## ORIGINAL RESEARCH



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## Ultrasonographic measurement of gastric volume in critically ill patients nourished with different enteral feeding protocols

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#### **Abstract**

Background: Gastric residual volume monitoring is still widely used in clinical practice for early detection of gastric intolerance. This study investigated the compatibility of gastric volume measurements by ultrasound with the free drainage method in intensive care patients receiving enteral nutrition with three different protocols. Methods: Patients were divided into 3 groups according to different nutrition protocols applied in our clinic. Gastric ultrasound was performed in the right lateral decubitus position. Antral cross-sectional area was calculated using the Bolondi formula and gastric volume was calculated using Perlas and Tacken formulas. After, the patient was placed supine, a nasogastric feeding catheter was placed in gravity/free drainage for one hour in all groups and gastric residual volumes were recorded. Results: 82 intensive care unit patients were included. Spearman's correlation analysis showed a correlation between gastric residual volume and the other three values (cross-sectional area, gastric volume obtained by Perlas and Tacken formula) (p < 0.05) in each feeding group. Based on the Bland-Altman analysis, Perlas seems to be a more reliable alternative to GRV due to the smaller differences and less variability. Conclusions: We found a positive correlation between gastric volume determined by gastric ultrasound and gastric residual volume determined by the drainage method, with no difference between the different feeding protocols. Using ultrasound assessment, gastric volume can be predicted without interruption of enteral nutrition in patients fed semi-solid feeding solutions. Clinical Trial Registration: NCT06700759.

## **Keywords**

Gastric ultrasonogrophy; Enteral nutrition; Gastric residual volume; Monitoring nutritional intolerance

## 1. Introduction

In intensive care unit patients, early (in 24-48 hours) enteral nutrition (EN) is recommended to maintain bowel function and improve prognosis [1]. The frequency of EN intolerance in these patients has been reported to vary between 31% and 46% [2]. Guidelines for monitoring nutritional intolerance; recommends daily monitoring of symptoms of vomiting, bloating, bowel movement, painful abdominal distension, and radiological evaluations [1]. Due to the non-specificity of these signs and symptoms, gastric residual volume (GRV) monitoring is widely used in clinical practice for early detection of gastric intolerance (GI) [3, 4].

It is common to use antral cross-sectional area measurement determined by gastric ultrasonography (GUSG) to estimate gastric emptying time, gastric content, and volume, especially for the evaluation of aspiration risk in the perioperative period [5]. With the help of different formulae using the measured antral cross-sectional area, it has been suggested that gastric volume (GV) measurements of critically ill patients can be a reliable, noninvasive, and clinically usable technique [6, 7].

The aim of this study was to investigate the compatibility of GV measurements by ultrasound with the free drainage method in intensive care patients receiving enteral nutrition with three different protocols.

### 2. Methods

This is a single-center, prospective and observational research in 3 multidisciplinary intensive care units with a total 45beds of a teaching hospital that serves about 1000 inpatients each year. This study was initiated with the approval of the Ethics Committee of University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital (2011-KAEK-25 2022/01-19; 12 January 2022) (ClinicalTrials.gov Identifier: NCT06700759). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patients or their legal heirs. The study conducted

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between 20 January 2022 and 20 June 2022.

Adult patients hospitalized in intensive care unit due to cerebrovascular disease and receiving mechanical ventilator treatment were included in the study. Patients who were started on semi-solid nutritional solution (standard enteral nutritional products of 1 kcal/1 mL according to the comorbidities of the patients) via nasogastric (NG) 14 F polyurethane (PU) feeding catheter (BAXEN Medical, Spain) and reached the full calorie goal for at least 24 hours were included. Full dose calorie calculation of the patients was performed using the Harris-Benedict method (for women:  $655.1 + (9.56 \times \text{weight} \text{ (kg)}) - (1.85 \times \text{height (cm)}) - (4.68 \times \text{age})$  for men:  $66.5 + (13.75 \times \text{weight (kg)}) + (5.03 \times \text{height (cm)}) - (6.75 \times \text{age})$ . Feeding tube placement was confirmed with an anteroposterior radiograph taken before inclusion in the study.

Patients with shock due to any cause, abdominal surgery, gastrointestinal bleeding, obstruction, suspected perforation, malabsorption syndrome, prokinetic use or diarrhea, patients with a body mass index (BMI) >35 according to last known height and weight, and pregnant patients (due to inability to achieve right lateral position) were excluded. In addition, patients who could not be imaged, whose feeding protocol was changed and whose vasopressor doses were increased were excluded from the study.

