

The relationship between glycaemic variability and cardiovascular autonomic dysfunction in patients with type 1 diabetes: a systematic review

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ABSTRACT

Rigorous glycaemic control – reflected by low HbA1c goals – is of utmost importance in the prevention and management of complications in patients with type 1 diabetes mellitus (T1DM). However, previous studies suggested that short-term glycaemic variability (GV) is important to consider as well, as excessive glucose fluctuations may have an additional impact on the development of diabetic complications. The potential relationship between GV and the risk for cardiovascular autonomic neuropathy (CAN), a clinical expression of cardiovascular autonomic dysfunction, is of increasing interest. This systematic review aimed to summarize existing evidence concerning the relationship between GV and cardiovascular autonomic dysfunction in T1DM. Electronic database search of Medline (Pubmed), Web of Science and Embase was performed, up to October 2019. There were no limits concerning year of publication. Methodological quality was evaluated with the Newcastle Ottawa Scale for observational studies. Six studies (four cross-sectional and two prospective cohorts) were included. Methodological quality of the studies varied from level C to A2. Two studies examined the association between GV and heart rate variability (HRV) and both found significant negative correlations. Regarding cardiovascular autonomic reflex tests (CARTs), two studies did not while two other studies did find significant associations between GV parameters and CART-scores. However, associations were attenuated after adjusting for covariates such as HbA1c, age and disease duration. In conclusion, this systematic review found some preliminary evidence supporting an association between GV and cardiovascular autonomic dysfunction in T1DM. Hence, uncertainty remains whether high GV can independently contribute to the onset or progression of CAN. The heterogeneity in methodological approach made it difficult to compare different studies. Future studies should therefore use uniformly evaluated CGM-derived parameters of GV, while standardised assessment of HRV, CARTs and other potential cardiac autonomic function parameters is needed for an unambiguous definition of CAN.

KEYWORDS Type 1 diabetes · Complications · Glycaemic variability · Cardiovascular autonomic dysfunction · Cardiovascular autonomic neuropathy · Cardiovascular reflex tests · Heart rate variability

ABBREVIATIONS

BRS	Baroreflex sensitivity	HBGI	High blood glucose index
CAN	Cardiovascular autonomic neuropathy	HRV	Heart rate variability
CART	Cardiovascular autonomic reflex test	LBGI	Low blood glucose index
CGM	Continuous glucose monitoring	MBG	Mean blood glucose
CONGA	Continuous overlap net glycaemic action	MAG	Mean absolute glucose difference
COV	Coefficient of variation	MAGE	Mean amplitude of glycaemic excursions
DAN	Diabetic autonomic neuropathy	MODD	Mean of daily differences
DCCT	Diabetes Control and Complications Trial	SD _T	Total standard deviation of glucose values
GV	Glycaemic variability	T1DM	Type 1 diabetes mellitus

RESEARCH IN CONTEXT BOX

What is already known?

- *Short-term* glycaemic variability (GV) is an integral component of glucose homeostasis in patients with type 1 diabetes (T1DM), next to HbA1c as a *long-term* measure of glycaemic control.
- In-depth assessment of these blood glucose fluctuations is of increasing interest in latest years since previous research suggested a role in the development of diabetic complications.
- Cardiac autonomic neuropathy is associated with higher morbidity and increased CVD mortality in type 1 diabetes and assessment of heart rate variability and cardiovascular autonomic reflex function can be used as early screening strategies.

What is the key question?

- Does higher glycaemic variability independently contribute to the development of cardiovascular autonomic dysfunction in type 1 diabetes?

What are the new findings?

- High short-term GV might negatively affect heart rate variability (HRV).
- The impact of GV on cardiovascular autonomic reflex function is less certain.

Possible impact on clinical practice in the future?

- Increasing attention should be pointed towards a glycaemic management that goes beyond HbA1c, aiming at a stable glucose homeostasis with less glycaemic fluctuations as it could be crucial in avoiding the vicious circle of deteriorating autonomic function, recurrent hypoglycaemia and its consequences.

However, there is an urgent need of more research concerning this matter, preferably long-term prospective studies.

INTRODUCTION

Strict glycaemic follow-up is of utmost importance in type 1 diabetes (T1DM) management to prevent or delay the onset of diabetic complications. Since chronic hyperglycaemia remains the main determinant of these complications, as proven by the Diabetes Control and Complications Trial (DCCT)¹, HbA1c is still considered as the gold-standard measure of glycaemic control with a primary target to lower HbA1c as much as safely possible². However, even if patients display similar HbA1c values and mean blood glucose, their glucose profiles may greatly differ³. The importance of these short-term fluctuations is of increasing interest in the past decade, which has led to a new concept: glycaemic variability (GV), a term referring to intra- and inter-day glycaemic fluctuations³⁻⁶.

Concerning the methods to assess GV, older studies used 7-point capillary blood glucose profiles. Nowadays, continuous glucose monitoring (CGM) is considered as the number one method for the determination of short-term GV, with numerous metrics that have been established yet^{3,7}. GV is generally subdivided in within-day and inter-day variability^{3,7,8}. To reflect *within-day* GV, total standard deviation (SD_T), coefficient of variation (COV%) and mean amplitude of glucose excursions (MAGE) are recommended and most widely used, while the mean of daily differences (MODD) has been suggested to reflect *between-day* variability^{7,8}. Besides, these measures can be complemented with the high and low blood glucose index (HBGI, LBGI), taking separately into

account the risk for hypo- and hyperglycaemic events⁹. Other metrics have also been proposed but are rarely used^{7,10-14}.

