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REVIEW

Assessment of the cardiovascular and gastrointestinal autonomic complications of diabetes

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Abstract

The global prevalence of diabetes mellitus is increasing; arguably as a consequence of changes in diet, lifestyle and the trend towards urbanization. Unsurprisingly, the incidence of both micro and macrovascular complications of diabetes mirrors this increasing prevalence. Amongst the complications with the highest symptom burden, yet frequently under-diagnosed and sub-optimally treated, is diabetic autonomic neuropathy, itself potentially resulting in cardiovascular autonomic neuropathy and gastrointestinal (GI) tract dysmotility. The aims of this review are fourfold. Firstly to provide an overview of the pathophysiological processes that cause diabetic autonomic neuropathy. Secondly, to discuss both the established and emerging cardiometric methods for evaluating autonomic nervous system function *in vivo*. Thirdly, to examine the tools for assessing pan-GI and segmental motility and finally, we will provide the reader with a summary of putative non-invasive biomarkers that provide a pathophysiological link between low-grade neuro inflammation and diabetes, which may allow earlier diagnosis and intervention, which in future may improve patient outcomes.

Key words: Autonomic nervous system; Gastrointestinal dysmotility; Neuroinflammation; Biomarkers; Diabetic

neuropathy

Core tip: Autonomic complications are common and bothersome long-term sequelae of diabetes. However, they are frequently under-diagnosed and sub-optimally treated. Arguably this is as a consequence of a lack of appreciation of the various testing options that are available, particularly for end organ dysfunction such as within the cardiovascular and gastrointestinal systems. Our review aims to provide a succinct review of the current investigational armamentarium that are available and also provide the reader with a summary of the cutting edge techniques that have the potential to influence clinical practice in the future.

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INTRODUCTION

The world prevalence of diabetes among adults will be 7.7%, affecting 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries[1]. This epidemic is potentially a consequence of changes in diet, lifestyle and the trend towards urbanization. Diabetes is associated with significant economic burden with healthcare costs estimated to be in the order of $132 billion annually in the United States and £10 billion in the United Kingdom[2,3]. Unsurprisingly, the prevalence of complications of diabetes reflects the increases in prevalence. Arguably amongst the most burdensome from a symptomatic point of view, yet frequently under-diagnosed, is the neuropathy that causes dysfunction of the autonomic nervous system (ANS), referred to as diabetic autonomic neuropathy (DAN), itself potentially leading to myriad of complications frequently manifest in the cardiovascular system and gastrointestinal (GI) tract. In addition to the bothersome nature of symptoms, Ewing *et al*[4] reported that in those with DAN, the survival rate at 5 years following diagnosis is as low as 47%.

The ANS is a bi-directional hierarchically controlled brain body interface that serves to integrate and modulate the internal milieu in response to the external environment thereby serving to maintain homeostasis. The ANS consists of the enteric nervous system and two broadly opposing branches referred to as the sympathetic (SNS) and parasympathetic nervous systems (PNS), having ubiquitous innervation throughout the body. The overall aim of this paper is to provide the reader with a contemporaneous and succinct review of the assessment of the autonomic complications of diabetes and discuss potential future biomarkers.

DIABETIC AUTONOMIC NEUROPATHY

One of the major microvascular complications of diabetes is development of neuropathy, defined as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes", which may deleteriously affect sensory, motor and autonomic nerve fibers[5].

Diabetes induced sensory and motor neuropathies affects C-fibers first and then progressively symmetrical affection of thick (Aβ) and thin (Aδ) - fiber neuropathy influencing axons of the distal lower extremities in a "glove and stocking" distribution. Interestingly, that despite comparable traditional risk factors, Asians with diabetes have substantially less large and small fiber neuropathy in comparison to matched Europeans[6]. Whilst clinicians would readily recognize that such symptoms represent a sensory neuropathy, symptoms related to DAN are often under appreciated, recognized and investigated. Diabetic sensorimotor polyneuropathies can be categorized according to the Toronto classification (Table 1)[5]. The traditional view of the pathogenesis od DAN is considered to be sequelae of vascular compromise, it has been more recently proposed that such complications represent the progression of systemic capillary dysfunction, which frequently are already present at diagnosis in those with type 2 diabetes[7]. Moreover, distinct differences in haemodynamic properties within the epineurium of *e.g.*, the sural nerve, have also been proposed to play an important role in the pathogenesis of painful diabetic neuropathy[8]. However, the order of these factors in the aetiopathogenesis of diabetic neuropathy has been challenged by Danish researchers, as changes in endoneurial capillary morphology and vascular reactivity apparently may predate the development of diabetic neuropathy in humans[7].

Considering that the pathophysiology is largely similar, DAN can be regarded as an entity not dissimilar to the aforementioned peripheral neuropathy[5]. DAN can be usefully regarded as both a structural and/or a metabolic disorder, and the clinical manifestation of which can be present with or without the presence of large fiber neuropathy. DAN may affect cardiovascular, GI sensorimotor, urogenital systems, and sudomotor function. The presence of DAN confers a heightened risk of mortality in diabetes and frequently co-exists with other peripheral polyneuropathies[9]. Evidence suggests that subclinical DAN can occur within the first year of onset of type 2 diabetes (T2DM), and within two years in type 1 diabetes (T1DM), although often unrecognized for a number of years after their onset[10].