The amount of EN products administered to the patients, age, gender, body weight, height, body mass index (BMI), comorbidities, vasopressor therapy and dose administered during imaging were recorded.

Our units have a physician-driven nutrition strategy, aiming for an adequate caloric and protein intake. Patients were divided into 3 groups according to implemented nutrition protocols. Ultrasonographic measurements were performed in 24-hour periods after the volume required for the target calorie intake.

Group 1: Patients who received 18 hours of infusion feeding, 6 hours of waiting and one hour of drainage;

Group 2: Patients who received 5 hours of infusion feeding, 1 hour of waiting and one hour of drainage;

Group 3: Patients who were fed with continuous infusion for 24 hours and drained for one hour.

## 2.1 Measurement of gastric volume

## 2.1.1 Measurement by GUSG

Using the GE LOQIC P6 (GE healthcare, Chicago, IL, USA) ultrasound device available in our clinic and a convex probe (2–5 MHz), the patient was placed in the right lateral position. To visualize the antrum, the probe was scanned in the sagittal plane in the epigastrium, sliding from left to right along the subcostal margins. The gastric antrum, aorta, superior mesenteric artery, and inferior vena cava were visualized just below the left lobe of the liver with the pancreas posterior to it. For the calculation of cross-sectional antral area (CSA), the anterior-posterior (AP) diameter and cephalo-caudal (CC) diameter (measured 3 times and averaged) were measured from serosa to serosa by capturing the best image during peristaltic movements at the level of the aorta or inferior vena cava. The antral area was calculated according to the formula CSA = (AP  $\times$  CC  $\times$   $\pi$ )/4 ( $\pi$  = 3.14) developed by Bolondi *et al.* [8].

Two different formulas were then used to calculate GV by CSA. The first formula was proposed by Perlas *et al.* [9]: Gastric volume (mL) =  $27 + (14.6 \times \text{right-lateral CSA}) - 1.28 \times \text{age}$ . The second formula was developed by Tacken *et al.* [10]: Gastric volume (mL) =  $79.38 + 13.32 \times \text{right-lat CSA}$ .

## 2.1.2 Measurement by drainage

After gastric volume was evaluated by ultrasonography (USG), the patient was placed in supine position, NG was connected to a drainage bag and gravity/free drainage was performed in all groups for one hour and the GRV values were recorded.

# 2.2 Statistical method and sample size calculation

The sample size of the study was calculated using G\*Power statistical power analyses for Windows 3.1.9 (G\*Power, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, NRW, Germany). In line with the a priori hypotheses, the amount of type I error in the study was taken as  $\alpha=0.05$ , the targeted power of the test  $1-\beta=0.85$  effect size was determined at a moderate level for all 3 groups with a pilot study, and the maximum sample size required for statistical analysis was determined as 26 with an effect size of 0.55. For each of the 3 groups, 26 patients were included in the study.

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 (IBM Corp., Armonk, NY, USA). Frequency and percentage for categorical data, median, minimum, and maximum for continuous data were given as descriptive values. Normality testing of the data was performed with the Kolmogorov-Smirnov Test. In intergroup comparisons, analysis of variance "(ANOVA) Test" was used for those with normal distribution, "Kruskal Wallis H Test" for those without normal distribution, and "Chi-Square Test" was used for the comparison of categorical variables. Spearman's correlation analysis and Bland-Altman used to evaluate the relationship between continuous variables. The results were considered statistically significant when the *p* value was less than 0.05.

## 3. Results

94 patients were included in the study. Eight patients were excluded because of unclear imaging with GUSG, and 4 patients were excluded because of increased vasopressor doses. 82 intensive care unit patients were included. The patients included in the study were 51.2% female and 48.8% male, aged 73 (22–101) years and weighed 75 (50–108) kg. The demographic and clinical characteristics of the patients who underwent three different feeding protocols are shown comparatively in Table 1 and no significant difference was observed between the groups.

The relationship between GRV determined by free drainage according to the feeding protocols, GV values obtained by Perlas and Tacken formulae and CSA is shown in Table 2. When the table is analysed; Spearman's correlation analysis showed that there was a significant positive correlation between GRV and the other three values (CSA, GV obtained by Perlas formula, GV obtained by Tacken formula) in each



TABLE 1. Distribution of demographic and clinical findings according to groups.