The role of GV in the development of diabetic complications is of increasing interest and should not be underestimated¹⁵⁻¹⁸, as some studies indicated that GV could independently contribute to microvascular complications¹⁹⁻²¹. A well-known complication is diabetic autonomic neuropathy (DAN), which affects the autonomic nervous system. Regarding the latter, damage to autonomic nerve fibres innervating heart and blood vessels can result in cardiovascular autonomic neuropathy (CAN), an important yet still underestimated problem^{22,23}. Pathogenesis of CAN is complex, multifactorial and still under debate. Prevalence was reported to be between 15-35%^{23,24} and the most important risk factors include diabetes duration, high HbA1c and the conventional CV risk factors (hypertension, hyperlipidaemia and smoking)²³⁻²⁵. Clinical manifestations of CAN may range from latent signs such as nocturnal hypertension with non-dipping²⁶ to signs of resting tachycardia, exercise intolerance and more severe signs such as orthostatic hypotension, ECG abnormalities or even silent myocardial infarction^{22,24,27}. Detection of CAN is crucial as it is associated with high morbidity and mortality²⁷⁻²⁹, and can be evaluated by simple non-invasive methods: analysis of heart rate variability (HRV), cardiovascular reflex tests (CARTs) or baroreflex sensitivity (BRS)^{22,23,27,30}.

Some researchers suggested that GV may independently contribute to neuronal damage leading to the development of CAN. While studies in T2DM have already indicated that GV might influence cardiovascular autonomic function³¹⁻³⁴, the evidence in T1DM is scarce. Given the previous research and gaps in existing literature, the purpose of this systematic review was to review the current evidence on the relationship between existing parameters of GV and cardiovascular autonomic dysfunction in patients with T1DM.

METHODS

This review was written according to the international PRISMA guidelines for reporting systematic reviews³⁵.

Eligibility criteria

All studies that examined the association between parameters of GV and cardiovascular autonomic function in patients with T1DM were included (PICOS format). No restrictions were applied for age or disease duration of the patient population. All parameters used in current literature to express a degree of GV were accepted^{3,6}. Considering cardiovascular autonomic function, several outcomes could be reported (HRV, CARTs,...). Articles could be written in English, French or Dutch and no limits were used concerning publication year of eligible studies.

Information sources and search strategy

Studies were identified by electronic database search of Medline (PubMed), Web of Science and Embase. No filters were applied to these initial search strategies. Final searches on these databases were carried out on November 6th, 2018. The three searches were combined and duplicates removed. Additionally, a hand-searching was performed by checking the reference lists of obtained articles and an automatic search alert feed on PubMed and WoS notified whether new articles were published based on our search strategy, up to October 21, 2019. An overview of MeSH terms, keywords and final search strategies can be found in Appendix 1 (*Table S1.*)

Study selection

The screening team consisted out of four researchers and was divided into two groups of two people, with two researchers that screened first half of the articles and the second group evaluating the other part. A first screening was executed on title and abstract and a second screening on full-text. When screening was finished, a meeting was organized to discuss the results whereat any disagreements were resolved by oral discussion and consensus.

Methodological quality assessment of individual studies

To evaluate the risk of bias in selected studies, the Newcastle-Ottawa Scale for observational studies was used (Table 1) ³⁶. All articles – both cohort and cross-sectional studies – were scored with the subscale for cohorts. This 8-item checklist is subdivided into three domains: selection, comparability and outcome. Two researchers (S.H., K.V.W.) independently completed the checklist and subsequently discussed their results. Next, a level of evidence was assigned to each study following the EBRO (Evidence-Based Guideline Development) method ³⁷. Each study was graded a level of evidence ranging from D up to A1, depending on EBM domain, study design and individual methodological quality. Consequently, the strength of conclusion (level 1-4) was assigned to the different outcome levels, taking into account the composition of the respective levels of evidence and consistency of their results ³⁷.

Data items and extraction

Two researchers (S.H., K.V.W.) individually read the included articles and extracted data using a pre-determined data extraction form. Differences in data forms were resolved by consensus and all relevant information from the included studies was gathered and consistently summarized in the evidence table. The following data were extracted: (1) year of publication, (2) study design and - if present - follow-up period, (3) population characteristics, (4) parameters of GV, (5) outcome measures of cardiovascular autonomic function and (6) results for each outcome.

RESULTS

Study selection

The overview with the number of hits for each database search is shown in Supplementary Table S2. The combination of the three search strategies yielded 2574 hits and the hand-searching resulted in 2 additional studies. This was reduced to 2054 articles after removing duplicates. After the first screening, only 27 articles remained. The second screening resulted in a final number of 6 articles to be included. (*Figure 1. Flowchart study selection*)

Study characteristics

This review contains six eligible studies, of which four cross-sectional³⁸⁻⁴¹ and two prospective cohort studies with a follow-up of 3 and 10 years^{42,43}. The sample size of the cross-sectional studies varied between 20-80 patients and the cohort studies included respectively 41 and 1375 patients^{42,43}. All six studies included adults, with a mean age range between 27-47 years. Mean HbA1c values were similar between different study populations, within a range of 64-75 mmol/mol (8-9%) and mean time since diagnosis varied from 2.6 to 17.5 years. Considering the methods for determination of GV, five studies used CGM³⁸⁻⁴² for a duration between 1-6 days, while one study used 7-point capillary blood glucose profiles at 3 months intervals⁴³. SD and MAGE of glucose values were the most studied parameters of GV, determined in five studies^{38,40-43}. Hypoglycaemic indices (LBGI, AUC hypo and %-time in hypo) were reported in three studies^{39,40,42}. COV glucose and M-value (two studies); CONGA, MODD and MAG (one study) were used less frequently. All studies used CARTs or HRV to assess cardiac autonomic function, except one that examined blood pressure dipping⁴². Three studies also determined whether or not CAN was present, all of them basing CAN diagnosis on the results of CARTs^{40,41,43}.

Risk of bias within studies

Since it is impossible for T1DM patients not to demonstrate GV as it is omnipresent in this population, the 'non-exposed cohort'-criteria were not assessed, i.e. it was not applicable to score questions 2 and 5 of the NOS scale ('Selection of non-exposed cohort' and 'Comparability of cohorts', respectively). Question 3 (Ascertainment of exposure) was scored positive for all studies that used CGM since a complete glucose data profile is obtained which objectively reflects GV. The most common positive scored question was 'Ascertainment of exposure' (five studies) and 'Assessment of outcome' (all studies). One included publication obtained level of evidence A2⁴³, one study level B⁴² because of lacking the required A2 criteria³⁷ and a level C was granted to all four cross-sectional studies since these were non-comparative³⁸⁻⁴¹. Results of risk of bias assessment are shown in Table 1. Consequently, a level of conclusion 3 was assigned to the two outcomes, HRV and CARTs (*Synthesis of results*).

