Title: [Gastrointestinal Complications in Patients with Myelodysplastic Syndrome or Acute Myeloid Leukemia Undergoing Treatment with 5-Azacitidine (GAS-COL)]
Titel: [Gastrointestinale Komplikationer hos Patienter med Myleodysplastisk Syndrom eller Akut Myeloid Leukæmi i Behandling med 5-Azacitidin (GAS-COL)]
Semester: [5. semester]
Semester theme: [Master's thesis/Kandidatprojekt]
Project period: [6/9 2021 - 6/1 2022]
ECTS: [30]
Language: English
Supervisor: [Christina Brock]
Project group: [63e21au5]

[Victoria Sarah Zaugg - 20156636]

[Ida Marie Nørum Wigh - 20164215]

AALBORG UNIVERSITET
STUDENTERRAPPORT

Words: [4911]
Page count: [22 pages]
Appendix: [31 pages]

English Abstract

Aims: We hypothesized that gastrointestinal symptoms in treatment with 5-azacitidine, including nausea, are associated with gastric emptying, and autonomic dysfunction coexists with altered gastrointestinal transit.
Background: Patients with myelodysplastic syndrome and acute myeloid leukemia can receive life prolonging treatment with 5-azacitidine. The patients often experience adverse effects from the gastrointestinal tract, particularly nausea and vomiting, affecting the quality of life. Causes of nausea are comprehensive and may be triggered both centrally and peripherally. No previous studies have investigated the underlying causes of nausea in patients treated with 5-azacitidine, and thus a knowledge gap exists.
Method: This study consisted of two study designs.

Study I; a follow up study with a baseline measurement in newly diagnosed and treatment naive patients and a second measurement after treatment initiation. One patient was included but only completed the baseline measurement.

Study II; a cross-sectional study with one measurement in patients undergoing treatment with 5-azacitidine and in a control group of 21 healthy controls. Two patients were included and underwent measurements.

Patients underwent; questionnaires, Wireless Motility Capsule, and autonomic measures including Vagus ™, Cardiac Vagal Tone, ePatch, orthostatic blood pressure measurement, and SUDOSCAN.
Results: No comparative tests were made due to a small sample size. The two patients both had prolonged gastric emptying time, one had a pathological Cardiac Autonomic Neuropathy-score and the other a borderline one. One had normal SUDOSCAN results and the other moderately decreased sudomotor function. One had orthostatic hypotension.
Conclusion: Underlying mechanisms involved in development of gastrointestinal complications in 5-azacitidine treatment are not yet fully understood, and the underlying causes may be multifactorial. However, the two examined patients undergoing treatment with 5-azacitidine have prolonged gastric emptying and they provide a new implication for the use of Wireless Motility Capsule in clinical research. Therefore, more extensive research, including more examined patients, is necessary, and more optimal research conditions are needed in order to provide the most optimal antiemetic treatment of these patients in the future.

Danish Abstract

Hypotese: Følgende hypotese blev opstillet; gastrointestinale symptomer relateret til 5-azacitidin-behandling, herunder kvalme, er associerede til ventrikeltømning, og autonom dysfunktion forekommer sammen med ændret gastrointestinal transit.
Baggrund: Patienter med myelodysplastisk syndrom og akut myeloid leukæmi modtager ofte livsforlængende behandling med lægemidlet 5-azacitidin. Mange patienter oplever bivirkninger i form af gastrointestinale symptomer, særligt kvalme og opkastninger forekommer, og kan påvirke patienternes livskvalitet. Patogenesen bag kvalme er kompleks og påvirkes både af centrale og perifere komponenter. Der er ikke tidligere lavet studier, hvori de underliggende årsager til kvalme i patienter, der modtager 5-azacitidin behandling, undersøges, og der eksisterer således et videnshul.
Metode: Indeværende studie bestod af to delstudier:

Studie I; et follow-up studie bestående af en indledende basismåling i nydiagnosticerede patienter, der endnu ikke har opstartet behandling, og en opfølgende måling i samme patient efter behandlingsstart. En patient blev inkluderet, men gennemførte kun basismålingen.

Studie II; et tværsnitsstudie med en enkelt måling i patienter, der allerede behandles med 5-azacitidin og sammenlignes med en kontrolgruppe bestående af 21 raske kontroller. To patienter blev inkluderet og gennemgik alle undersøgelser.

Undersøgelser omfattede; spørgeskemaer, Wireless Motility Capsule og autonome mål inklusiv Vagus ™, Cardiac Vagal Tone, ePatch, ortostatisk blodtryksmåling og SUDOSCAN.
Resultater: Der var for få data til at foretage en sammenlignende analyse. Begge patienter i studie II havde forlænget tømningstid af ventriklen, en havde en patologisk Cardiac Autonomic Neuropathy-score, og en havde en grænsende patologisk score. En patient havde normale SUDOSCAN-resultater, mens en patient havde moderat nedsat sudomotor-funktion. En patient havde også ortostatisk hypotension.
Konklusion: De underliggende mekanismer involveret i udvikling af gastrointestinale komplikationer under 5-azacitidin-behandling kendes stadig ikke fuldt ud, og årsagen kan være multifaktoriel. Dog havde de to undersøgte patienter forlænget ventrikeltømning, og der er således en implikation for at anvende Wireless Motility Capsule i klinisk forskning. Der er behov for mere dybdegående forskning, inklusiv flere målinger fra patienter og bedre undersøgelsesforhold, for at optimere behandlingen af kvalme i denne patientgruppe.

Index

Gastrointestinal Complications in Patients with Myelodysplastic Syndrome or Acute Myeloid Leukemia Undergoing Treatment with 5-Azacitidine (GAS-COL)

Abbreviations

ATT	Antroduodenal transit time
ANS	Autonomic nervous system
AML	Acute myeloid leukemia
CAN	Cardiovascular autonomic neuropathy
CTT	Colonic transit time
CVT	Cardiac vagal tone
ENS	Enteric nervous system
GCSI	Gastroparesis Cardinal Symptom Index
GET	Gastric Emptying Time
GI	Gastrointestinal
GSRS	Gastrointestinal Symptom Rating scale
HRV	Heart rate variability
MDS	Myelodysplastic syndrome
SBTT	Small Bowel transit time
WGTT	Whole gut transit time
WMC	Wireless motility capsule

1| Introduction

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are hematological disorders affecting the myeloid cell lines. MDS is characterized by disruption of hematopoiesis resulting in cytopenias and hypercellular bone marrow[1–6]. AML is a malignant disorder characterized by clonal proliferation derived from primitive hematopoietic stem cells. Both disorders often affect elderly patients, the median age of AML- diagnosis is 68 years[7]. Furthermore, MDS possesses a risk of transforming into secondary AML in 30-40% of the cases[1,4,6]. Patients often present with vague symptoms including fatigue, bleeding, infection and shortness of breath, and the disorders are associated with poor prognosis severely affecting the patients' quality of life[2–4,8].
Due to advanced age and comorbidities, many patients are not candidates for the regimen of intensive chemotherapy and allogeneic stem cell transplantation[3,9,10]. Consequently, 5-azacitidine is offered as a life prolonging treatment[1,3,7].
5-azacitidine (Vidaza) is a pyrimidine nucleoside analogue (antimetabolite) with a direct cytotoxic effect on abnormally proliferating cells and an ability to cause hypomethylation of DNA. This hypomethylation counteracts the hypermethylation of the tumor suppressor genes which has been hypothesized to be involved in the pathogenesis in MDS and AML[2,4,10,11].
Most common patient reported adverse effects of 5-azacitidine are hematological and gastrointestinal (GI), and GI-symptoms include nausea, vomiting, diarrhea and constipation. Nausea and vomiting have been reported in up to 83% of patients, however the underlying pathophysiology is incompletely understood[1–4,10].