Variables	Total $(N = 82)$	18 hours feeding $(n = 27)$	5 hours feeding $(n = 27)$	24 hours feeding $(n = 28)$	<i>p</i> -value
	n (%) or Median (Min–Max)	n (%) or Median (Min–Max)	n (%) or Median (Min–Max)	n (%) or Median (Min–Max)	
Age (yr)	73 (22–101)	78 (23–95)	75 (33–95)	71.5 (22–101)	0.390
Gender					
Female	42 (51.2)	13 (48.1)	16 (59.3)	13 (46.4)	0.589
Male	40 (48.8)	14 (51.9)	11 (40.7)	15 (53.6)	0.389
Height (cm)	165 (150–190)	165 (150–180)	165 (155–190)	170 (150–180)	0.600
Weight (kg)	75 (50–108)	70 (50–108)	75 (50–100)	75 (60–90)	0.842
Calories delivered/Volume (mL)	1536.5 (1142–2344)	1526 (1148–2223)	1456 (1142–2043)	1611 (1275–2344)	0.137
Comorbidities					
Hypertension	50 (61.0)	17 (63.0)	15 (55.6)	18 (64.3)	0.776
Diabetes Mellutis	28 (34.1)	13 (48.1)	7 (25.9)	8 (28.6)	0.169
Coronary Artery Disease	16 (19.5)	3 (11.1)	5 (18.5)	8 (28.6)	0.260
Heart Failure	17 (20.7)	1 (3.7)	6 (22.2)	10 (35.7)	0.013
Dysrhythmia	9 (11.0)	2 (7.4)	4 (14.8)	3 (10.7)	0.683
Kidney failure	13 (15.9)	3 (11.1)	5 (18.5)	5 (17.9)	0.711
Sedoanalgesia	25 (30.5)	4 (14.8)	10 (37.0)	11 (39.3)	0.095
Vasopressor support	26 (31.7)	11 (40.7)	7 (25.9)	8 (28.6)	0.458
Gastric volume measurements					
Free drainage volume (GRV)	10 (0-250)	0 (0-250)	0 (0-150)	20 (0-160)	0.247
CSA	5.8 (0.7–21.8)	6.1 (1.6–21.5)	5.6 (0.7–19.0)	5.7 (2.2–21.8)	0.607
GV obtained by Perlas formula	22.6 (-54.4-263.4)	21.7 (-27.0-263.4)	18.2 (-54.4-212)	33.6 (-7.4-240.8)	0.430
GV obtained by Tacken formula	156.6 (88.1–370.2)	160.9 (97.2–365.1)	154.1 (88.1–333.1)	155.8 (108.8–370.2)	0.699

Min: Minimum; Max: Maximum; GRV: Gastric residual volume; CSA: Cross-sectional area; GV: Gastric volume.

feeding group. This indicates that GUSG can accurately reflect the GRV measured by the standard drainage method.

We conducted a Bland-Altman analysis comparing the Perlas (Fig. 1) and Tacken (Fig. 2) methods to GRV. The average difference between GRV and Perlas is approximately -10.76, indicating a small but noticeable systematic difference between the two methods. However, this difference is relatively minor. The standard deviation of the differences is 27.94, suggesting that the variation is also fairly low. In contrast, the average difference between GRV and Tacken is much larger, at -143.18, indicating a significant discrepancy between these two methods. The standard deviation for Tacken is 30.29, slightly higher than Perlas, which points to more variation in the differences.

The highest GRV value measured by drainage was 250 mL. In addition, no clinical findings that could be significant in terms of enteral nutrition intolerance were found in the patients.

## 4. Discussion

Contrary to guidelines, GRV is still measured in patients receiving EN [11]. GRV monitoring by aspiration or drainage may interfere with the achievement of calorie and protein targets in patients on EN and may also lead to loss of gastric fluid content. The use of GUSG seems reasonable to minimize the interruption during GRV monitoring by aspiration or drainage. In this study, in which we aimed to minimize the interruption, we found that 3 different values obtained with USG in the right lateral decubits (RLD) position were positively correlated in the determination of GRV by drainage method in patients receiving different enteral nutrition protocols.