Summary of individual study results

An overview of all individual studies is shown in Table 2 and their key findings are presented in Table 3.

Synthesis of results: Relationship between GV and cardiovascular autonomic function in T1DM

Association between GV and HRV parameters. Two studies examined the relationship between short-term GV (both based on CGM) and HRV and both found significant negative associations^{38,39}. Significant negative associations between higher hypoglycaemic indices and LF power as a frequency-domain measure of HRV (LBGI-LF power: $R^2= 0.351$; AUC hypo-LF power: $R^2= 0.316$), were reported in a population without evidence of diabetic complications³⁹. Other research found significant negative correlations between COV-RR (time-domain HRV) and intraday GV parameters ($r=$ from -0.38 to -0.65), which was especially pronounced during night-time³⁸. Thus, high short-term GV might negatively affect HRV (level of conclusion 3).

Association between GV and CART parameters. Four studies evaluated CART parameters, as determinants of cardiovascular autonomic function or to assess whether or not CAN was present^{39-41,43}, but showed ambiguous results (level of conclusion 3). One study did not find significant associations between hypoglycaemic indices and CART-ratios³⁹, and the prospective cohort study based on data from the DCCT found no significant association between within-day GV (from 7-point capillary blood glucose) and the risk for CAN after adjusting for MBG⁴³. Two more recently published studies (2018) both reported significant associations between GV and CAN, however, they did not completely confirm each other as they used different covariates in statistical analysis. The study of Njiraty *et al.* found a tendency of higher GV in the CAN group ($n=10/20$), as well as significant positive correlations between two GV parameters (SD, MAG) and CAN-scores (based on CARTs; $r=$ from 0.47 to 0.62), but only one remained significant (SD glucose – SBP fall; $r= 0.49$) after adjusting for HbA1c, age and disease duration⁴¹. On the other hand, the study of Jun *et al.* in 80 patients found that several parameters of GV (SD, COV, MAGE, LBGI, HBGI, %-time in hypoglycaemia and AUC hypo) were independently associated with CAN, all of this after adjusting for MBG and clinical risk factors. Level 2 hypoglycaemia (<54 mg/dl) showed the highest relative contribution to this association in this study (standardized weight= 0.4135; $p= 0.001$)⁴⁰.

DISCUSSION

Statement of principal findings: summary of evidence

This systematic review examined the association between GV – a concept increasingly gaining attention – and CAN, a clinical expression of cardiovascular autonomic dysfunction and an underestimated problem in T1DM. The scarcity of data (only 6 studies), together with the null finding from prospective studies, makes it hard to draw solid conclusions and points to an urgent need for more research into this relationship. Concerning the impact of GV on HRV, as a measure of CAN, high short-term GV might negatively affect parameters of HRV as shown by two cross-sectional studies (level of conclusion 3)^{38,39}. Regarding the association between GV and the results of CARTs as determinants of cardiovascular autonomic function, ambiguous results were reported by included studies⁴⁰⁻⁴³. While two studies did not find significant associations between GV indices and CART-parameters after adjusting for mean blood glucose (MBG)^{39,43}, two more recent studies however, did find significance^{40,41}. Hence, uncertainty remains about the impact of GV on cardiovascular autonomic reflex function (level of conclusion 3).

Methodological considerations of GV and CAN assessment – Strengths and weaknesses of included studies

Heterogeneity in methodological approach – both for GV and CAN assessment – makes it difficult to fully compare the different study results and therefore hard to draw solid conclusions about their relationship.

Cardiovascular autonomic function. Regarding HRV, assessment should take place under well-standardised conditions as recommended by the Task Force on HRV⁴⁴. Spectral analysis of RR-intervals (for determination of frequency-domain HRV) is most valid and reliable if monitored under resting conditions (concept of *stationarity*), preferably after ten minutes of supine rest in a darkened room and abstained from nicotine, caffeine or pharmacologic agents that interfere with HR or blood pressure⁴⁴. Once again, this is often not reported in studies. HRV assessment should comprise both time- and frequency-domain measures. This was not the case in two studies, with the evaluation of only time- or frequency-domain HRV, respectively^{38,39}. From the existing multiplicity of time-domain measures, the Task Force recommends four of them (SDNN, SDANN, HRV triangular index and RMSSD)⁴⁴. Furthermore, it is not useful to compare time-domain measures obtained from recordings of different durations. Duration should be standardized and a short-term five-minute resting evaluation or an entire 24h-recording are both useful^{44,45}. Also for CARTs, studies should strictly follow recommendations on how to perform and interpret these tests since several confounding factors have been identified^{46,47}.

CAN diagnosis: clear cut case or tricky business? Next to the heterogeneity in HRV analysis and CART evaluation, the diagnosis of CAN is not unambiguous as well⁴⁷. Moreover, this differed between included studies as other criteria were used for CAN diagnosis. All studies based CAN diagnosis on the results of CARTs, however with different interpretations of these tests^{40,41,43}. Although different criteria for CAN are found in literature, studies should adhere to one clear algorithm⁴⁷. CARTs, if executed correctly, are considered the gold-standard in CAN diagnosis since they are safe, non-invasive and can be well-standardized^{23,24}. The traditional classification in

early, confirmed and severe CAN remains useful⁴⁷⁻⁴⁹ but an 'autonomic neuropathy score' – obtained by scoring the results of CARTs – is advised because of its dual advantage of evaluating the progression of CAN and providing an overall quantitative result⁴⁶. Secondly, HRV monitoring is a valuable tool for assessing cardiovascular autonomic function, with some researchers even claiming that it is the most sensitive and specific method^{50,51}. HRV could detect early changes in myocardial autonomic innervation and thus CAN in sub-clinical stages^{23,52,53}, even in young patients⁵⁴. In our opinion, both HRV analysis and CARTs should be performed in order to have extensive insight into patients' cardiovascular autonomic function and potential CAN diagnosis^{23,24,47}. Moreover, by evaluating both HRV and CARTs, the cardiovascular branches of the ANS are examined at rest as well as under stress conditions, as resting HRV reflects the sympathetic and parasympathetic HR modulation while the CARTs mainly evaluate the capacity of the cardiovascular reflexes to react on stimuli. Moreover, assessment of cardiovascular autonomic function can also comprise other aspects, such as evaluation of baroreflex function measured by baroreflex sensitivity (BRS). As BRS was found to be reduced in T1DM⁵⁵⁻⁵⁷ and able to differentiate patients with CAN from those without⁵⁸, it could be a valuable adjunct in reflecting cardiovascular autonomic function^{58,59}. BRS can be estimated non-invasively through analysis of spontaneous beat-to-beat fluctuations of BP and R-R interval lengths on ECG⁵⁹.