In humans it is widely accepted that GI-motility is regulated in a complex bidirectional interaction between the central nervous system, the ANS, the enteric nervous system (ENS) and endocrine pathways, and similarly generation and maintenance of nausea is complex and encompasses motility disturbances, autonomic- and central regulation, hormones and external factors (e.g. chemotherapy)(Prashant et al. 2016). Pathways are summarized in figure 1. Nausea and motility disturbances are combined in gastroparesis, defined as a motility disorder with delayed gastric emptying characterized by symptoms like nausea, vomiting, bloating and abdominal pain[13]. Gastric emptying has not been investigated in patients treated with 5-azacitidine, but animal studies suggests that 5-azacitidine alters gastric function through decreased secretion and delayed gastric emptying causing increased weight of the ventricle[14,15]. Segmental transit, including gastric emptying and motility disturbances, can be assessed non-invasively using a Wireless Motility Capsule (WMC) to measure pH, temperature and pressure throughout the GI-tract[16–18].
A component of nausea is, as described, disruption of the nervous system, and a long-term adverse effect of chemotherapy is chemotherapy-induced neuropathy that has previously been reported during treatment with 5-azacitidine[19,20].
The parasympathetic branch of the ANS and its bi-directional coordination of conveying sensory information from the gut to the brain, the gut-brain axis, possesses a stimulatory effect on the ENS and enhances motility. Autonomic GI-function is difficult to assess, and thus the evaluation of cardiometrically derived autonomic function is often used as a surrogate marker of the abdominal vagal function[21,22].

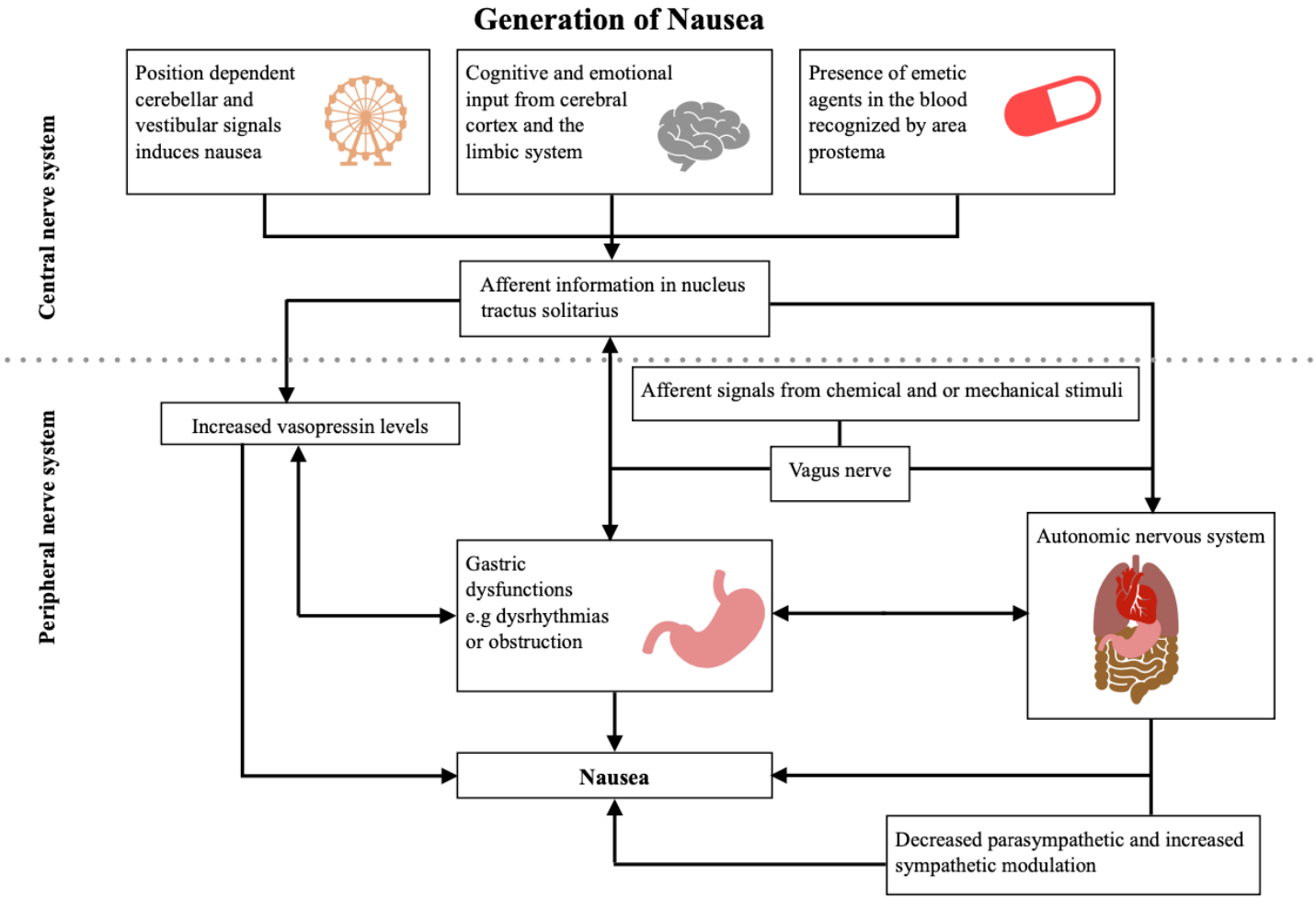

Figure 1: *Overview of known causes of nausea with central and peripheral pathways based on the review by Prashant et al.* (Prashant et al. 2016). *Efferent information from nucleus tractus solitarius is transmitted to the autonomic nervous system (ANS) via the vagus nerve to mediate intensity of nausea and accompanying characteristic pathophysiological changes. Other factors such as gastric dysfunction and vasopressin levels are also associated with nausea though further studies are required to clarify the mechanisms.*

There is a knowledge gap in exploring 5-azacitidine induced GI-complications, and, to the best of our knowledge, segmental transit time and autonomic measures are yet to be investigated. Thus, we hypothesized that GI-symptoms, including nausea, are associated with gastric emptying, and autonomic dysfunction coexists with altered GI-transit. Consequently, the aims of this study are:

1) To assess severity of patient reported GI-symptoms using validated questionnaires.
2) To assess gastric emptying time (GET) in patients treated with 5-azacitidine using WMC.
3) To assess patient autonomic function using validated autonomic measures.

2 | Materials and Methods

2.1 | Study Design

The protocol consisted of two separate studies: Study I and study II. Both studies were approved by the North Denmark Region Committee on Health Research Ethics (GAS-COL, N-20210017), and all patients gave informed consent prior to investigation.
Study I: A clinical follow-up study, a baseline measurement was made before initiation of 5-azacitidine treatment. This was compared to the follow-up measurement after a minimum of six weeks corresponding to having received a second round of treatment.
Study II: A cross-sectional study, included patients who were in treatment with 5-azacitidine. Measurements could be conducted at any point during treatment.

2.2 | Study Population

Study I: Two patients were screened, and one was included.
Study II: Twenty-two patients were screened and 5 were included.
Healthy controls: Were obtained from a former study, all participants gave informed written consent, and the study was approved by the North Denmark Region Committee on Health Research Ethics (TODINELI, N-20130077).

2.2.1 | Inclusion and Exclusion Criteria

Patients were included if they were > 18 years old, could read, speak and understand Danish, and had verified MDS or AML. The patients were either scheduled for or already undergoing treatment with 5-azacitidine.
Exclusion criterias included known coeliac disease, GI-abnormalities, known neuropathy or disease in the neural system (e.g Multiple Sclerosis, Guillain-Barré, diabetic polyneuropathy ect.). Pregnancy and breastfeeding and present or previous abuse of alcohol, euphoriants or medicine were exclusion criterias as well. In addition, patients who had received chemotherapeutic treatment for a previous cancer and who had participated in other clinical trials less than three months prior to inclusion, were excluded, unless participation in these was judged to have no influence on the recordings.

2.3 | Patient reported outcome

GI-symptoms were assessed using two standardized questionnaires.

2.3.1 | Upper GI-symptoms

The Gastroparesis Cardinal Symptom Index (GCSI) questionnaire was used to assess GI-symptoms through 14 days. The questionnaire consists of nine questions from PAGI-SYM, divided into three subgroups (nausea/vomiting, postprandial fullness, and bloating) and is a valid tool for measuring symptom severity in patients with gastroparesis. The GCSI-scale is scored from 0 (no symptoms) to five (very severe symptoms).

2.3.2 | Lower GI-symptoms

Were assessed using The Gastrointestinal Symptom Rating Scale (GSRS). Patients were asked to assess the severity of GI-symptoms throughout the preceding week. The GSRS consists of 15 items divided into five symptom clusters; abdominal pain, reflux syndrome, diarrhea syndrome, indigestion syndrome and constipation syndrome. The GSRS has a seven-point scale ranging from one (no symptoms) to seven (very troublesome symptoms)[23,24].