Nevertheless, the formal diagnosis of DAN is frequently delayed, the causes of which are most certainly multifactorial but arguably includes the non-specificity of presenting symptoms, the lack of clinician appreciation

Table 1 Toronto classification of diabetic sensory neuropathy[5]

Definition of minimal criteria for diabetic sensorimotor polyneuropathy	Clinical features
Possible	Reduced sensation, positive neuropathic sensory symptoms (burning pain in the distal lower extremities), symmetrical reduction in distal sensation and/unequivocally decreased or absent ankle reflexes
Probable	A combination of two or more of the following: Neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes
Confirmed	Decreased nerve conduction on objective testing with signs and symptoms as above
Subclinical	Decreased nerve conduction on objective testing in the absence of signs or symptoms

and the limited availability of specialized diagnostic services. Cardiovascular autonomic neuropathy is frequent, which can results in life threatening complications such as arrhythmias, silent myocardial ischemia and sudden death. However, DAN can potentially affect any portion of the ANS, and should therefore be considered a systemic disorder[11]. Evidence suggests that up to 10% of those with diabetes are at risk of developing DAN which may manifest as a variety of troublesome symptoms including orthostatic hypotension, aberrant GI motility and erectile dysfunction all of which can lead to a diminution in quality of life[5].

Hitherto, the focus of assessment for DAN has been derived from measures such as heart rate variability (HRV) and sudomotor function. However, over the recent past there have been considerable advances in measuring the "downstream" effects of DAN on both the cardiovascular system and the GI tract.

PATHOPHYSIOLOGY OF DIABETIC AUTONOMIC NEUROPATHY

Hyperglycemia induced macro- and microvascular complications

After 20 years of diabetes, neuropathy can be objectively demonstrated in up to 40%-50%[12]. The pathophysiology of neuropathy is multifactorial with structural and metabolic alterations having been described within axons, Schwann cells, and microvascular elements within the endoneurium and extracellular matrices[13]. Newer findings suggest that changes in the endoneurial capillary morphology and vascular reactivity are present before development of diabetic neuropathy in humans[7]. In addition, the authors found an association between the level of endoneurial hypoxia and reductions in nerve conduction velocity, in diabetes patients with manifest neuropathy.

Using experimental models of diabetes, reduced levels of neurotrophic support, including nerve growth factor and insulin like growth factor, have been implicated in reducing endoneurial blood flow thereby leading to neuronal damage[14]. In addition, such impairments in blood flow also result in alterations in Na^+/K^+ ATP-ase activity and nitric oxide metabolism. Animal studies suggest that altered Na^+/K^+ pump function may occur due to C-peptide deficiency, resulting in the shunting of glucose through the polyol pathway, thereby leading to increased levels of sorbitol and alterations of the nerve excitability recovery cycle which further contribute to neuronal damage[15,16].

Peripheral and autonomic neurons, as well as their interconnections, are particularly vulnerable to hyperglycemia[17]. The mechanisms that underlie this vulnerability can be considered to both direct, as a consequence of heightened influx of extracellular glucose and indirectly through a plethora of other biochemical pathways. Examples of such indirect metabolic pathway are summarized in Figure 1 and include, but are not limited to, the following.

Polyol pathway: In the polyol pathway intracellular glucose is converted to sorbitol by the rate limiting enzyme aldose reductase, in an energy dependent manner *via* nicotinamide adenine dinucleotide phosphate[18]. The activation of this pathway may result in osmotic damage and diminution of Na^+/K^+ -ATPase activity[19]. These processes lead to increased intracellular oxidative stress[20].

Hexosamine pathway: The hexosamine biosynthesis pathway is a minor branch of glycolysis, where fructose-6-phosphate is converted to glucosamine-6-phosphate, catalyzed by the rate-limiting enzyme: Glutamine: Fructose-6-P-amidotransferase.

Formation of reactive oxygen species: In diabetes, reactive oxygen species (ROS) play an important role in the development of cardiovascular diseases, through excessive formation of oxidants, decreased bioavailability of nitric oxide, and decreased antioxidant capacity in the vasculature and kidneys[21]. These processes are initiated and amplified during chronic hyperglycemic conditions[22].

Increased diacylglycerol and protein kinase C pathways: Increased activation of the polyol pathway may cause a decrease in the activity of (Na^+/K^+) ATPase, and studies have suggested that this drop may activate diacylglycerol and protein kinase C (PKC) pathways[23]. Activation of PKC pathways increase cytosolic phospholipase A2 activity and produces a pro-inflammatory mediators such as prostaglandin E_2, which inhibits cellular (Na^+/K^+) ATPase[24].