Non-dipping blood pressure: phenomenon needing attention. The clinical importance of this aberrant BP pattern is well-known since non-dippers showed a higher incidence of cardiovascular events and target organ damage^{60,61}. There is still uncertainty about the exact pathogenesis of this loss of normal nocturnal BP decrease. Hypertension, renal function impairment and altered nocturnal sympathovagal balance are often linked to the aetiology of non-dipping^{61,62} and non-dipping is sometimes considered as a surrogate measure of CAN⁶³⁻⁶⁶. Concerning the role of GV, higher postprandial BG excursions were found to be related to non-dipping in T2DM, with both endothelial and autonomic nervous dysfunction (sympathetic overdrive) proposed as potential mechanistic linkages⁶⁴. In this review, only one study examined the association between non-dipping and GV but did not find significance, not at baseline nor after follow-up⁴². However, this was only for three years, which might have been too short to detect any significant association. Still, the authors stated that GV can act as a potential risk factor driving non-dipping, which may lead to silent CVD⁴². In the future, mechanisms of non-dipping, as well as the associations between abnormal diurnal variation in BP and blood glucose, need further research.

Assessment of glycaemic variability. Only CGM provides a full picture of short-term glucose fluctuations and consequently captures the full degree of GV, as CGM-outputs comprise 5-min interval glucose values from which all existing parameters can be calculated^{67,68}. The 7-point capillary BG profile on the other hand, used in the study of Lachin *et al.* with data from the DCCT⁴³, is insufficient to characterize GV correctly, as the validity of this method is strongly dependent on the timing and number of finger prick measurements throughout the day and often many data points are missing⁶⁹⁻⁷¹. However, multiple imputation methods were used in the study of Lachin

et al., making their results somewhat more reliable⁴³. Regarding the use of CGM in research, the system should be used for at least seven days⁶⁸, which conflicts with two studies that only implemented CGM for two and three days, respectively^{38,40}. Besides this, to get a reliable picture of the everyday GV of a patient, standardised instructions are needed. Patients should retain their normal activities of daily living including usual eating habits and physical activity level, which is often not specified in the methodology section of included studies.

The plethora of existing GV parameters: bless or burden? Many different parameters were used in the included studies to reflect GV, but one should be cautious when interpreting or comparing results of studies since not all indices are equivalent as they can represent different concepts⁷². Some of these parameters are highly correlated and actually comprise the same information, while others do not⁷³. A combination of the most relevant indices is necessary to effectively describe GV. For example, COV is more valid than SD in reflecting *overall* GV⁷⁴ and more sensitive in predicting hypoglycaemia^{9,74}, while the MAGE index mainly reflects mealtime-related GV⁷. The importance of avoiding excessive post-meal glucose excursions is evidently acknowledged since this substantially contributes to hyperglycaemic exposure and thus MBG⁷⁵. Therefore, the advantage of MAGE is that it only assesses major glucose swings and excludes minor ones, being considered as a measure of unstable diabetes⁷⁶. Also between-day GV – assessed by the MODD – needs further investigation. In this review, however, only one study reported this parameter³⁸. In conclusion, future studies should focus on standardizing the methods for GV assessment and its two most important components: amplitude and timing^{68,77}. A uniform set of GV parameters is needed and studies should separately report the results per parameter. Fabris *et al.* showed that more than 25 parameters could be reduced to a set of 10, comprising all necessary information on GV⁷⁸. More studies are needed to examine which of these GV parameters offer advantage in predicting diabetic complications⁷². Importantly however, most of aforementioned parameters (SD, MAGE, MODD) are still dependent on mean glycaemia. Simply adjusting for MBG might not be enough to exclude the possibility that the observed association actually comes from the difference in MBG. COV and MAG at the contrary are not dependent on MBG and have been recommended by DeVries *et al.* to reflect true glucose variability⁷⁹. Taking this into consideration and looking closer at included studies, we evaluated the impact of excluding the mean-dependent CGM-parameters. Iwasaki *et al.*³⁸ assessed *only* mean-dependent parameters (SD, MAGE, M-value, MODD), which makes this small cross-sectional study somewhat less robust. Also for the study of Njiraty *et al.*⁴¹, uncertainty increased if we exclude SD glucose as parameter for GV, as this was the only remaining significant correlation in the multiple regression model. On the other hand, interpretation of the study results of Jun *et al.*⁴⁰ (assessing SD, MAGE and COV) did not change, as COV was significantly associated with CAN risk in all regression models.

Hypoglycaemia as underlying disturbing factor. Lowering patient's MBG is only possible if GV is constrained, otherwise BG fluctuations would inevitably enter the range of hypoglycaemia⁸⁰. Increased hypoglycaemia due to excess GV has been demonstrated in several studies, showing GV to be a predictor of hypoglycaemic events^{78,81,82}.

Therefore, it is interesting to explore whether the increased hypoglycaemia is more important than GV itself and whether it is independently associated with the outcomes of interest. Hypoglycaemia was found to be associated with lengthening of the QTc interval on ECG^{83,84} and reduction of cardiac vagal outflow with diminished HRV⁸⁵⁻⁸⁷. Hence, the impact of hypoglycaemia on cardiac autonomic regulation should not be neglected⁸⁸. In this review, two studies evaluated parameters describing hypoglycaemia and both found significant results^{39,40}. However, several parameters can characterise hypoglycaemia: area under the curve (AUC hypo), %time spent in hypoglycaemia, number of hypoglycaemic events or the LBGI (risk index). The study of Jun *et al.* found significant results for all of these hypoglycaemic parameters⁴⁰. Once again, these indices are not equivalent as they reflect different aspects. The AUC hypo gives complete insight into the *severity* of hypoglycaemia since there is a contribution from both time of exposure and level of hypoglycaemia; while time spent in hypoglycaemia (%) only reflects total *duration* but not severity. Separate monitoring of time in level 2 hypoglycaemia (<54 mg/dl) showed the highest contribution to the reported associations and can provide a solution for this issue⁴⁰. Hence, future studies should further elucidate the importance of (level 2) hypoglycaemia, next to parameters of GV.