Bouvet *et al.* [12] suggested that CSA can also determine GV in the supine position in intensive care patients, but the right lateral position may be better. The reason for this may be that the stomach has the capacity to expand and semisolid enteral nutrition products used especially in intensive care patients such as ours tend to collect in the fundus region of the stomach [5]. In addition, in the pioneering study of Perlas [13] in the use of GUSG to evaluate hunger before

TABLE 2. Distribution of the relationship between measurements according to groups.

Groups	Measurements	Spearman's Correlation Analysis	Gastric volume	
1st Grou	ıp (18/6)			
	CSA	Correlation coefficient	0.774	
	CSA	<i>p</i> -value	< 0.001	
	Perlas	Correlation coefficient	0.733	
	renas	<i>p</i> -value	< 0.001	
	Tacken	Correlation coefficient	0.774	
	Tackell	<i>p</i> -value	< 0.001	
2nd Gro	up (5/1)			
	CSA	Correlation coefficient	0.719	
		<i>p</i> -value	< 0.001	
	Perlas	Correlation coefficient	0.837	
		<i>p</i> -value	< 0.001	
	Tacken	Correlation coefficient	0.751	
	Tacken	<i>p</i> -value	< 0.001	
3rd Grou	up (24/0)			
CSA	Correlation coefficient	0.682		
	CSA	<i>p</i> -value	< 0.001	
	Perlas	Correlation coefficient	0.707	
	renas	<i>p</i> -value	< 0.001	
	Tacken	Correlation coefficient	0.685	
	таскен	<i>p</i> -value	< 0.001	

CSA: Cross-Sectional Area.

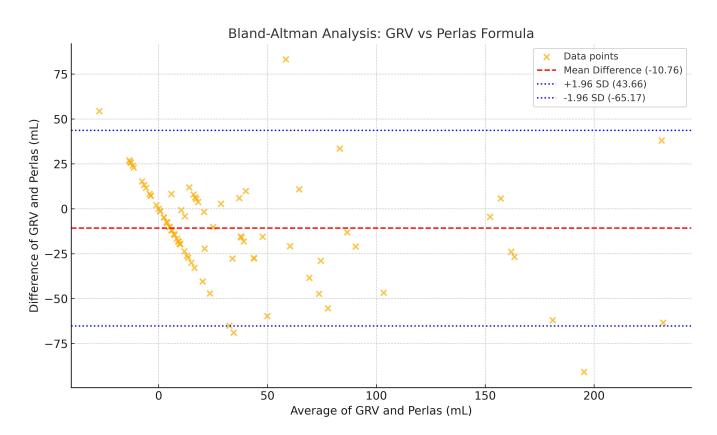


FIGURE 1. Bland-Altman analysis of GRV vs. Perlas formula. GRV: gastric residual volume; SD: Standard deviation.

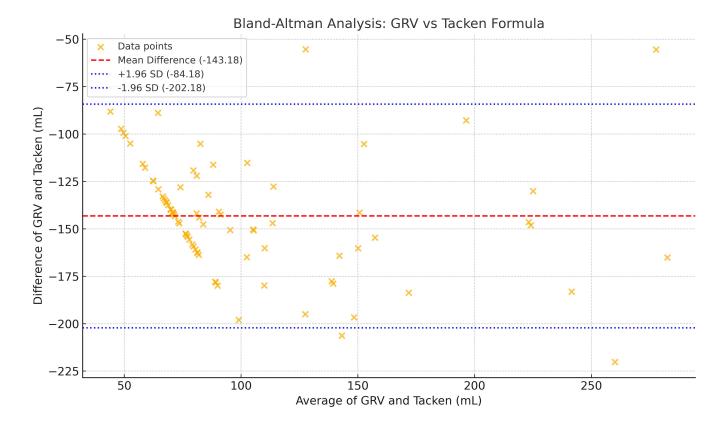


FIGURE 2. Bland-Altman analysis of GRV vs. Tacken formula. GRV: gastric residual volume; SD: Standard deviation.

anesthesia, it was reported that the stomach could be seen empty in the supine position and the stomach contents could be visualized in the right lateral position. In our initial evaluations before we started the study, we encountered difficulties in visualizing the gastric antrum in supine patients due to gas interposition. Therefore, we thought that the right lateral position would be more appropriate and evaluated our patients in this position. Despite this, we could not obtain a clear antrum image in 8 patients, so we excluded them from our patient groups. In general, although there are difficulties in positioning intensive care patients due to some limitations, patients with favorable conditions are frequently positioned during routine care. Unlike Bouvet's [12] and especially Taşkın's [14] patients with sepsis and shock, since our patient group included neurological diseases, the patients were easily positioned, and the measurements were terminated in a healthy manner.