2.4 | Assessment of GI-function

GET was measured using a WMC (SmartPill, Medtronic, Minneapolis, USA) which has previously been used to determine GI-transit. The WMC is a minimally invasive device, consisting of a non-digestible capsule measuring 26 mm x 13 mm and weighing 4.5 g. The capsule measures temperature, pH and pressure throughout the GI-tract. Sensors can obtain temperature ranging from 25 to 49 C°, pH ranging from 0.5 to 9 pH units and pressure ranging from 0 to 350 mmHg. Following activation, calibration, and swallowing, the data was continuously collected and transmitted to an external data receiver worn by the patient. After capsule expulsion, the data was downloaded to a computer using a docking station.

2.4.1 | Wireless Motility Capsule Study Protocol

Following at least a six hour fast, patients ingested a standardized meal, SmartBar (Medtronic, Minneapolis, USA, 260-kcal, composed of 3% fat, 21% protein and 75% carbohydrate, 3% of which is fiber) with two glasses of water. Immediately after finishing the meal, the patients swallowed the WMC. To improve data quality, patients were instructed to keep the data receiver close to their bodies until the receiver showed loss of signal in relation to a bowel movement, indicating capsule expulsion. They were also instructed to register specific events by pressing the event button on the receiver; food ingestion, bowel movement, going to bed, and waking up. The time and character of each marked event were reported in a personal diary. Following ingestion, patients were instructed to fast for the following six hours. After six hours they could resume their individual daily diet.

2.4.2 | Definition of Anatomical Landmarks

The downloaded WMC data was analyzed using the MotiliGI 2.2 computer software package (Medtronic, Minneapolis, USA). To enhance internal validity, the data was analyzed by two investigators, who reached consensus.

According to the method proposed by Sarosiek et al., it was possible to divide the GI-tract into three distinct regions[25]. As the WMC transits through the GI-tract, these anatomical regions could be determined based on concomitant changes in temperature, pH, and pressure curves, see figure 2.

The gastric region is marked by ingestion of the capsule followed by a temperature increase to approximately 37 C° and a pH-drop greater than 2 pH units. A sudden rise in pH reflects the transit from the gastric region into the more alkaline duodenum.

The small bowel region is characterized by a gradual increase in pH until a sudden, persistent drop in pH equivalent to > 1 pH unit which represents passage across the ileocaecal junction into the colon.

The colonic region is defined from the ileocaecal junction to expulsion of the capsule causing either a sudden drop in temperature or loss of signal.

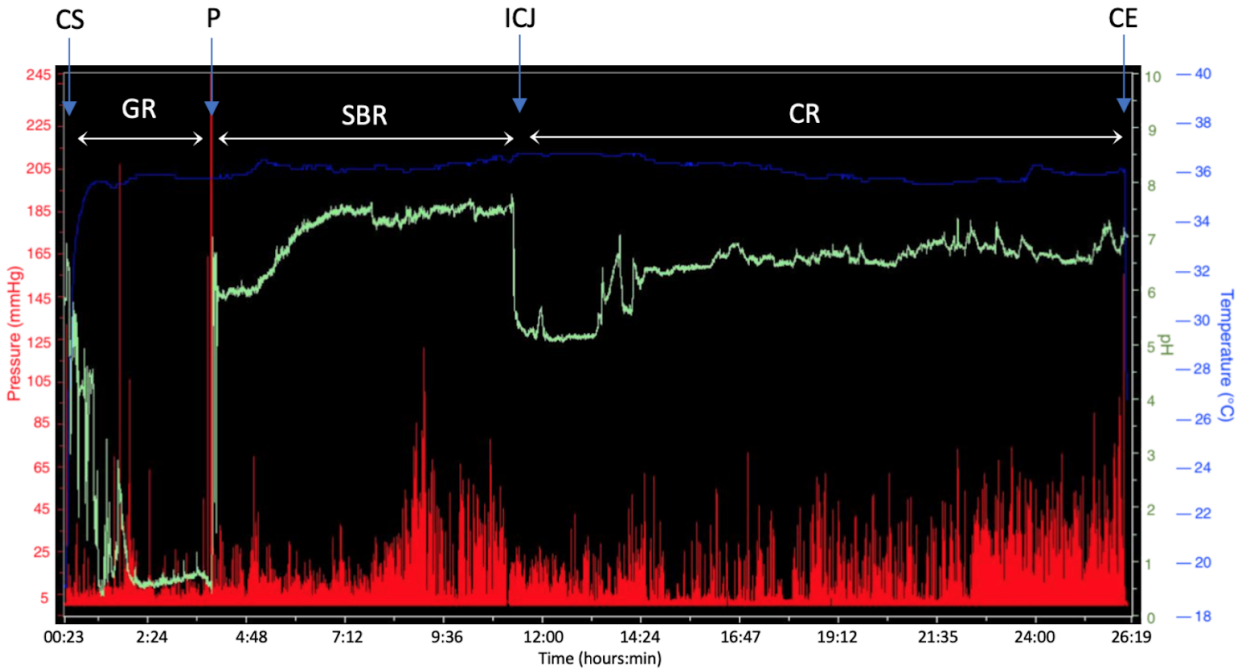

Figure 2: *Example of a characteristic WMC-trace from a healthy participant. The x-axis represents time, and the y-axis represents temperature in C° (blue), pH in units (green), and pressure in mmHg (red). The three regions of the GI-tract are illustrated: The gastric region (GR), the small bowel region (SBR), and the colonic region (CR). Capsule swallowing (CS), pylorus (P), ileocaecal junction (ICJ), and capsule expulsion (CE).*

2.5 | Autonomic Measurements

2.5.1 | Assessment of Cardiovascular Autonomic Neuropathy (CAN)

All measurements were performed in a quiet lab to minimize inputs, and the patients rested for 10 minutes prior to the investigation. Cardiovascular autonomic reflex test is the standard for measurement of CAN-score and can be tested using a handheld device, Vagus™ (Medicus Engineering ApS, Aarhus, Denmark) which measures heart rate variability during standardized stressors using a one led electrocardiography recording. Heart frequency recordings were measured during: Rest in a supine position for five minutes, postural change from supine to standing position, inspiration-ratio during deep breathing for one minute, and Valsalva's-ratio which is exhalation with a fixed resistance of 40 mmHg followed by one minute of rest. The Vagus™ device has previously been validated with high diagnostic concordance compared to stationary equipment and has been used for testing CAN-score in patients with diabetes. If one cardiovascular autonomic reflex test was not completed, the CAN-score was estimated from the remaining procedures[26–28]. Scores are age-dependent and a CAN-score of two or more indicates an abnormal test, a score of one is borderline and zero is normal[28,29].

2.5.2 | Assessment of Cardiac Vagal Tone (CVT)

CVT is an index of parasympathetic efferent tone and an indicator of brainstem modulation of the efferent signals to the heart[28,30]. Heart rate is regulated through a combination of baroreceptors, parasympathetic vagal innervation and sino-atrial depolarization, and adjustments are made in response to altered interior or exterior conditions e.g., changes of postural positioning[28,31,32].
CVT is based on two parameters: Cardiac cycle in the form of a R-R interval in resting individuals and vagal efferent modulation. The relationship between the two parameters resembles linearity, and data can be quantified in a linear vagal scale. A linear vagal scale = 0 represents a completely atropinized state, and measures > 0 represents efferent vagal tone. High values indicate a healthy parasympathetic function[28,30].
CVT was measured using a small, non-invasive, three led electrocardiography recorder, eMotion Faros™ 180 (Bittium, Oulu, Finland). The three electrodes (Ambu Blue Sensor P, Denmark) were placed in the right and left midclavicular line below the clavicle and over the apex of the heart. The cardiac monitor recorded 8 kHz for five minutes from which CVT and heart rate variability were calculated using the commercially available biosignal acquisition system from ProBiometrics (Neurozoid, ProBiometrics, London, UK). In order to minimize artifacts, changes between to succeeding QRS complexes exceeding 15 beats pr. minute, e.g. caused by moving, were defined as artifacts and five heartbeats before and after were removed. If the number of edited heartbeats exceeded 20%, the file was discharged[28].