Covariates in the relationship between GV and CAN. Lastly, as with many studies, study populations were rather heterogeneous: from adolescents⁸⁹ to complication-free patients^{39,40,42} and patients with established complication status^{38,41}. The question remains if results can be generalised for the entire patient spectrum since the effects of GV could vary throughout the broad range of disease duration and complication status. These aspects should be as much as possibly adjusted for in statistical analysis but varied widely between included studies. While Jun *et al.* used age, HbA1c and disease duration as covariates⁴⁰, the study of Njiraty *et al.* adjusted for smoking, BMI and SBP⁴¹ and Virk *et al.* also adjusted for body height and socio-economic status⁸⁹. Future studies should uniformly adjust only for the *known* risk factors of CAN (as pointed out previously in the introduction of this review) as covariates in their analyses and thereby decrease the risk for overfitting.

Role of GV in diabetic complications: time to shed clarity on this unanswered question?

Regarding the underlying pathophysiology, the frequent occurrence of both excessive glucose decrements and increments increases the amount of oxidative stress and inflammatory markers, which was reported to be the key mediator in the detrimental effect on vascular and nerve function^{8,18,90-94}. While some *in vitro* studies found that acute glycaemic swings can induce endothelial cell damage via excessive formation of free oxygen radicals⁹⁵, *in vivo* research did not confirm this association between high GV and increased oxidative stress (by urinary excretion rates of 8-iso-PGF2 α) in T1DM patients⁹⁶. The term *Pyramid of the Risk* was introduced after the finding that GV – next to the classic glucose triad of mean, fasting and postprandial BG – may also have deleterious effects on the development of diabetic complications⁹⁷. Previous reviews concluded that GV has if any, only minimal impact on the development of diabetic complications, however, most of the studies in these reviews re-analysed data from the DCCT in which GV was determined using 7-point capillary BG profiles and not CGM^{15,16}. A recent review from The Lancet concluded that it has not yet been *definitively* confirmed whether GV is an

independent risk factor for diabetes complications⁶. Concerning the relationship of GV with CAN in T1DM in particular, only a few studies focused on this association, all of them included in this review.

Causality and importance of the relationship between GV and cardiovascular autonomic dysfunction. The cross-sectional nature of many studies prevents inferring a causal relationship. Furthermore, one can also question the *direction* of this possible association, i.e. ‘*what is cause and consequence and vice versa?*’. While GV may negatively impact sympathovagal balance by increasing oxidative stress as mentioned before^{22,98}, autonomic dysfunction on the other hand may reciprocally affect GV due to disturbed glucose counter-regulation and decreased hypoglycaemic awareness^{88,99}. These latter two are often present in long-standing T1DM and may result in even more pronounced fluctuations and hypoglycaemic events^{100,101}. In summary, the direction of the association remains to be elucidated, but is perhaps bidirectional as it is not clear whether autonomic dysfunction drives GV or the other way around.

Strengths and limitations of this review

This systematic review comprises a comprehensive literature search up to 2019 with extensive search strategies on three major databases. The methodological quality of included studies was carefully and critically assessed with levels of evidence based on the risk of bias. Finally, we present a thorough discussion of important methodological considerations as well as implications for further research. Potential limitations of this review could include the limited amount of studies – with four cross-sectional and two prospective studies – and inclusion of only articles written in English, French or Dutch.

CONCLUSION

This systematic review found some preliminary evidence supporting the hypothesis of an association between GV and cardiovascular autonomic dysfunction in T1DM. The scarcity of data (only 6 studies), together with the null finding from prospective studies, makes it hard to draw solid conclusions and points to an urgent need for more research into this relationship. At this moment, uncertainty remains whether high GV can independently contribute to the onset or progression of CAN. The heterogeneity in methodological approach makes it difficult to compare different studies. Future studies should therefore use uniformly evaluated CGM-derived parameters of GV, while standardised assessment of HRV, CARTs and other potential cardiac autonomic function parameters is needed for an unambiguous definition of CAN.

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STUDY (DESIGN)	CRITERIA						INDIVIDUAL STUDY QUALITY: LEVEL OF EVIDENCE
	1	3	4	6	7	8	
Jaiswal <i>et al.</i> (2014) (cross-sectional)	-	+	/	+	/	/	C
Iwasaki <i>et al.</i> (2015) (cross-sectional)	+	+	/	+	/	/	C
Lachin <i>et al.</i> (2017) (prospective cohort)	+	-	+	+	+	+	A2
Nyiraty <i>et al.</i> (2018) (cross-sectional)	+	+	/	+	/	/	C

Jaiswal <i>et al.</i> (2018) (prospective cohort)	-	+	?	+	?	+	B
Jun <i>et al.</i> (2018) (cross-sectional)	+	+	/	+	/	/	C
<p>Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies</p> <p><u>Selection of cohort(s):</u> 1: Representativeness of the exposed cohort; 3: Ascertainment of exposure; 4: Demonstration that outcome was not present at start of the study;</p> <p><u>Outcome assessment:</u> 6: Assessment of outcome; 7: Follow-up long enough for outcomes to occur; 8: Adequacy of follow-up of cohorts</p> <p>“+”: yes; “-”: no; “?”: unknown, not mentioned or uncertain; “/”: not applicable to score</p> <p><i>NOTE: Since it is impossible not to demonstrate glycaemic variability, there was no ‘non-exposed cohort’ present in the included studies, making it not applicable to score questions 2 and 5 of the NOS scale (‘selection of the non-exposed cohort’ and ‘comparability of the cohorts’, respectively).</i></p>							

TABLE 1. Risk of bias assessment in individual studies.