Formation of advanced glycation end products: Hyperglycaemia results in the formation of advanced glycation end products, comprising of proteins or lipids

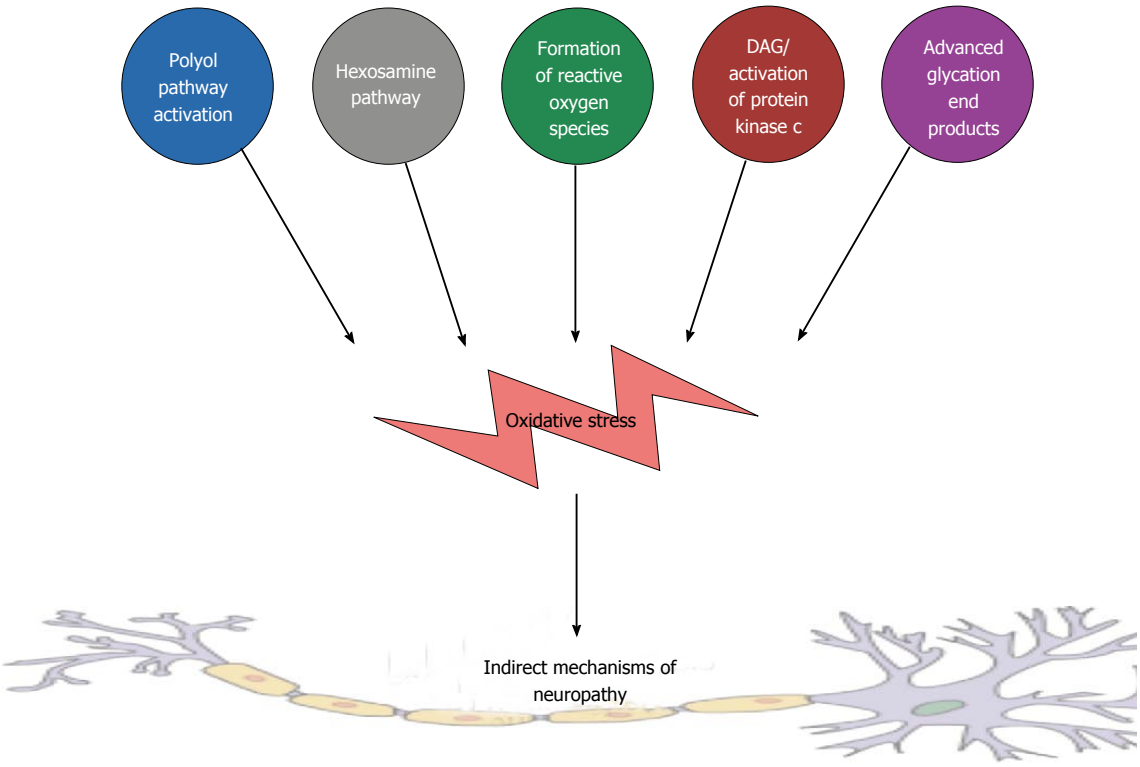

Figure 1 A highly schematic summary of the proposed indirect mechanisms of neuropathy. DAG: Diacylglycerol.

that become glycated after prolonged exposure to sugars[25]. This results in a diminished redox capacity of the neuron leading to enhanced vulnerability to ROS.

Cumulatively, these biochemical pathways, in conjunction with activation of the complement system[26], coalesce to form a cumulative indirect cascade that can initiate and summate neuro-inflammation, as is observed in DAN.

ASSESSMENT OF AUTONOMIC DYSFUNCTION

The last three decades have witnessed the increasing recognition of the pivotal role of the ANS in the pathophysiology of a number of disorders including diabetes. Although ANS function can be measured directly, using a needle recording of the peroneal nerve for instance, such methods are invasive and time consuming. Therefore, indirect, or proxy measures of ANS function have been developed, the most popular and widely utilised being HRV and are summarised in Figure 2.

HEART RATE VARIABILITY

The clinical relevance of HRV was first appreciated in 1965 when Lee *et al*[27] demonstrated that foetal distress was preceded by alterations in the inter-beat intervals between successive R waves in the electrocardiogram (ECG), before any appreciable changes occurred in heart rate (HR) *per se*. This epiphenomenon in the oscillations in the interval between successive heartbeats is known as "heart rate variability". In deriving physiologically salient measures from HRV, there are three broad methods, time domain, power spectral analysis and beat-to-beat measures.

TIME DOMAIN ANALYSIS OF HEART RATE VARABILITY

Since HR is controlled within a negative feedback loop influenced by both the SNS and PNS, the examination of beat-to-beat periodicities can provide an insight into their relative influences. Such variations in HR may be examined using time domain analysis. In a continuous ECG recording, the interval between consecutive normal QRS complexes on the ECG is known as the normal-to-normal (NN) interval. From the NN interval, statistical time domain measures can be derived and are divided into two classes, firstly those derived from the direct measurement of NN intervals and secondly those derived from the difference between NN intervals. The simplest variable is the standard deviation of normal-to-normal (SDNN RR intervals), which reflects the cyclic components of variability within the recording. Other commonly used measures are detailed in Table 2. The major disadvantage of these methods is the limited statistical power for the evaluation of short-term recordings of less than five minutes. Time domain analysis has been widely used to characterize autonomic neuropathy in diabetes, and has shown to be associated with the degree of sensorimotor neuropathy and also influences symptom generation peripherally within the GI tract[28]. Finally, reduced HRV