2.5.3 | Assessment of Heart Rate Variability (HRV)

HRV was assessed using the ePatch which is a small, body-worn sensor that adheres to the skin and records up to five days of electrocardiography. The ePatch consists of a rechargeable sensor and a three-led single use electrode. The device was placed two finger breadths under the upper margin of manubrium and continuously recorded with a sampling rate of up to 1024 samples pr. second pr. channel and stored locally on the device. Data was retrieved through an USB interface (Philips B, e-Patch), and the HRV was derived from R-R intervals using CardiScopeTM to prepare and filter recordings (HASIBA Medical GmbH, Graz, Austria)[33].
In Accordance with recommendations from the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, following data was collected: Heart rate (HR) and the following four time dependent HRV-indexes:

1. Standard deviation of normal-to-normal intervals (SDNN).
2. Standard deviation of the average normal-to-normal intervals for each five-minute segment of the recording (SDANN).
3. Mean of the standard deviation of all the normal-to-normal intervals for each five-minute segment of the recording (SDNNI).
4. Root mean square of successive R-R interval differences (rMSSD).

In addition, four markers of frequency dependent HRV were found:

1. VLF: absolute power of the very-low-frequency band (0.0033–0.04 Hz).
2. LF: absolute power of the low-frequency band (0.04–0.15 Hz).
3. HF: absolute power of the high-frequency band (0.15–0.4 Hz)
4. LF:HF-ratio.

The content of VLF and LF are traditionally thought to represent the sympathetic activity, while HF and rMSSD are considered to represent parasympathetic control. LF:HF-ratio is considered to reflect the sympato-vagal balance[28].

2.5.4 | Orthostatic Blood Pressure

Orthostatic blood pressure is a measurement of sympathetic innervation of the blood vessels. Blood pressure was measured non-invasively on the upper arm in supine position, in upright position, immediately after standing up, one, two, three, four, five and 10 minutes after standing up using a commercially available device (Omron M4, Hoofddorp, Netherlands).

There is no consensus or clear cut-off limits for orthostatic hypotension. The American Consensus Comity in 1996 defined a decrease of > 20 mmHg systolic or > 10 mmHg diastolic within three minutes of standing as orthostatic hypotension, but a cutoff value of ≥ 30 mmHg systolic and ≥ 10 mmHg diastolic have also been used[32,34,35]. Therefore, local instructions for Aalborg University Hospital were used; decrease of 25-27 mmHg systolic, 10 mmHg diastolic, pulse increase ≥ 30 bpm, or symptoms of or fainting (Region Nordjylland PRI: Orthostatisk Blodtryksmåling).

2.5.5 | Assessment of Sudomotor Function

Function of the small nerves innervating the sweat glands was noninvasively measured using SUDOSCAN (Impeto Medical Inc., Paris, France) through measurement of electrochemical skin conduction in hands and feet represented by the reaction of the chloride ions in the sweat glands after electrochemical stimulation[33]. The palmar and plantar side of the hands and feet were placed on four electrodes and a four-volt current was sent through releasing chloride ions in response. The ions reacted with the stainless steel on the electrodes. Data was provided as an ESC readout for all four extremities[28,36].

2.5.6 | Autonomic Symptoms

Were assessed using the Composite Autonomic Symptoms Score (COMPASS-31) which is a self-assessed quantitative measure consisting of 31 questions formed into six subgroups including orthostatic intolerance, vasomotor, secretomotor, GI, bladder and pupillomotor with a score from 0 to 100, a higher score indicates more severe autonomic neuropathy[37].

2.6 | Statistical Analysis

Tests of normality, histograms and Q-Q plots were made for all healthy controls revealing the individual differences in data distribution and a Shapiro-Wilk test of normality was conducted. Data from the included patients was presented in a joint table. In addition, a correlation table comparing symptoms and GET was made in both groups using a linear regression model. All statistical tests were performed using the program STATA (StataCorp LLC, version 17.0).

2.6.1 | Justification of Sample Size

The goal was to include 14 patients in Study I and 14 patients in Study II. The sample size was based on the following GET in the normal elderly population (mean 190 minutes ±60 min). We hypothesized that the GET was prolonged by 50% (285 minutes). With a significant alpha-level of 0.05 and 80% power, 14 completed data sets were needed in both groups.

2.6.2 | Pending Data Analysis

2.6.2.1 Study I: In order to compare baseline measurements with the second measurement, parametric data will be compared using a paired t-test, and a Wilcoxon's rank sum test will be used for non-parametric data.

2.6.2.2 Study II: In order to compare healthy controls and patients, an unpaired t-test will be conducted for parametric data and a Mann-Whitney U-test for non-parametric data.

3 | Results

3.1 | Study I

One baseline recording was made but the patient withdrew consent due to the severity and worsening of the illness. Consequently, only data from study II was analyzed.

3.2 | Study II

Out of five included patients, two completed all measurements. Further details and characteristics about this group are presented in figure 2.

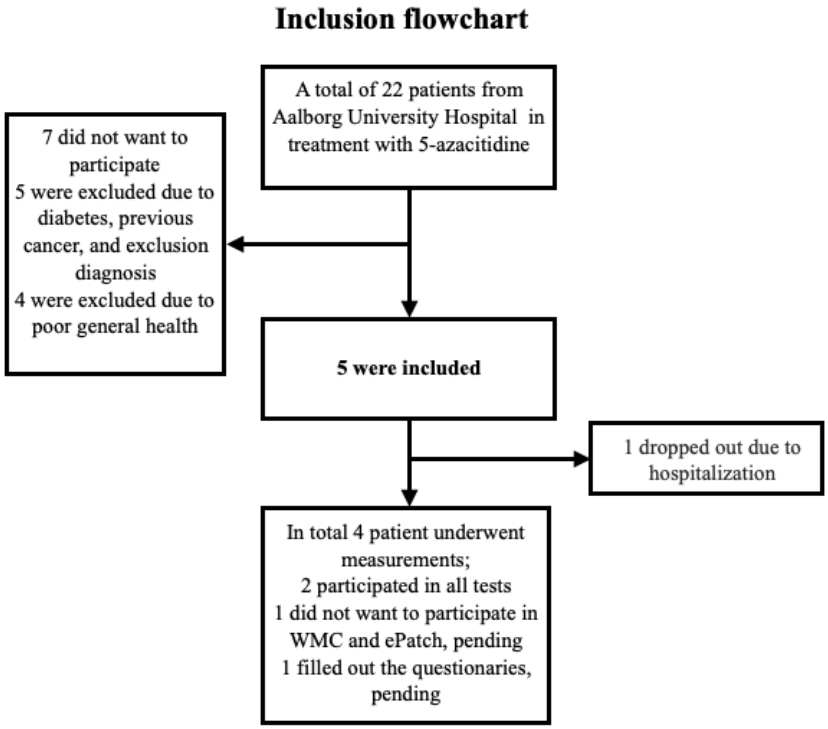

Figure 2: *Flowchart showing in- and exclusion of patients for the case-control study. Wireless Motility Capsule (WMC).*

3.2.2 | Patient Data

Due to the limited size of this group, it was not possible to test for normal distribution or perform a comparative test. Instead, patient data from the two patients is presented in a joint table, see table 1, divided into baseline characteristics, GI-symptoms, GI-transit, and autonomic measures. Both patients were male and 71 and 69 years old respectively, and both had AML. One patient had a GCSI-score of 1.92 scoring 1 in nausea/vomiting, 2.75 in postprandial fullness and 2 in bloating. The other patient had a GCSI-score of 0, while the GSRS-scores were 2.83 and 1 respectively. Noticeably, both patients had a delayed GET (defined as GET > 4 hours) measuring 18.4 and 5.14 hours. Antroduodenal transit times (ATT) were 1 and 3 minutes. Due to signal loss colonic transit time (CTT) and whole gut transit time (WGTT) could not be measured. In addition, one patient had a CAN-score of 1 which is borderline, and one patient had a score of 2 which is pathological. One patient had moderately decreased sudomotor function, and the other patient had orthostatic hypotension.