First author (year of publication)	Study design (Follow-up)	Population characteristics	Glycaemic variability: method of assessment & parameters	Cardiovascular autonomic function: outcomes	Results
Saiswal <i>et al.</i> ³⁹ (2014)	Cross-sectional analysis baseline (/)	n= 44 (17 M, 27 F) Age: 34 ±13 yrs (range:18-65) DD: 13 ± 6 yrs (all >5 yrs) BMI: 26 ± 4 HbA1c: 64 mmol/ mol (8.0 ± 1.2%) TDI: 0.7 ± 0.2 U/kg/day	CGM for 5 days <u>Indices of hypoglycemic stress</u> <ul style="list-style-type: none"> ▪ LBGI ▪ AUC for hypoglycaemia (<70 mg/dl) 	<u>HRV (rest and during CART)</u> LF power HF power <u>CARTs</u> Deep breathing: E:I-ratio Valsalva ratio Standing: 30:15 ratio	<u>Correlations between hypoglycaemic indices and HRV</u> → LBGI: Significant correlations with LF power (r=-0.47; p=0.002**) and HF power (r=-0.37, p=0.018*) → AUC: Significant correlations with LF power (r=-0.43; p=0.005**) and HF power (r=-0.38; p=0.015*) <u>Linear regression:</u> <u>Unadjusted model: all associations significant</u> → LBGI – LF power: R ² =0.220; LBGI – HF power: R ² =0.135 → AUC hypo – LF power: R ² =0.185; AUC hypo – HF power: R ² =0.143 <u>Adjusted models:</u> → All associations with LF power persisted after adjusting for HbA1, age, DD, BMI (LBGI: R ² =0.351; AUC hypo: R ² =0.316) → Associations with HF power remained significant in adjusting model for HbA1c (LBGI: R ² =0.199; AUC hypo: R ² =0.207), but disappeared after adjusting for other covariates (NS) → No significant associations between LBGI/AUC and CART ratios
Iwasaki <i>et al.</i> ³⁸ (2015)	Cross-sectional (/)	n= 31 (13 M, 18 F) Hospitalized Age: 47.2 ± 16.4 yrs DD: 18.6 ± 12.8 yrs BMI: 22.4 ± 3.7 HbA1c: 64 mmol/mol (8.0 ± 1.5%) MDI: n=20, CSII: n=11	CGM for >24 h (56.5 ± 15.3h) <u>Intraday GV</u> <ul style="list-style-type: none"> ▪ SD glucose ▪ MAGE <u>Hybrid: MBG & GV</u> <ul style="list-style-type: none"> ▪ M-value <u>Inter-day GV (2days)</u> <ul style="list-style-type: none"> ▪ MODD 	<u>HRV</u> COV of RR-interval	<u>Correlations between COV-RR and GV measures</u> → Significant correlations with whole day SD (r=-0.50; p=0.007**), MAGE (r=-0.47; p=0.011*), M-value (r=-0.38; p=0.048*) and MODD (r=-0.59; p=0.001***) → Significant correlations with nighttime SD (r=-0.59, p=0.001***), MAGE (r=-0.47; p=0.011*), M-value (r=-0.53; p=0.004**) and MODD (r=-0.65; p=0.0003***) → Significant correlations with daytime SD (r=-0.44; p=0.019*) and MAGE (r=-0.50; p=0.006**); No significant correlation with daytime M-value and MODD (NS)
Lachin <i>et al.</i> ⁴³ (2017)	Prospective cohort	n= 1375 (M/F: almost equal) Age: 27 ± 7 yrs	7-point capillary blood glucose measurement,	<u>CAN - based on: CARTs</u> Deep breathing: ΔRR-int.	<u>Longitudinal logistic regression model for repeated measures, using Generalized Estimating Equations for CAN over 2, 4, 6 and 8 yrs follow-up</u>

	1983 - 1993 (10 yrs)	DD: 1-15 yrs (all) <i>Primary prevention group</i> : 2.6 ± 1.4 yrs <i>Secondary prevention group</i> : 8.7 ± 3.8 yrs HbA1c: 74 mmol/mol (8.9%) TDI: 0.62-0.71 ± 0.23 U/kg/day respectively No CAN at baseline	Every 3 months <u>Within-day GV</u> <ul style="list-style-type: none"> ▪ SD glucose ▪ MAGE ▪ M-value 	Valsalva ratio Standing: DBP drop CAN if <predefined cut-offs on at least one CART CAN assessment: at baseline and after 2,4,6,8 years of follow-up	→ Unadjusted model: CAN significantly associated with MAGE (p=0.017*) and M-value (p=0.046*) → After adjusting for MBG: None of the measures of within-day GV (SD glucose, MAGE, M-value) remained significantly associated with CAN (p=0.20; p=0.06; p=0.10)
Nyiraty <i>et al.</i> ⁴¹ (2018)	Cross-sectional (/)	n= 20 (6 M, 14 F) Age: 39.5 ± 3.4 yrs DD: 17.5 ± 2.5 yrs BMI: 22.3 ± 0.8 HbA1c: 65 mmol/mol (8.1 ± 0.7%) TDI: 42.8 ± 2.9 U All treated w/ MDI	CGM for 6 days <ul style="list-style-type: none"> ▪ SD glucose ▪ MAGE ▪ CONGA ▪ MAG 	<u>CAN & CAN-score (/10) - based on: CARTs</u> <u>CARTs</u> Deep breathing: ΔHR Valsalva ratio Standing: 30:15 ratio Standing: SBP drop Handgrip: DBP rise CAN if score ≥2/10	<u>Between-groups-analysis: CAN vs no CAN-group</u> → No significant difference in GV parameters between 2 groups; although tendency of higher GV in CAN group (n=10) <u>Correlations between GV measures and CARTs/CAN</u> → SD glucose: significant correlations with CAN-score (r=0.47; p<0.05*) and SBP drop (r=0.51; p<0.05*) → MAG: significant correlations with CAN-score (r=0.62; p<0.01**), 30:15 ratio (r=-0.50; p<0.05*) and SBP-drop (r=0.59; p<0.01**) → MAGE & CONGA: no sign. correlations with CAN-score or CARTs <u>Multivariate linear regression</u> → After adjusting for HbA1c, age and DD → one correlation remained significant: SD glucose – SBP drop (r=0.49; p<0.05*)
Jaiswal <i>et al.</i> ⁴² (2018)	Prospective cohort (3 yrs)	n= 41 (16 M, 25 F) Age: 34 ± 13 yrs (range:18-65) DD: 13 ± 6 (All DD >5 yrs) BMI: 26 ± 4 HbA1c: 64 mmol/mol (8.0 ± 1.2%) TDI: 0.7 ± 0.2 U/kg/day	CGM for 5 days Every 3 months <ul style="list-style-type: none"> ▪ COV glucose ▪ MAGE ▪ LBGI & HBGI ▪ AUC for hypoglycaemia ▪ AUC for hyperglycaemia 	Blood pressure dipping (%) ABPM assessment: every year	<u>Between-groups-analysis at baseline: Non-dipping group (n=4) vs Dipping group (n=37):</u> → No significant differences in GV measures <u>Correlations at baseline</u> → No significant correlations at baseline between BP dipping-% and GV measures <u>Between-groups-analysis after 3 yrs: Progressors group (n=24) vs Non-progressors group (n=17):</u> → No significant differences in GV measures
Jun <i>et al.</i> ⁴⁰ (2018)	Cross-sectional (/)	n= 80 (35 M, 45 F) Age: 39.9 ± 14 yrs	CGM for 3 days, of which middle 48h used	<u>CAN - based on: CARTs</u> Deep breathing: EI-ratio	<u>Between-groups-analysis: CAN-group (n=36) vs No CAN-group (n=44):</u> → Higher SD (p<0.001***), COV (p=0.007**) and MAGE (p=0.003**)