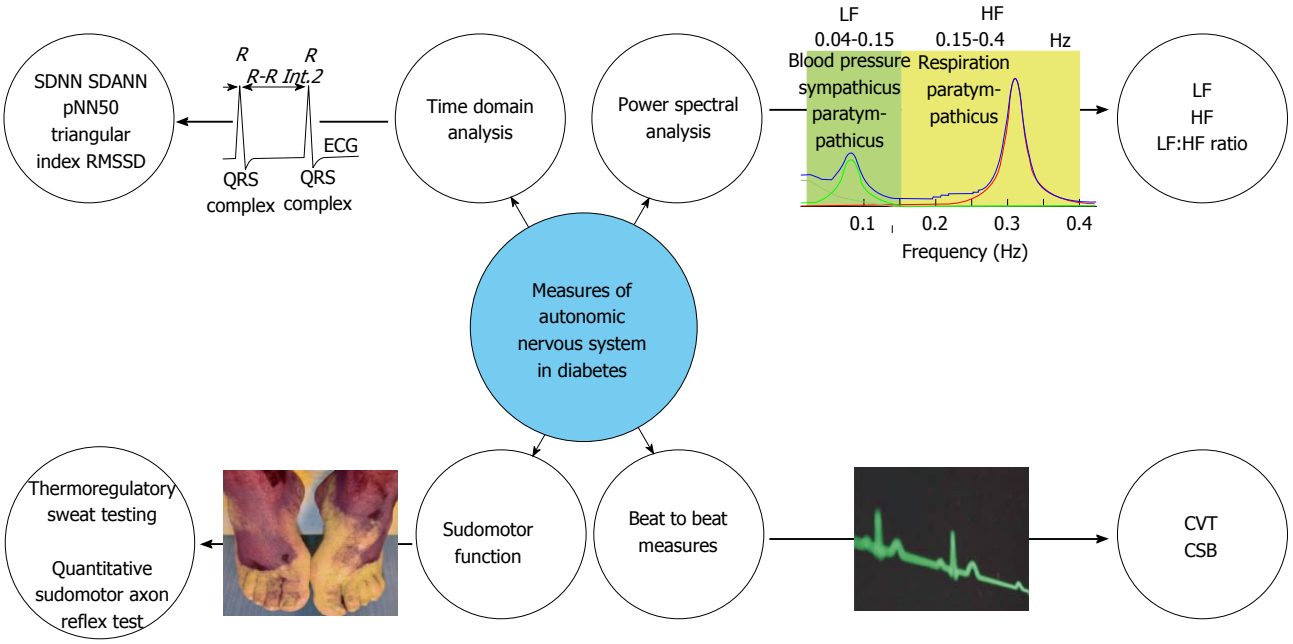

Figure 2 A summary of the autonomic testing options. SDNN: Standard deviation of normal-to-normal; ECG: Electrocardiogram; HF: High frequency; LF: Low frequency.

Table 2 Time domain analysis variables and their physiological relevance

Variable (units)	Description	Physiological relevance
SDNN (ms)	Standard deviation of the normal RR (NN) interval reflecting all of the cyclic components responsible for variability in the period of recording	An overall estimate of HRV, but does not indicate the contribution of any particular influence
SDANN (ms)	Standard deviation of the averages of NN intervals calculated over a short period of time, usually less than five minutes	Reflects the influence of circadian rhythms on autonomic function
pNN50 (%)	The proportion of NN intervals having a difference of > 50 ms	Reflects predominant vagal influence on variability
Triangular index (ms)	The integration of the density distribution of all the NN intervals as a function of the maximum density	Overall estimate of HRV similar to SDNN
RMSSD (m/s)	The square root of the means squared differences in successive NN intervals	Estimate of the short-term components of HRV

SDNN: Standard deviation of normal-to-normal; NN: Normal-to-normal; HRV: Heart rate variability.

also were associated with altered central processing within the operculum-insular network, underlining the systemic influence of diabetic neuropathy[29].

POWER SPECTRAL ANALYSIS OF HEART RATE VARIABILITY

The ANS activity that influences HRV is periodic in its nature, with sympathetic and parasympathetic components oscillating at distinct frequencies. The purpose of the frequency domain analysis of HRV (spectral analysis) is to dissect HRV into its specific frequency components, which defines the energy per unit time, which is often referred to as "power", contained in each frequency component. Power spectral analysis has become the prevailing model for exploring HRV, and therefore autonomic function, within the literature. When considering short-term recordings obtained in resting conditions, the HRV spectrum is characterised by three major components at high (HF), low (LF) and very low frequency. The HF band represents respiratory sinus arrhythmia, as this is generally considered to represent vagal output to the heart, and this is termed cardiac vagal control, whereas the LF band is considered to represent sympathetic activity. Thus by examining the ratio between LF and HF power, sympathovagal balance can be derived. However, there are a number of methodological challenges of using HRV, notwithstanding assumptions concerning a relative constant respiratory rate and depth, referred to as respiratory stationarity, and limited temporal resolution such that these measures are not validated for time epochs of less than five minutes[30,31]. Such a shift in the sympathovagal balance has been proposed to be the underlying mechanism of symptom improvement in patients suffering from gastroparesis, who were treated with gastric electrical stimulation.

BEAT-TO-BEAT MEASURES

In attempting to overcome these methodological chall-

enges, beat-to-beat measures of ANS "tone" have been recently developed and validated such as cardiac vagal tone and cardiac sensitivity to the baroreflex which measure efferent and afferent vagal tone respectively. In a preliminary study, we have demonstrated in 14 T1DM patients that lower cardiac vagal tone and cardiac sensitivity to the baroreflex were associated with disease duration, which was independent of glycaemic control and age[32]. Therefore, such novel autonomic indices may offer a longitudinal biomarker, which may aid in the prediction of autonomic neuropathy. Given their relative ease of use, and the lack of need for expert interpretation, these parameters could be useful as near patient screening tools in the future.