Table 1: *Summarizes the baseline characteristics, GI-symptoms, WMC measures, and autonomic measures for two patients, GX001 and GX002.*

	GX001	GX002
Baseline characteristics		
Age (Years)	71	69
Smoker (Yes, No, Prior)	No	Prior
Gender (Male)	Male	Male
BMI	27.8	20.1
Diagnosis (AML, MDS)	AML	AML
Assessment of Gastrointestinal Symptoms		
Gastroparesis Cardinal Symptom Index		
GCSI-score	1.92	0
Nausea/vomiting	1	0
Postprandial fullness	2.75	0
Bloating	2	0
Gastrointestinal Symptom Rating Scale		
GSRS-score	2.83	1
Abdominal pain	2.67	1
Reflux syndrome	1	1
Diarrhea syndrome	5	1
Ingestion symptoms	2.5	1
Constipation syndrome	3	1
Gastrointestinal Transit Times		
Gastric emptying time (hours)	18.4*	5.14
Antroduodenal transit time (minutes)	1	3
Small bowel transit time (hours)	4.15	38.27
Colonic transit time (hours)	MV	MV
Whole gut transit time (hours)	MV	MV
Autonomic Measures		
Cardiovascular autonomic neuropathy-score	1	2
Cardiac Vagal Tone	3.79	3.28
Heart rate variability - 24-hours		
Heart rate (bpm)	64	66
Sinus rhythm (%)	99	99.2
R-R interval (ms)	937.5	909.09
SDNN	109.6	117.2
SDANN	95	103
rMSSD	30.2	23.4
LF-Interval (Hz)	505	311
HF-Interval (Hz)	301	158
LH:HF-ratio	1.68	1.97
Blood pressure		
Systolic blood pressure (mmHg)	110	158
Diastolic blood pressure (mmHg)	65	87
Pulse (bpm)	70	80
Orthostatic hypotension (Yes, No)	No	Yes
Sudomotor function		
Left foot (μS)	68	83
Right foot (μS)	72	84
Left Hand (μS)	48	84
Right Hand (μS)	44	82
Composite Autonomic Symptom Score		
Orthostatic Intolerance	20	12
Vasomotor	0	1.7
Secretomotor	6.4	0
Gastrointestinal	13.4	5.4
Bladder	1.1	0
Pupillomotor	1	0

** The WMC recording suggested that the patient ate before 6 hours. However, the WMC remained in the stomach for 9.4 hours during overnight fasting.*
MV = Missing value

3.2.3 | Healthy Controls

In the control group, 21 participants were included. The mean age was 51.2 (±6.42) years, and 15 were males while 6 were females. None of the controls had any known medical conditions. The healthy controls had a median GCSI-sore of 0.33 [0;0.42] including median nausea/vomiting of 0, mean postprandial fullness of 0.55 (± 0.51) and a median bloating of 0. The healthy controls had a median GSRS-score of 1.12 [1.05;1.33]. All data is presented in table 2.
In the control group data was tested for normality using histograms, QQ-plots and a Shapiro-Wilk test of normality, and GET (mean 3.11 ±0.85 hours), SBTT (mean 4.43 ±1.11 hours) and ATT (2 ±1.25) were normally distributed, while CTT (median 16.24 hours [13.25;25.51]) and WGTT (median 24.6 hours [22.3;32.37]) were not. However, two traces were invalid due to a broken file and major signal loss and were not included in the analysis of transit times.

Table 2: *Summarizes the baseline characteristics, gastrointestinal symptoms, transit times, and autonomic measures. Data are shown as mean standard deviation (±), median [interquartile range], or percentage.*

	Healthy Controls N = 21
Baseline characteristics	
Age (Years)	51.19 (± 6.42)
Smoker (Yes)	14 %
Gender (Male)	71 %
BMI	25.6 [23.7;28]
Assessment of Gastrointestinal Symptoms	
Gastroparesis Cardinal Symptom Index	
GCSI-score	0.33 [0;0.42]
Nausea/vomiting	0 [0;0]
Postprandial fullness	0.55 (±0.51)
Bloating	0 [0;0.5]
Gastrointestinal Symptom Rating Scale	
GSRS-score	1.12 [1.05;1.33]
Abdominal pain	1 [1;1.33]
Reflux syndrome	1 [1;1]
Diarrhea syndrome	1 [1;1]
Ingestion symptoms	1.25 [1;1.5]
Constipation syndrome	1 [1;1.33]
Gastrointestinal Transit Times	
Gastric emptying time (hours)	3.11 (±0.85)
Antroduodenal transit time (minutes)	2 (±1.25)
Small bowel transit time (hours)	4.43 (±1.11)
Colonic transit time (hours)	16.24 [13.25;25.51]
Whole gut transit time (hours)	24.55 [22.3;32.37]
Autonomic measures	
Cardiac Vagal Tone	4.33 (±1.67)
Blood pressure	
Systolic blood pressure (mmHg)	128.9 (±14.73)
Diastolic blood pressure (mmHg)	75.8 (±10.91)
Pulse (bpm)	66 (±6.81)
Parametric data is given in mean (± standard derivation) *Non-parametric data is given in median [upper, lover quartile]*	

3.2.3.1 | Association Between GI-Symptoms and GI-Transit in Healthy Controls

GI-transit times in healthy controls were correlated with GI-symptoms using linear regression as data was both parametric and nonparametric; results are presented in table 3. GET was positively correlated to GSRS (p = 0.017), see figure 3 for visual representation. No correlation was found between the remaining transit times and symptoms (GCSI, nausea/vomiting, postprandial fullness, bloating and GSRS).

Table 3: *Summarizes regression analysis between GI-transit times and GCSI, upper intestinal symptoms (nausea/vomiting, postprandial fullness and bloating) and GSRS.*

Transit Time	**Gastric Emptying Time**			**Small Bowel Transit Time**			**Colonic Transit Time**			**Whole Gut Transit Time**		
	Coef	95 % (CI)	P = value	Coef	95 % (CI)	P = value	Coef	95 % (CI)	P = value	Coef	95 % (CI)	P = value
GCSI-score	0.04	-0.2;0.28	0.728	0.02	-0.16;0.21	0.788	0.005	-0.003;0.01	0.229	0.005	-0.003;0.01	0.217
Nausea/vomiting	0.01	-0.03;0.06	0.616	-0.003	-0.04;0.03	0.851	-0.0003	-0.002;0.001	0.727	-0.0003	-0.002;0.001	0.735
Postprandial fullness	0.01	-0.3;0.31	0.956	-0.01	-0.25;0.22	0.910	0.009	-0.001;0.02	0.080	0.009	-0.001;0.02	0.079
Bloating	0.10	-0.4;0.60	0.677	0.09	-0.3; 0.47	0.638	0.006	-0.01;0.02	0.486	0.006	-0.01;0.02	0.462
GSRS-score	-0.16	-0.28;-0.03	0.017*	-0.04	-0.15;0.07	0.485	0.003	-0.003;0.008	0.313	0.002	-0.003;0.007	0.376