R. Sharma *et al.* [15] reported that the estimation of GV by GUSG in the right lateral position using the Perlas formula was found to be higher than the aspiration method, but still effective in intolerance follow-up. On the contrary, V. Sharma *et al.* [6] stated that the GV estimated using GUSG performed in a 30-degree head-up supine position and using only CSA was correlated with and close to the aspirated volume. In addition, Taşkın *et al.* [14] also reported that the aspirated volume was similar to the measurement of only CSA in 30 degrees supine position The reason why R. Sharma [15] found the GV calculated according to aspiration to be high may be due to the Perlas formula they used instead of the measurement positions. Similar to R. Sharma's study, when we used the

Perlas formula in our study, we found that the GV values were higher than the values obtained by drainage. However, as age increased, we observed that the values were correlated but low. We think that this is caused by the age variable. When Perlas' formula is analysed, it can be predicted that gastric volume will decrease as the patient's age increases. In addition, Tacken et al. [10] suggested that a different formula could be used because they had a similar opinion. They suggested that while the Perlas formula can make closer predictions in clear liquids, its sensitivity decreases in viscous nutritional solutions, such as those used in intensive care. We found that our gastric volume calculations using the formula of Tacken et al. [10] were higher than the values obtained by drainage contrary to Perlas' formula. While the Perlas formula has been proposed for the assessment of gastric volume in clear liquids, the Tacken formula is suggested for thick liquids, such as nutritional products used in intensive care. However, in the Bland-Altman analysis, it has been observed that the Perlas formula shows better agreement with GRV measurements compared to the Tacken formula. The Tacken et al. [10] formula has been tested in healthy volunteers and has not yet been validated in large numbers of intensive care patients. Our study is the first in this field and was not designed to validate this formula.

Bouevet et al. [12] stated in their study that determination of GRV by aspiration would be incomplete in critically ill patients. However, in this study, the findings obtained with gastric USG were not compared with a third method such as computerized tomography (CT) scan or magnetic resonance imaging (MRI), which can better indicate GRV. This makes it difficult to evaluate which method is superior [16]. Gastric

residual volume monitoring may be affected by the diameter of the gastric tube, the size of the syringe and the position of the patient. Uysal *et al.* [17] reported that intermittent and slow aspiration method may cause larger gastric residual volume measurement. In our study, a 14F PU feeding catheter, which is routinely used in our clinic, was used. Contrary to many studies, free drainage was performed for one hour instead of aspiration with a syringe, both to prevent collapse of the soft structure of this catheter during aspiration and because we anticipated that free drainage would give closer results.

The clinical interpretation of gastric residual volume (GRV) often involves normalization to body weight (mL/kg) or comparison against critical thresholds associated with aspiration risk. However, the primary aim of this study was to assess the agreement between ultrasonographic estimations and measured GRV using established formulas, rather than to define or evaluate such thresholds. Consequently, metrics like mL/kg and specific critical values were not incorporated into the analysis.

Despite this, the clinical relevance of GRV monitoring cannot be overlooked. Prior literature suggests that GRV values exceeding 200–500 mL or normalized thresholds in mL/kg may indicate increased aspiration risk or feeding intolerance. While these thresholds were beyond the scope of this study, our findings provide a methodological foundation for future investigations. Ultrasonographic GRV assessments demonstrated reliable agreement with standard methods, underscoring their potential for safer, non-invasive monitoring in critically ill patients.

GRV evaluation in critically ill patients in intensive care unit (ICU) can be performed in different situations such as central catheterization, emergency interventions and airway control such as intubation-extubation, in addition to determining nutritional intolerance. In these patient groups, GRV may be an important marker especially in determining the risk of vomiting and aspiration. In our study, we evaluated the GRV with USG in terms of estimation of GRV in continuous and intermittent feeding methods applied by different clinicians. In this direction, approximately how many mL of feeding solution the patients received was known and evaluations were made accordingly. No intolerance findings such as vomiting and distension were observed in our patients and GRV above 250 mL was not determined. Since our study included patients who were not on prokinetics, the fact that the maximum GRV measured by aspiration was 250 cc may be explained by the good gastric emptying time of the patients. However, we believe that the targeted results could not be achieved due to deviations in the formulas. Because when the calculated values were analyzed, it was observed that Perlas formula calculated lower than expected and Tacken formula calculated higher values. It may be more reasonable to evaluate patients with CSAs rather than formula-based volume estimation before such interventions and procedures.