SUDOMOTOR FUNCTION

Patients with diabetic neuropathy typically have decreased sweating in the feet, which is associated with dry skin, itching and foot ulceration. Sweat glands are innervated by the sudomotor, postganglionic, unmyelinated cholinergic sympathetic C-fibers. Several methods have been developed to assess sudomotor function and contribute to the detection of autonomic dysfunction in diabetic peripheral neuropathy. The thermoregulatory Sweat testing (TST) evaluates the integrity of central and peripheral sympathetic sudomotor pathways[33]. The core body temperature is artificially raised to 38 ℃, by increasing the ambient room temperature within a chamber, and a maximal sweat response is detected by a change in an indicator dye colour. Abnormal sweating patterns can therefore be recorded and provides a general index of severity of the autonomic failure. Nevertheless TST is limited by the fact that it cannot differentiate pre- from post-ganglionic lesions, is time consuming, requires special equipment, research facilities and patient preparation[33]. The quantitative sudomotor axon reflex test (QSART) evaluates postganglionic sympathetic cholinergic sudomotor function. Sweat glands are stimulated with a cholinergic agent and the sweat production is measured as an increase of humidity through a hygrometer. QSART is capable of detecting neuropathy with a sensitivity of > 75%[5]. However, QSART is unable to detect preganglionic lesions, requires special equipment and is not widely available[5]. By combining QSART with TST sensitivity is improved to 98% and it furthermore provides the clinician with the possibility to localize the lesion[34]. A relatively novel non-invasive rapid screening test, the Sudoscan, has been introduced, which provides sensitivity of 65% and specificity of 80% in correct classification of DAN. Furthermore, the test showed strong association between foot and hand electric skin conductance and nerve conduction tests[35]. Another user-friendly technique is the visual indicator test, referred to as the Neuropad. The Neuropad has high sensitivity but moderate specificity against large fibre neuropathy assessments. However the receiver operator characteristics of Neuropad is significantly improved, when used in combination with corneal nerve fibre length (< 14 mm/mm^2) with a sensitivity and specificity of 83% and 80%, respectively[36].

ASSESSMENT OF GASTROINTESTINAL TRACT DYSFUNCTION

GI symptoms, maybe divided into those arising in the foregut, including the oesophagus and stomach, and those limited to the mid and hindgut. Although intuitively, considering that diabetes is a systemic disorder, a considerable degree of overlap between these three distinct anatomical areas would be expected. Up to 50% of patients with diabetes have experienced disabling GI symptoms, including nausea, vomiting, bloating, early satiety, and abdominal pain and are thought to be sequelae of GI dysmotility[37]. GI dysmotility includes delayed gastric emptying, gastroparesis, rapid gastric emptying and other motor dysfunctions, such as impaired distention within the gastric fundus.

Gastroparesis, *i.e.,* the pathological delay in the emptying of contents from the stomach into the small bowel, is one of the most frequently encountered GI complications. However, the degree of gastric emptying and symptom burden is often poorly correlated[38]. Up to 12% of patients with diabetes report symptoms consistent with GI dysmotility and such symptoms may result in nutritional compromise, diminished quality of life and poor glycaemic control as a result of impaired nutritional delivery into the small bowel[39].

GI motility is regulated and coordinated in a complex bidirectional interaction between the central nervous system, the ANS, the enteric nervous system and various endocrine and hormonal pathways. As its name suggests, the vagus nerve, which innervates the entire GI tract, apart from the distal third of the colon, has a stimulatory effect on the enteric nervous system and thus enhances GI motility, an effect that is broadly antagonized by sympathetic fibres. The interplay between these and changes in cellular level is largely unknown, but the pathophysiological mechanisms leading to gastroparesis are multifactorial in nature. However, the ANS is likely to be of critical importance. For instance, similar GI symptoms to those reported by patients with diabetes are seen in non-diabetics following truncal vagotomy, a previously frequently used surgical intervention for peptic ulcer disease in the pre-proton pump inhibitor era. These observations gave rise to the initial assumption that gastric dysmotility reflects irreversible damage to the vagal nerve. Currently, as there is a paucity of investigations to directly assess GI autonomic function directly, vide infra, the evaluation of cardiometrically derived autonomic function is often used as surrogate marker of the function of the abdominal vagus. However, the reported correlations to date have been relative weak, and in other studies, no relationship between gastric emptying and autonomic function has been

Table 3 A comparison of the various contemporaneous techniques for the measurement of gastrointestinal motility[43]

Technique	Area of the GI tract evaluated	Length of stay required in clinic/office	Acceptability to the patient	Radiation exposure	Physiological conditions of measurement	Standardization of test	Measurement of propagating contractions	Availability/expense of test	Ease of interpretation of the result
Gastric emptying scintigraphy	Stomach	c.5 h	High	Yes	Yes	No	No	Widely/moderately expensive	Moderate
Whole gut scintigraphy	Pan-GI	c.8 h	High	Yes	Yes	Yes	No	Very limited/very expensive	Difficult
Radio - opaque marker study	Stomach colon	30 min to	High	Yes	Yes	No	No	Widely/inexpensive	Easy
^{13}C octanoic acid breath test	Stomach	c.4 h	High	No	Yes	Yes	No	Very limited/ inexpensive	Relatively easy
Wireless motility capsule	Pan-GI	c.30 min	High	None	Yes	Yes	No	Limited/currently moderately expensive	Relatively easy

GI: Gastrointestinal.

shown[40]. The prevalence GI dysmotility is likely to be associated with the duration of diabetes, and thus attributable to an increased prevalence of autonomic neuropathy. The prevalence of GI dysmotility, and specifically gastroparesis, also appears to be higher in females than in males for uncertain reasons, but potentially suggesting a hormonal effect on the disease process.