** Correlation significant when p ≤ 0.05*
Coefficient (Coef)

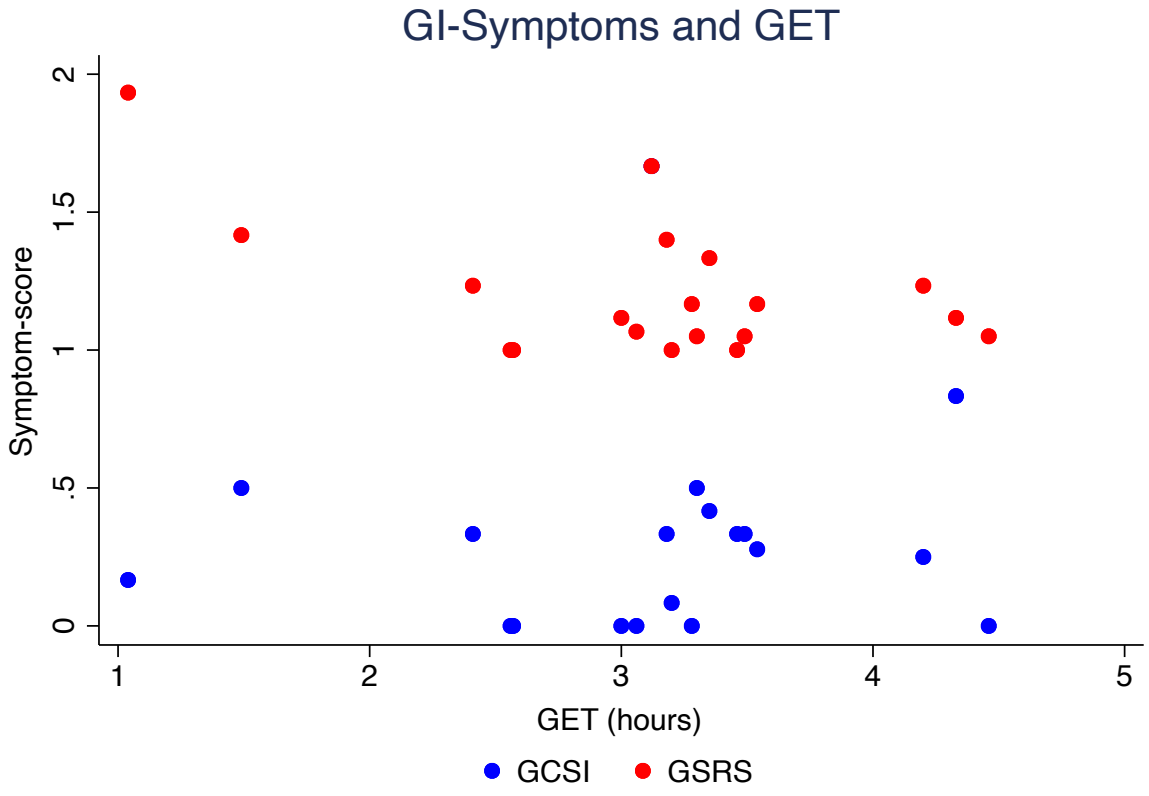

Figure 3: *Scatterplot showing correlation between gastric emptying time (GET) and symptoms in healthy controls; Gastroparesis Cardinal Symptom Index (GCSI) and Gastrointestinal Symptom Rating Scale (GSRS). The x-axis represents time in hours and the y-axis represents GCSI/GSRS symptom score.*

3.3 | GET in Healthy Controls and Patients

In order to visually portray GET for healthy controls and patients, a box plot was made and data from the two patients, shown as dot plots, in a joint figure. Both patients had a GET higher than the upper quartile of the controls, see figure 4.

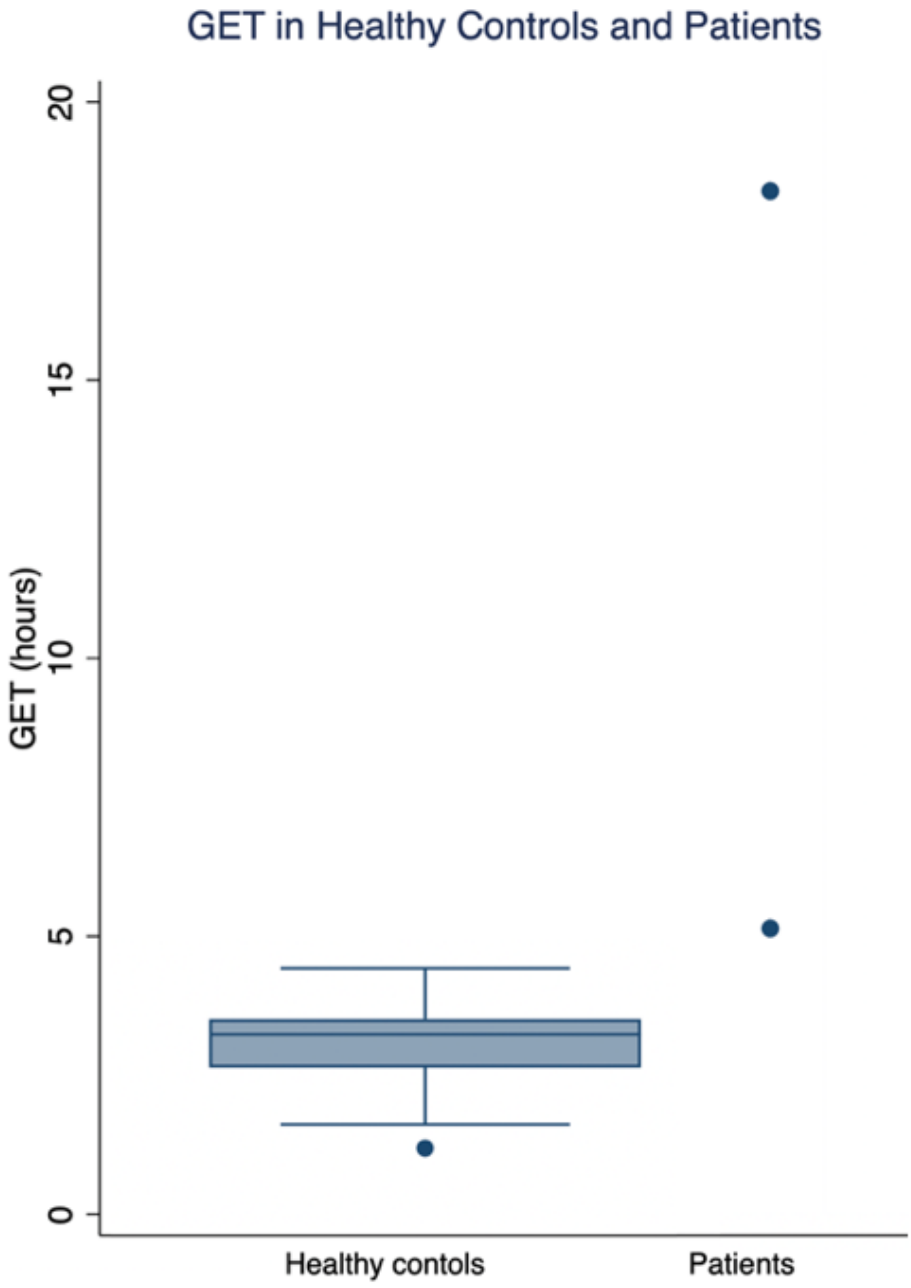

Figure 4: *Box plot showing gastric emptying time (GET) in healthy controls and dot plot showing GET in the two individual patients.*

4 | Discussion

In the current study it was not possible to include 14 patients as required, and thus no definitive comparative statistical test could be made in either study I or study II, however the procedures have proven feasible, and as the study is ongoing, we believe that data will be obtained.
GI-adverse effects related to treatment with 5-azacitidine are common and especially nausea is well documented, compromising the patient's quality of life. In the current study, symptoms were measured using standardized questionnaires, and in a previous study by Brock et al. it was found that healthy individuals had GCSI-scores of 0 (nausea), 0.2 ± 0.3 (postprandial fullness) and 0.3 ± 0.9 (bloating) which correlates with the current findings in healthy controls, and, hopefully, can provide a standard of reference in future studies[21]. The patient reported symptom-scores exhibited variation and due to small sample size and individual approaches to symptom and illness perception, no conclusion can be made.

The approval of 5-azacitidine by the European Medicines Agency is based on two studies including AML, MDS and chronic myelomonocytic leukemia patients, where the drug proved to prolong survival in patients who were unlikely or unfit to undergo stem cell transplantation. It is also suggested to treat the patients with antiemetic medicine during treatment since nausea is a common side effect, however it is not elaborated further[38]. To our knowledge, no previous studies have investigated the underlying causes of GI-symptoms in humans, but animal studies have revealed the effect of 5-azacitidine on gastric function in rats. Chihák et al. reported decreased gastric secretion and emptying, consequently leading to increased weight of the stomach, indicating food retention as well as lower acidity in the stomach[14,15]. Even though these studies are older, they reveal that GI-complications and prolongation of GET in response to 5-azacitidine treatment are prevalent and well-recognized which justifies the need for the current study. The two clinical cases in the current study showed prolonged GET which could indicate gastric dysrhythmia or dysmotility but no general conclusion can be made.
Animal studies can, however, not be translated to humans. The advantage of animal studies is full control over the test subjects when it comes to ingestion, interventions, and the possibility to extract and examine organs which for obvious reasons cannot be recreated in human studies. In addition, the symptoms in animals cannot be evaluated, and thus the findings cannot be related to severity of GI-symptoms. In our study a different method is used to measure gastric function, and although WMC cannot measure weight of the stomach, the method has shown to provide information about transit and pH-level of the stomach in healthy individuals and diabetic patients in a non-invasive and more ethical manner, making it feasible for further investigation in AML and MDS patients.

