid has generally also been associated with risk factors of CVD showing that as sialic acid increases, so do markers

for the presence of CVD, indicating that sialic acid could supplement existing markers such as LDL and h-CRP, as well as risk factors such as periodontitis and menopause.

Diabetes is an area of interest due to the fact that elevated sialic acid serum concentration is indicative of type 2 diabetes, it is not indicative of type 1 diabetes. It is currently unclear why this is the case and as such more research on this topic is required to determine the difference in the diseases that leads to this discrepancy. Contrary to this, complications of both type 1 and 2 diabetes have been shown to be correlated with sialic acid, not only the presence of these vascular complications, but predicting early onset by up to 3 years allowing for early diagnosis, treatment and prevention of these conditions.

An array of different sialic acid markers have been found to be indicative of the presence of malignant tumours across different cancer types with elevated sialic acid found in patient groups versus healthy controls (table 9). However, there is a drawback that while it is a marker for presence of tumours, sialic acid is not able to differentiate between different types of cancer, for example, a test for elevated sialic acid levels could not allow for the identification of a specific type of tumour without further analysis of relevant markers, physical examinations or procedures such as biopsies of suspect tissues. Sialic acid does show promise in terms of tumour staging - once a tumour is identified, sialic acid levels could be effectively utilised, and compared to existing markers, to differentiate between early and late stage cancers. One area where sialic acid excels as a marker under the umbrella of cancer treatment is that sialic acid levels are shown to change when a tumour is successfully treated and will fall as the tumour regresses. This could allow for a simple, and minimally invasive, test to determine the effectiveness of a treatment.

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7. Future Perspective

Sialic acid offers potential as a diagnostic tool for different diseases which exhibit overexpression of sialic acid. Current diagnostic methods for CVD can be unreliable, relying on risk calculation algorithms or markers such as cholesterol and blood pressure that can be affected by factors other than the presence of CVD. Cancer diagnosis generally requires uncomfortable and invasive procedures or identification through markers that can produce false positive results. Replacing or combining sialic acid analysis with these less reliable

markers or invasive diagnostic procedures with a simple blood test for sialic acid could allow for fast, accurate and minimally invasive diagnosis of CVD or cancer.

There is also potential for sialic acid to offer interesting insight when monitoring the progress of diseases that have already been diagnosed. The pathogenesis of CVD, or the stage of malignant tumours, has an effect on sialic acid concentrations and these can be tracked. This could allow for the opportunity to track treatments, their efficacy and patient responsiveness by measuring sialic acid levels, with decreasing sialic acid showing response to the treatment

However, using sialic acid as a biomarker is not appropriate at present when comorbidities exist as it is not currently possible to differentiate between the presence of CVD, diabetes or cancer within one partient, as each condition results in an increase in TSA. However, TSA is not just one marker; while a majority of sialic acid is found as *N*-acetylneuraminic acid, there are over fifty different sialic acids in the body. Acetylation, methylation and sulfaction at each hydrolxyl position yield a large number of different sialic acid derivatives. Investigating these individual derivatives may pave the pay to a better marker than TSA. A prime candidate for such investigation might be the *O*-acetylated derivative of sialic acid, Neu5, $9Ac_2$. Research into Neu5, $9Ac_2$ has indicated that it could be a potential marker for different cancers such as breast cancer[95] and leukemia,[96] offering potential as a more specific marker for these diseases over Neu5Ac.

8. Executive Summary

Introduction

- Elevated concentrations of sialic acid in biological fluids have been shown to be markers for the presence and pathogenesis of cardiovascular diseases, diabetes, diabetic complications and cancer.
- Elevated sialic acid has been observed in different biological fluids: serum, plasma, urine and saliva.
- Sialic acid is found as the terminating unit on glycans which decorate the surface of cells and proteins. Glycosylation changes are observed under certain conditions, such as in the presence of inflammation.

Sialic acid concentration and its association with cardiovascular disease (CVD) and markers of CVD

- Individuals with elevated plasma sialic acid levels are at greater risk of CHD and stroke compared to those with lower levels of serum sialic acid.
- Elevated sialic acid in serum and plasma is observed in patients with atherosclerosis, CHD, CHF, myocardial infarction and hypertension when compared to healthy controls.
- Sialic acid has been associated with risk factors such as a serum cholesterol and hs-CRP, CIMT and lipoprotein (a).

Sialic acid concentration and its association with type 1 and type 2 diabetes and diabetes complications

- Elevated serum sialic acid has been associated with type 2 diabetes, but not type 1 diabetes.
- Diabetic neuropathy, nephropathy and retinopathy are associated with elevated serum sialic acid levels.
- Elevated serum sialic acid levels indicated presence of albuminuria, with sialic acid levels increasing as the conditions became more severe. Raised sialic acid levels could also predict the onset of the condition by up to three years.

Sialic acid concentration and its relation to various cancers, cancer stages and classifications

- Elevated sialic acid in serum and urine indicated the presence of both early and late stage cancers. Late stage cancers showed greater concentrations of sialic acid than early stage cancers.
- Sialic acid levels decrease with successful treatment of the cancer and do not change in those who do not respond to treatment, offering opportunities for monitoring the effectiveness of cancer therapies.

Conclusion

- Sialic acid is a biomarker for the presence and pathogensis of different diseases, however it cannot be used to differentiate between different diseases.
- Sialic acid may offer good opportunities as a tool for monitoring the effectiveness of treatmentsand the staging of cancers.

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