As mentioned, GI symptoms *per se* whilst occurring frequently in diabetes are not strongly predictive of physiological abnormalities on objective testing. Therefore, the use of patient reported tools is insufficient to establish a formal diagnosis but are useful in establishing symptom severity. There are a number of methods for objectively evaluating GI motility and these are summarised in Table 3.

SCINTIGRAPHIC ASSESSMENT OF GASTROINTESTINAL MOTILITY

Scintigraphic gastric emptying

The current gold standard for the diagnosis of diabetic gastroparesis is the scintigraphic evaluation of gastric emptying[38]. It is generally recommended that prior to testing patient should (1) have serum glucose levels that are stable; (2) avoid medications that influences gastric emptying for 48-72 h prior to testing; and (3) avoid nicotine exposure during the test period as these factors can confound interpretation of the test results. Patients are fed a standardized meal of ^{99m}Tc-sulfur colloid-containing eggs, following which serial imaging over 4 h is undertaken using a gamma camera. Gastroparesis is considered to be present if > 60% of the isotope activity remains in the stomach 2 h after the test ingestion - or if at least 10% of the initial activity is still detected after 4 h[37].

Scintigraphic small bowel and colonic motility

Small bowel and colonic transit can be measured in a similar manner to gastric emptying, although serial imaging is prolonged. Delayed small bowel transit is diagnosed if < 40% of total small bowel of the isotope activity has accumulated in the terminal ileum-cecum at 6 h. To assess colonic transit, images of the colon are acquired at 24, 48, and 72 h after ingestion of the radiolabelled meal, with subsequent calculation of a metric referred to as the geometric centre. The geometric centre is an average of the intra colonic, weighted by segment colonic region, and intra-faecal distribution of the isotope.

However, scintigraphy is relatively expensive, associated with radiation exposure and still not standardized across centres. Moreover, due to is significant radiation burden, scintigraphy limits its application in children, women of child bearing potential, and subjects undergoing repetitive measurements of gastric emptying in a short period of time.

RADIO-OPAQUE MARKERS

An alternative method for measuring GI motility is to use indigestible radio-opaque markers (ROMs) coupled with standard radiography/fluoroscopy.

ROM gastric emptying

ROM is given together with a standard meal. Emptying of ROM is followed with fluoroscopy every hour until all ROMs are emptied or for a maximum of 6 h. When compared to scintigraphic method, the ROM method in diabetic patients has comparable specificity albeit with less sensitivity[41]. In on other words, this means a normal ROM test does not exclude delayed gastric emptying, and if the clinical suspicion of gastroparesis remains, scintigraphy should be performed. However, the ROM method may represent a reasonable "screening test" for delayed gastric emptying as it is inexpensive and a widely available.

Radio-opaque marker colonic transit

Colonic transit can be measured using a ROM technique, although there is a current lack of standardization, for instance more than 10 different testing protocols have been published. In broad terms, a patient's ingests a known quantity of ROM and then subsequent has a plain abdominal radiograph undertaken at a defined time point, usually a number of days post ingestion, to define whether the transit is normal or delayed.

GASTRIC EMPTYING BREATH TESTING

Gastric emptying tests uses non-radioactive forms of carbon incorporated in safely ingestible food or liquid products. The substrate is ^{13}C-octanoic acid, which is labelled to a standardized meal, which is absorbed in the small intestine and metabolized to $^{13}CO_2$, which is then expelled from the lungs during respiration. The rate-limiting step in this conversion is gastric emptying. The breath test correlates well with scintigraphic findings, it has been proposed as a non-invasive, reliable test for measuring gastric emptying, without recourse to the use of ionizing radiation[42]. Additionally, the breath test is less expensive and easier to perform than scintigraphy and offers the added advantage of being able to be undertaken in the office environment and shipped to a laboratory for analysis[42].

WIRELESS MOTILITY CAPSULE

The wireless motility capsule (WMC) is an indigestible single-use capsule which provides a further option in which gastric emptying, small bowel transit and colonic transit times can be concurrently measured[43]. The WMC consists of a wireless transmitting capsule, a portable receiver worn by the patient for the duration of the test as well as analysis software. Following an overnight fast, the patient consumes a standardized meal of known fat and calorific content, which initiates postprandial motility patterns. Immediately after the meal, the patient ingests the WMC after which they are free to leave the clinical setting.