In the current study, self-reported symptoms were the primary outcome as patients with AML and MDS treated with 5-azacitidine most frequently suffer from nausea. Nausea is generally accepted to reflect upper GI-disturbances. GET was considered the secondary outcome because it may be regarded as a net-result of motility disturbances, altered secretion and hormonal imbalances. Delayed gastric emptying is a sign of gastroparesis which, as mentioned previously, is characterized by symptoms like nausea and vomiting. Thus, there is a symptomatic overlap between gastroparesis symptoms and adverse effects reported by patients in treatment with 5-azacitidine. This may indicate that the symptoms arise due to gastric retention. It is noteworthy, that gastric electrical stimulation significantly can decrease nausea in idiopathic and diabetic gastroparesis without influencing the GET[39]. The findings indicate that dysmotility may not be the driving component in symptom development, and it may therefore be plausible that GET alters the sympatico-vagal balance and thereby influences the central neural component to symptom generation. In continuation of this observation, it is interesting to examine whether the patients with prolonged GET have prolonged ATT indicating pylorus spasms, antral hypomotility and retrograde movement of food bolus[40]. However, in the two current study cases, none had prolonged ATT, indicating normal pyloric coordination.
It is relevant to examine segmental transit times, considering that symptom- and anatomical location in the GI-tract often do not correlate. For example, nausea can be a sequlea to constipation, and in addition it can be argued that 5-azacitidine, among other antimetabolites, can have a more widespread effect the GI-tract. Studies have shown that 5-azacitidine and other antimetabolites contribute to decreased intestinal absorption,

increased permeability with higher excretion of lactulose and lower nutritional status. This indicates that the chemotherapeutics cause an impaired regulatory influence on the GI-function[41,42].
Unfortunately, the patient´s WMC recordings did not allow retrieval of CTT and WGTT. In healthy participants, we found that GET and SBTT were normally distributed in contrast to CTT and WGTT. This is not unexpected as WGTT has previously been examined in a larger study population of healthy individuals, and a specific pattern was found in CTT and WGTT with a peak after about 24 hours and again after 48 hours[43]. This could indicate that events such as bowel movement have more of a circadian variation and are more dependent on individual habits compared to GET.

The tertiary outcome concerns autonomic measurements which relate to GI-complications as generation of nausea is closely connected to the ANS through vagal mediation as described earlier by Prashant et al.[12]. As a proxy for the autonomic component in GI-function, we analyzed the cardiac autonomic reflex test, and observed that both patients had positive CAN-scores (borderline or pathological), one had orthostatic hypotension, and one had a moderately reduced sweat production which taken together indicated dysautonomia and could be a sign of autonomic neuropathy. Chemotherapy induced neuropathy is often an overlooked but frequent side effect of cancer treatment, and Magge et al. have described that many therapy types used in treatment of hematological neoplasms, including antimetabolites, can cause both central and peripheral neuropathy[44,45]. There is, however, a paucity in data describing autonomic nerve function which is concerning since presence of CAN is a predictor of all-cause mortality in diabetic patients and it could be similar in other patient groups[46]. Thus, clinicians should be aware of peripheral, autonomic and central neuropathies when treating patients with chemotherapy, because it is likely that the side effects are existing but have not yet been reported. Moreover, it has previously been described how paraneoplastic syndromes and direct nerve affection in AML can cause neuropathy e.g., in the case report by Lee et al where a patient developed orthostatic hypotension due to paraneoplastic autonomic neuropathy[47]. This reflects how the disease can affect all parts of the nervous system which could be a possible confounder to be considered in patients who are severely bothered by neuropathic symptoms. GI-manifestations as a direct result of AML have also been reported and it cannot be excluded that some of the patient-reported GI-complications, including nausea, can be attributed to the disease per se, as AML has also shown to affect nutritional status and intestinal immunity and cause increased inflammation in the GI-tract[8,41,48].

These considerations regarding the origin of the GI-symptoms and gastric function in this group of patients reflect a knowledge gap, and it is plausible the cause is multifactorial; however, the preliminary results of the current study suggest that the WMC method and autonomic measurements could contribute to a better understanding of why these symptoms arise and whether gastroparesis or autonomic neuropathy are contributing factors. Such considerations are important when choosing a therapeutic approach as several antiemetic medications can target both central and peripheral receptors, providing an important clinical knowledge[12].

4.1 | Limitations

Firstly, despite the relevance of the study, the inclusion of patients has shown to be difficult due to the severity and unpredictability of the illness which leaves the patient with limited resources. Thus, study I has been expanded to run in parallel with study II, including all patients treated with 5-azacitidine at Aalborg University Hospital. Currently the number of patients treated with 5-azacitidine is 22, and therefore recruitment would benefit if referral was expanded to other regions e.g., Region Midt in order to include more patients.
Secondly, even though study I has a more valid design in terms of causality, it is vulnerable for participation because treatment-naive patients just have received their potentially fatal diagnosis and consequently often are in a life-crisis. In contrast, study II may accommodate more flexibility as the measurements can be made at any point during treatment and besides requiring only one measurement.
Thirdly, it should be discussed that primarily the patients with high function levels wanted to participate, making the study less representative and causing a level of selection bias.
In addition, even though more feasible, the cross-sectional study is associated with confounding factors in the form of: Patients having undergone previous chemotherapeutic treatments in relation to their AML or

MDS e.g. with cytarabine arabinoside, patients having undergone stem cell transplantation or having received numerous blood transfusions which can affect e.g. nerve or organ function. In addition, treatment duration is variating in the cross-sectional study and can affect symptom severity. These confounders could be minimized in the follow up study design, as the patients are naive to treatment, and thereby increase the internal validity of the study. Overall, the most optimal study design is likely the combination of the two.

Even though the patients had time to adjust to having been diagnosed and were familiar with the treatment, the cross-sectional study has faced inclusion problems as well. The experience is that the patients do not want to spend unnecessary time at the hospital or feel too ill to participate.
Fourtly, unforeseen events such as hospital admissions, antibiotic treatment, and need for ICU-treatment made inclusion unstable and two patients had to drop out after inclusion as proceeding with examinations was not ethically justified. In future studies it should consequently be considered how to make participation more convenient, e.g., exclusively investigate using WMC and questionnaires, or split the test day in two so the time spent at the hospital will be minimized or even accommodate the patients completely and do the tests at home.
Henceforward it is important to include a more representable study population regarding gender but also age, social status and type of diagnosis must also be taken into consideration.

In summary, this study is in-conclusive for a comparative analysis. Subjective GI-complications are well documented and well known as a consequence of treatment with 5-azacitidine, but the underlying multifactorial mechanisms are not yet fully understood. However, the two examined patients undergoing treatment with 5-azacitidine had prolonged GET and they provide a new implication for the use of WMC in clinical research. Therefore, more extensive research including more patients is necessary and more optimal research conditions are needed in order to provide the most optimal antiemetic treatment of these patients in near future.

5 | Acknowledgements

First and foremost, thanks to our main supervisors Christina Brock and Marianne Tang Severinsen, for this opportunity and guidance during this project. Furthermore, thanks to Anne-Marie Wegeberg. We would also like to thank the Mech-Sense team, University Hospital of Aalborg, Denmark who provided WMC-data and for valuable inputs.

6 | References

1. Anargyrou K, Vassilakopoulos TP, Angelopoulou MK, Terpos E. Incorporating novel agents in the treatment of myelodysplastic syndromes. *Leukemia Research*. 2010;34(1):6-17.
2. Barkate H v, Sejpal JJ, Belagali YR. A comprehensive overview of the role of Azacitidine in the management of myelodysplastic syndromes and acute myeloid leukaemia. *European Oncology and Haematology*. 2014;10(2).
3. Cataldo VD, Cortes J, Quintás-Cardama A. Azacitidine for the treatment of myelodyplastic syndrome. *Expert Review of Anticancer Therapy*. 2009;9(7):875-884.
4. Sullivan M, Hahn K, Kolesar JM. Azacitidine: A novel agent for myelodysplastic syndromes. *American Journal of Health-System Pharmacy*. 2005;62(15):1567-1573.
5. Vigna E, Recchia AG, Madeo A, et al. Epigenetic regulation in myelodysplastic syndromes: Implications for therapy. *Expert Opinion on Investigational Drugs*. 2011;20(4):465-493.
6. Ziemba A, Hayes E, Freeman BB, Ye T, Pizzorno G. Development of an oral form of azacytidine: 2′ 3′ 5′ triacetyl-5-azacytidine. *Chemotherapy Research and Practice*. 2011;2011.
7. Agarwal S, Kowalski A, Schiffer M, Zhao J, Bewersdorf JP, Zeidan AM. Venetoclax for the treatment of elderly or chemotherapy-ineligible patients with acute myeloid leukemia: a step in the right direction or a game changer? *Expert Review of Hematology*. 2021;14(2):199-210.