	<p>DD: 10.1 ± 7.3 BMI: 22.6 ± 3.2 HbA1c: 66 mmol/mol (8.2 ± 1.7%) TDI: not mentioned MDI: n=60; CSII: n=3; only basal: n=17 Smoking: n=5</p>	<p><u>GV:</u></p> <ul style="list-style-type: none"> ▪ SD glucose ▪ COV glucose ▪ MAGE ▪ LBGi & HBGI <p><u>Hypoglycaemic stress:</u></p> <ul style="list-style-type: none"> ▪ AUC for hypoglycaemia ▪ AUC for hyperglycaemia ▪ %-time hypoglycaemia ▪ %-time hypoglycaemia ▪ %-time nocturnal hypoglycaemia 	<p>Valsalva ratio 30:15 ratio Standing: SBP drop CAN if: 2/3 abnormal parasympathetic CARTs or orthostatic hypotension</p>	<p>→ More extreme peak and nadirs were observed in CAN group → Higher %-time in level 2 hypo (p=0.001***), AUC for level 2 hypo (p=0.001***), %-time in nocturnal level 2 hypo (p=0.001***), number of nocturnal level 2 hypo episodes (p=0.008**) and LBGi (p=0.004**) → Higher AUC for level 2 hyper (p=0.008**) and HBGI (p=0.003**)</p> <p><u>Multivariate logistic regression: Associations CGM parameters and CAN risk</u></p> <p><i>Model 1: adjusting for age, sex, BMI, SBP, HbA1c, LDL, current smoking status, DD and use of antihypertensive drugs</i></p> <p><i>Model 2: further adjusting for MBG</i></p> <p style="text-align: center;">↓</p> <p>GV/CGM parameters that increase risk for CAN: → SD (OR=1.05; p=0.005**), COV (OR=1.11; p=0.001***) and MAGE (OR=1.02; p=0.019*) → AUC for level 2 hypo (OR= 3.19, p=0.001***), %-time in level 1 and 2 hypo (OR= 7.55, p=0.020*; OR= 38.60, p=0.001***), %-time in nocturnal level 1 and 2 hypo (OR=7.55, p=0.020*; OR= 113.24, p=0.001***) → LBGi (OR= 1.29; p=0.001***) and HBGI (OR= 1.23; p=0.01**)</p> <p><u>Predictive value of CGM parameters for CAN after adjusting for MBG (model 2)</u></p> <p>→ %-time in level 2 hypo and nocturnal level 2 hypo highest predictive value for CAN (p<0.001***); AUC for level 2 hypo & LBGi follow with also strong predictive value (p<0.001***); SD, COV, MAGE and HBGI also significant (p=0.003**; p=0.001***; p=0.017*; p=0.012*)</p> <p><u>Relative contribution of CGM parameters (dominance analysis)</u></p> <p>→ %-time in level 2 hypo (model 1) and %-time in nocturnal level 2 hypo (model 2) highest ranked variables (standardized weight 0.4135 and 0.3636)</p>
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Level of significance: *: p<0.05; **: p<0.01; ***: p<0.001; (NS): not significant

Table legend:

n: sample size; M/F: male/female; yrs: years; DD: diabetes duration (time since diagnosis, yrs); MBG: mean blood glucose; BMI: body mass index (kg.m⁻²); TDI: total daily insulin dose; MDI: multiple daily injections of insulin; CSII: continuous subcutaneous insulin infusion (insulin pump); CVD: cardiovascular disease; CKD: chronic kidney disease; CGM: continuous glucose monitoring; GV: glycaemic variability; SD: standard deviation; COV: coefficient of variation (SD/MBG); MAGE: mean amplitude of glycaemic excursions; MODD: mean of daily differences; LBGi/HBGI: low/high blood glucose index; AUC: area

under the curve; CAN: cardiac/cardiovascular autonomic neuropathy; HRV: heart rate variability; LF/HF: low/high frequency; CART: cardiovascular autonomic reflex test; E:I-ratio: expiration:inspiration-ratio; SBP/DBP: systolic/diastolic blood pressure; ABPM: ambulatory blood pressure monitoring; OR: odds ratio

TABLE 2. Evidence table: Results of included studies assessing the relationship between glycaemic variability and cardiovascular autonomic function in type 1 diabetes.