The WMC records pH, pressure and temperature as it transverses the GI tract. Gastric emptying time is reflected by an abrupt change in pH as the capsule moves from the acidic environment of the stomach to the alkaline environment of the duodenum. Small bowel transit time is the time from exit of the stomach to an abrupt pH drop of at least 1 pH unit around the ileocaecal junction. Colonic transit time is defined as time between caecal entry of capsule and its exit from the body. The whole gut transit time is the combined transit time of gastric emptying time, small bowel transit time and colonic transit time and is defined as delayed when greater than 73 h (Figure 3)[44]. Thus the WMC offers a minimally invasive alternative to the measurement of regional and whole gut transit. The capsule does not require any radiation, is standardized and can be carried out in most clinical settings. However, the WMC measures gastric emptying indirectly through the use of a physiologic meal. The pressure profiles are based on non-stationary, single point pressure measurements throughout the GI tract, which limits it utility in comparison to traditional manometric testing[45,46].

FUTURE DEVELOPMENTS

Trans-abdominal ultrasonography

Ultrasonography represents a simple non-invasive technique to evaluate gastric function. Although operator dependent, ultrasonography provides information on gastric emptying, with a high correlation with scintigraphic techniques.

Magnetic resonance imaging

Magnetic resonance-techniques offer a potentially exciting non-invasive method for evaluating segmental and global motility within the GI tract, although protocols are currently limited to the research sphere. Nevertheless, given the widespread distribution of magnetic resonance imaging scanners across many clinical centres, it is likely that this method of imaging may become the method of choice in the future. However, further work is needed to standardize protocols and testing conditions.

BIOCHEMICAL MARKERS OF NEUROINFLAMMATION IN DIABETES

Since the measurement of nerve conduction velocity *per se* is resource intensive, both in terms of equipment and specialist neurophysiological interpretation, the development and validation of non-invasive biomarkers remains an important priority. Considering the putative pathophysiological link between low-grade inflammation and diabetes[47], we shall highlight some novel biochemical biomarkers, which have the potential to complement

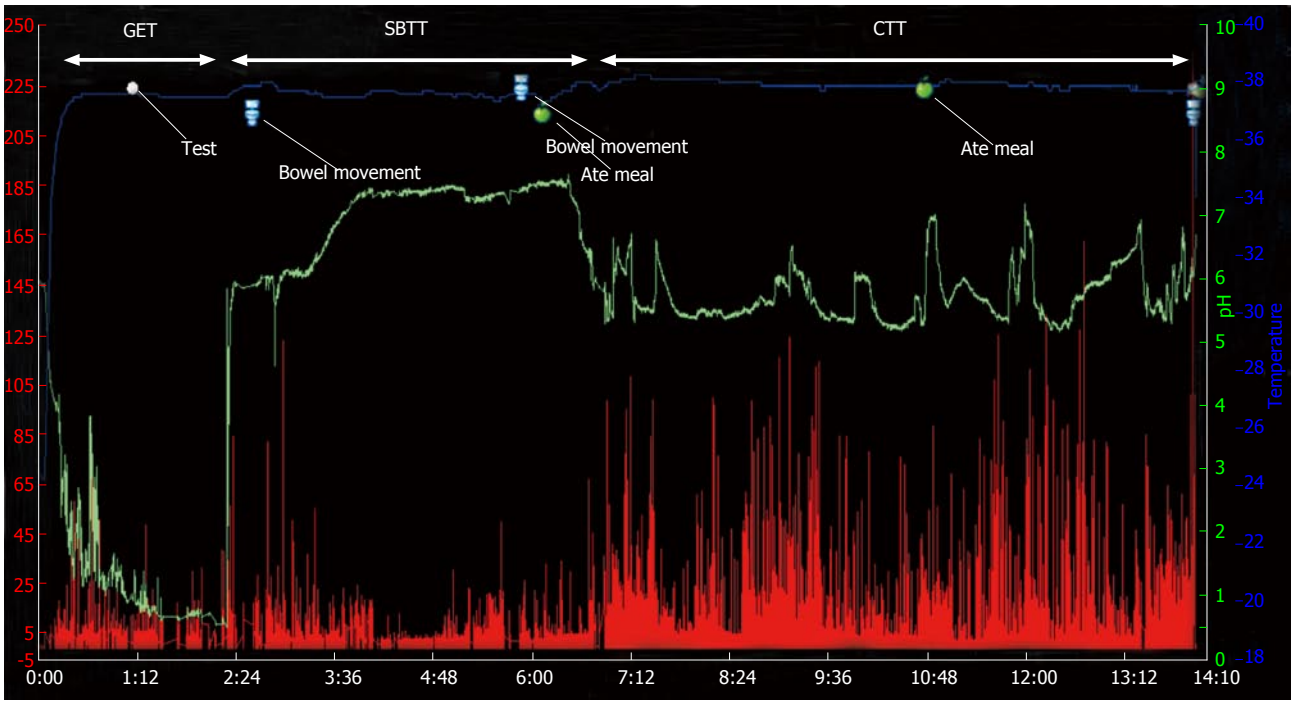

Figure 3 A typical tracing from a wireless motility capsule recording with time along the X-axis, pressure on the y1-axis (red line) and pH on the y2-axis (green line) and temperature (blue line). Gastric emptying time (GET), small bowel transit time (SBTT) and colonic transit time (CTT) are illustrated. Whole gut transit time is derived from the addition of GET, SBTT and CTT.

detailed neurophysiological testing in the future.