8. Ebert EC, Hagspiel KD. Gastrointestinal manifestations of leukemia. *Journal of Gastroenterology and Hepatology (Australia)*. 2012;27(3):458-463.
9. Crossnohere NL, Richardson DR, Reinhart C, et al. Side effects from acute myeloid leukemia treatment: results from a national survey. *Current medical research and opinion*. 2019;35(11):1965-1970.
10. Derissen EJB, Beijnen JH, Schellens JHM. Concise drug review: Azacitidine and decitabine. *Oncologist*. 2013;18(5):619-624.
11. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *International Journal of Cancer*. 2008;123(1):8-13.
12. Prashant Singh SSY and BK. Nausea: a review of pathophysiology and therapeutics | Enhanced Reader. 2016.
13. Waseem S, Moshiree B, Draganov P v. Gastroparesis: Current diagnostic challenges and management considerations. *World Journal of Gastroenterology*. 2009;15(1):25-37.
14. Cihák A, Korková L, Kohout J, Reutter W. Lower gastric secretion and higher incorporation of orotic acid into liver RNA in rats treated with 5-azacytidine and cycloheximide. *Neoplasma*. 1978;25(3):317-327.
15. Cihák A, Korbová L. Effects of N4-methyl and N4,N4-dimethyl derivatives of 5-azacytidine on liver RNA synthesis and gastric secretion in rats. *Neoplasma*. 1985;32(3):293-298.
16. Saad RJ. The Wireless Motility Capsule: a One-Stop Shop for the Evaluation of GI Motility Disorders. *Current gastroenterology reports*. 2016;18(3):1-7.
17. Cassilly D, Kantor S, Knight LC, et al. Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*. 2008;20(4):311-319.
18. Tran K, Brun R, Kuo B. Evaluation of regional and whole gut motility using the wireless motility capsule: Relevance in clinical practice. *Therapeutic Advances in Gastroenterology*. 2012;5(4):249-260.
19. Kaley TJ, Deangelis LM. Therapy of chemotherapy-induced peripheral neuropathy. *British Journal of Haematology*. 2009;145(1):3-14.
20. Pratt RW, Weimer LH. Medication and toxin-induced peripheral neuropathy. *Seminars in Neurology*. 2005;25(2):204-216.
21. Brock C, Søfteland E, Gunterberg V, et al. Diabetic autonomic neuropathy affects symptom generation and brain-gut axis. *Diabetes Care*. 2013;36(11):3698-3705.
22. Adams SC, Schondorf R, Benoit J, Kilgour RD. Impact of cancer and chemotherapy on autonomic nervous system function and cardiovascular reactivity in young adults with cancer: A case-controlled feasibility study. *BMC Cancer*. 2015;15(1).
23. Dimenäs E, Glise H, Hallerbäck B, Hernqvist H, Svedlund J, Wiklund I. Well-being and gastrointestinal symptoms among patients referred to endoscopy owing to suspected duodenal ulcer. *Scandinavian Journal of Gastroenterology*. 1995;30(11):1046-1052.
24. Dimenäs E, Carlsson G, Glise H, Israelsson B, Wiklund I. Relevance of norm values as part of the documentation of quality of life instruments for use in upper gastrointestinal disease. *Scandinavian Journal of Gastroenterology, Supplement*. 1996;31(221):8-13.
25. Sarosiek I, Selover KH, Katz LA, et al. The assessment of regional gut transit times in healthy controls and patients with gastroparesis using wireless motility technology. *Alimentary Pharmacology and Therapeutics*. 2010;31(2):313-322.
26. Ejskjaer N, Fleischer J, Fleischer J, Jacobsen PE, Poulsen PL, Nygaard H. A pocket-size device to detect autonomic neuropathy. *Journal of Diabetes Science and Technology*. 2008;2(4):692-696.
27. Fleischer J, Nielsen R, Laugesen E, Nygaard H, Poulsen PL, Ejskjaer N. Self-Monitoring of Cardiac Autonomic Function at Home Is Feasible. *Journal of Diabetes Science and Technology*. 2011;5(1):107.
28. Wegeberg AM, Lunde ED, Riahi S, et al. Cardiac vagal tone as a novel screening tool to recognize asymptomatic cardiovascular autonomic neuropathy: Aspects of utility in type 1 diabetes. *Diabetes Research and Clinical Practice*. 2020;170.

29. Spallone V, Bellavere F, Scionti L, et al. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy *. *Nutrition, Metabolism & Cardiovascular Diseases*. 2011;21:69-78.
30. Ruffle JK, Coen SJ, Giampietro V, Williams SCR, Aziz Q, Farmer AD. Preliminary report: parasympathetic tone links to functional brain networks during the anticipation and experience of visceral pain. 7/9/2018.
31. Little CJL, Julu POO, Hansen S, Reid SWJ. Real-time measurement of cardiac vagal tone in conscious dogs. *American Journal of Physiology - Heart and Circulatory Physiology*. 1999;276(2 45-2).
32. Pop-Busui R. Cardiac autonomic neuropathy in diabetes: A clinical perspective. *Diabetes Care*. 2010;33(2):434-441.
33. Huikui. Measurement of heart rate variability: a clinical tool or a research toy? | Elsevier Enhanced Reader. Published 1999. Accessed November 14, 2021.
34. Schatz IJ, Bannister R, Freeman RL, et al. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. *Neurology*. 1996;46(5):1470.
35. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation*. 2007;115(3):387-397.
36. Selvarajah D, Cash T, Davies J, et al. SUDOSCAN: A simple, rapid, and objective method with potential for screening for diabetic peripheral neuropathy. *PLoS ONE*. 2015;10(10).
37. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: A refined and abbreviated composite autonomic symptom score. *Mayo Clinic Proceedings*. 2012;87(12):1196-1201.
38. European Medicines Agency. EMA; Vidaza. EPAR summary for the public. Published online 2016. Accessed January 5, 2022. www.ema.europa.eu/contact.
39. Shanker A, Bashashati M, Rezaie A. Gastric Electrical Stimulation for Treatment of Refractory Gastroparesis: the Current Approach to Management. *Current Gastroenterology Reports*. 2021;23(2).
40. Brock C, Liao D, Wegeberg AM, Mohr Drewes A. The antroduodenal transition time is prolonged in adults with type 1 diabetes. *Neurogastroenterology and Motility*. 2021;33(11).
41. Chen TS. Effects of arabinosyl cytosine and 5-azacytidine on the intestinal absorption of nutrients. *Toxicology and Applied Pharmacology*. 1982;62(1):73-80.
42. Souza NCS, Simões BP, Júnior AAJ, Chiarello PG. Changes in intestinal permeability and nutritional status after cytotoxic therapy in patients with cancer. *Nutrition and Cancer*. 2014;66(4):576-582.
43. Wang YT, Mohammed SD, Farmer AD, et al. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: Influence of age, gender, study country and testing protocol. *Alimentary Pharmacology and Therapeutics*. 2015;42(6):761-772.
44. Nand S, Messmore HLJ, Patel R, Fisher SG, Fisher RI. Neurotoxicity associated with systemic high-dose cytosine arabinoside. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1986;4(4):571-575.
45. Magge RS, DeAngelis LM. The double-edged sword: Neurotoxicity of chemotherapy. *Blood Reviews*. 2015;29(2):93-100.
46. Maser RE, Mitchell BD, Vinik AI, Freeman R. The Association Between Cardiovascular Autonomic Neuropathy and Mortality in Individuals With DiabetesA meta-analysis. *Diabetes Care*. 2003;26(6):1895-1901.
47. Lee S, Chary M, Petersen B, Mascarenhas JO. Paraneoplastic orthostatic hypotension associated with acute myeloid leukemia. *European Review for Medical and Pharmacological Sciences*. 2015;19(15):2934-2937.
48. Yin XR, Liu P, Xu X, et al. Elevated plasma phage load as a marker for intestinal permeability in leukemic patients. *Medical Microbiology and Immunology*. 2020;209(6):693-703.