First author (year of publication)	Study design (Follow-up)	Key findings & Main conclusions
Jaiswal <i>et al.</i> (2014) ³⁹	Cross-sectional analysis at baseline	<i>Increased GV promoting hypoglycemic stress is associated with reduced HRV, independent of glycemic control as assessed by HbA1c. Inverse associations persisted after adjusting for HbA1c but attenuated by age, DD and BMI.</i>

Iwasaki <i>et al.</i> (2015) ³⁸	Cross-sectional	<i>COV-RR, as a parameter of HRV, is closely associated with GV, showing negative correlations with whole-day, daytime and – especially – nighttime variables of GV.</i>
Lachin <i>et al.</i> (2017) ⁴³	Prospective cohort 1983 – 1993 (10 yrs)	<i>Overall, within-day GV (determined from quarterly glucose profiles) does not play an apparent role in the development of CAN (based on three CARTs) beyond the influence of MBG. Adjusted for MBG, no measure of within-day variability was associated with any outcome.</i>
Nyiraty <i>et al.</i> (2018) ⁴¹	Cross-sectional	<i>GV (on CGM) correlates with both parasympathetic and sympathetic dysfunctions (CAN score, orthostatic SBP fall, CARTs). Increasing values of SD and MAG are observed in the presence of more severe CAN.</i>
Jaiswal <i>et al.</i> (2018) ⁴²	Prospective cohort 2014-2017 (3 yrs)	<i>No significant correlations were found at baseline between non-dipping nocturnal blood pressure and measures of GV, and GV did not explain differences in the risk for progression to non-dipping over time.</i>
Jun <i>et al.</i> (2018) ⁴⁰	Cross-sectional	<i>CGM-defined GV is associated with CAN (CARTs, orthostatic hypotension) independent of MBG and other clinical risk factors for CAN. The GV parameters that describe level 2 hypoglycaemia (<54 mg/dl) were the strongest contributors to this association.</i>
Table legend:		
<i>GV: glycaemic variability; HRV: heart rate variability; MBG: mean blood glucose; DD: diabetes duration (time since diagnosis, years); CGM: continuous glucose monitoring; SD: standard deviation; COV: coefficient of variation; CAN: cardiac/cardiovascular autonomic neuropathy; CART: cardiovascular autonomic reflex test.</i>		

TABLE 3. Main conclusions of included studies.

The relationship between glycaemic variability and cardiovascular autonomic dysfunction in patients with type 1 diabetes: a systematic review

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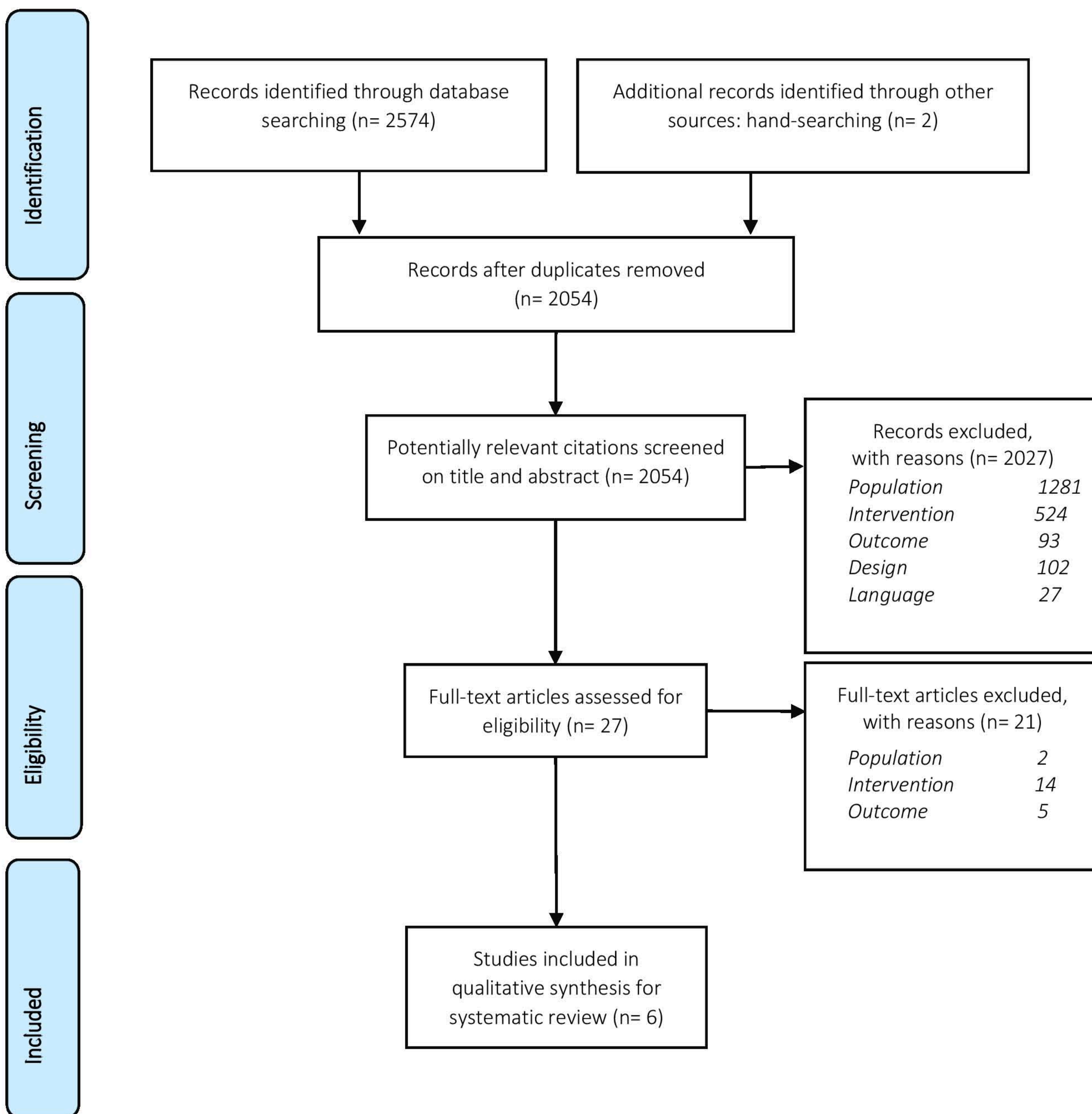


Fig. 1. Flowchart study selection.