ANTIBODIES TO NEURAL STRUCTURES

There is increasing evidence that the immune system may play a role in the genesis and maintenance of diabetic neuropathy. Therefore, autoantibodies directed towards neuronal structures have received considerable attention, although reports are conflicting. For instance, Zanone *et al*[48] reported an association between autoantibodies directed against sympathetic ganglia, vagal afferents and the adrenal medulla in T1DM patients with symptomatic autonomic neuropathy.

In contrast however, Husebye *et al*[49] did not demonstrate any quantitative differences in autoantibodies binding to adrenal medulla in T1DM or T2DM in comparison to health controls. Nevertheless, the objective demonstration of a causal pathway where the identification of antigens directed towards specific end organ neuronal targets, which can be reversed with neutralising antibodies, remains a prerequisite step.

OXIDATIVE STRESS

Oxidative stress is considered a central facet in the development of diabetes and associated micro- and macrovascular complications. DNA and RNA oxidation have been linked to several diseases including diabetes. Whilst tissue specific levels of oxidation represent a single time point within a certain organ or cell system, urine excretion of 8-Oxo-2′-deoxyguanosine and 8-hydroxyguanine gives a more global measure of oxidative stress. Not unsurprisingly therefore, it has been argued that in multi-system disorders, such as diabetes, such measures of global oxidative stress are of more pertinence. While an association between increased excretion of 8-Oxo-2′-deoxyguanosine in both diabetic retinopathy and nephropathy has been shown, there remains a paucity of data concerning in those patients with neuropathy. However, increased levels of 8-Oxo-2′-deoxyguanosine have been demonstrated in neurodegenerative disorders, such as Alzheimer's disease, and therefore such markers warrant further objective evaluation in patients with diabetic neuropathy.

TUMOUR NECROSIS FACTOR-ALPHA

The influential cytokine theory of disease posits that a number of cytokines are involved in the maintenance of health and homeostasis within the peripheral, central and autonomic nervous systems. Cytokines are produced by cells from the immune system including mast cells, Schwann cells, fibroblasts and sensory neurons. Tumour necrosis factor-alpha (TNF-α) is a potent systemic pro-inflammatory cytokine and is a central component of the inflammatory response, in various immune mediated inflammatory diseases. It is a pathophysiological feature of such disorders, such as rheumatoid arthritis, which are characterised by chronic inflammation. TNF-α is produced in Schwann cells and has a role in peripheral nerve regeneration and regulation of apoptosis. Elevated concentrations of TNF-α and heightened disease activity in immune mediated inflammatory disorders is well described, however, more recently a similar associa-

tion has been reported with neuropathy in diabetes T1DM and T2DM[49]. As such, TNF-α in diabetes may play a role in the pathogenesis and development of diabetic neuropathy and therefore could represent a candidate biomarker for the presence, severity and progression of diabetic neuropathy. Recent evidence provides further support for this proposition, as it has been showed that T2DM patients with neuropathy had higher levels of TNF-α in comparison to patients without neuropathy and healthy controls[50]. In addition, an animal model of painful diabetic neuropathy showed that treatment with anti-TNF-α monoclonal antibody exhibited a neuroprotective effect[51]. Finally, Yamakawa *et al*[52] demonstrated that a single dose of anti-TNF-α attenuated the electrophysiological and biochemical deficits associated with diabetic neuropathy for at least 1 mo. To the best of our knowledge there are no reports in the literature of anti-TNF therapy being utilised in patients with established diabetic neuropathy, although it is plausible that it would benefit clinical symptoms in selected patient groups, although single case reports exists, which describes mixed sensorimotor neuropathies as a consequence of anti-TNF therapy.

SOLUBLE CD163

CD163 is an endocytotic receptor for haptoglobin-haemoglobin complexes, which is expressed exclusively in macrophages and monocytes. The extracellular portion of CD163 is soluble (sCD163) and circulates in the peripheral blood. Although the absolute function of sCD163 is incompletely understood, an association is observed between increased circulating levels of and chronic inflammatory states[53]. Interestingly, increased levels of sCD163 have been reproducibly demonstrated in diabetes[54]. Furthermore, sCD163 has been shown to be associated with insulin resistance in T2DM; an association that is independent of TNF-α[55]. In addition, a trend towards increased levels of sCD163 has very recently been demonstrated in cerebrospinal fluid in a preliminary study of patients with T2DM with established diabetic polyneuropathy as compared to matched controls without neuropathy[56]. Taken together, these data provide an interesting rationale for the further evaluation in larger prospective studies of sCD163 as a candidate biomarker, particularly as it links both inflammatory and neuropathic processes.

Although there is a current paucity of non-invasive diagnostic and prognostic biomarkers for diabetic neuropathy, there are a number of promising candidates. Whilst singularly each has their respective limitations, in combination a higher clinical utility may be derived in the future.

CONCLUSION

DAN remains an under-recognized complication, yet its symptomatic sequelae are troublesome and combine to reduce the quality of life and worsen prognosis in patients with diabetes. Although biomarkers for early identification of DAN and testing for ANS dysfunction and its specific end-organ complications, such as in the GI tract, remain in their infancy, further objective evaluation is warranted to improve detection rates and diagnostic accuracy, which may potentially lead to improved patient outcomes.

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