The study by Jahreis *et al.* [18] emphasizes the importance of measuring gastric residual volume (GRV), particularly in evaluating aspiration risk during enteral feeding. The study highlights that elevated GRV values may increase the risk of aspiration. Similarly, in our study, the accurate estimation of GRV using ultrasound could provide significant advantages in

clinical practice. As a non-invasive method, ultrasound allows continuous monitoring of gastric volumes during enteral feeding, which can facilitate the early detection of complications such as gastric intolerance or aspiration risk. The effects of different feeding protocols on GRV could provide valuable insights for clinical practice. For instance, differences between intermittent and continuous feeding may result in varying gastric emptying rates and volumes. This suggests that GRV measurements monitored via ultrasound could be a useful tool for evaluating the impact of feeding regimens on patients.

The observed correlations between measured values (CSA, Perlas, Tacken) and gastric residual volume (GRV) provide valuable insights into the potential utility of ultrasonographic methods in clinical practice. While this study did not directly evaluate patient outcomes such as aspiration risk or nutritional tolerance, the strong agreement between ultrasonographic estimates and measured GRV suggests that ultrasound may offer a reliable, non-invasive alternative for GRV assessment. This has implications for improving patient safety by minimizing the need for invasive procedures and potentially enabling more continuous monitoring of gastric volumes during enteral nutrition

This study aimed to evaluate gastric residue in three different feeding schedules to investigate whether variations in feeding regimens could influence the accuracy and reliability of ultrasonographic measurements of gastric volume. Different feeding schedules may affect gastric motility, gastric emptying rates, and distribution of semi-solid nutritional content within the stomach, potentially altering the accuracy of ultrasonography in estimating gastric residual volume (GRV). Specifically, intermittent feeding schedules with defined drainage periods might create transient changes in gastric content distribution, impacting ultrasonographic measurements, whereas continuous feeding schedules may result in a more stable gastric volume. Understanding these dynamics is essential for assessing whether ultrasonography can be reliably used across diverse feeding protocols in critically ill patients.

Patients with increased vasopressor use were excluded from this study to ensure the homogeneity of the study population and minimize potential confounding factors. Vasopressors can significantly influence gastric motility and perfusion, potentially leading to alterations in gastric emptying and volume. These changes might interfere with the accuracy and reproducibility of ultrasonographic measurements of gastric volume, introducing variability unrelated to the feeding protocols under investigation. By excluding these patients, we aimed to isolate the effect of feeding regimens on gastric volume and ensure the reliability of the measurements.

This study has several limitations that may affect the generalizability and interpretation of its findings. Our study does not focus on the necessity of GRV assessment, but rather aims to explore how to perform it easily and effectively if GRV monitoring is conducted. In our study, USG was performed by an intensive care specialist rather than a radiologist. However, gastric USG is widely used in point of care ultrasonography (POCUS) applications and is considered a straightforward procedure that can be successfully learned with minimal preliminary training. A significant portion of our patient group consisted of individuals with intracranial



events, which may represent a limited population. However, cerebrovascular diseases are commonly encountered in the ICU. Excluding conditions like shock was intended to create a more homogeneous group and improve protocol adherence. Additionally, the ease of positioning these patients was also taken into account. As a result, our findings may not be applicable to all critically ill patients, particularly those with complex comorbidities. We used the Perlas formula, which was originally developed in different patient populations and clinical settings. This may limit its accuracy when applied to critically ill patients receiving enteral nutrition, as we did not validate the formula within our specific patient population. Additionally, the study was conducted using three specific enteral feeding protocols from our clinic, which may not reflect the diversity of feeding practices seen in other ICU settings. This restricts the generalizability of our findings, as variations in feeding protocols could influence gastric volume and tolerance to enteral nutrition. Moreover, the free drainage method we used is not considered the gold standard, and the absence of comparison with more definitive imaging techniques, such as computed tomography (CT) or magnetic resonance imaging (MRI), limits our ability to definitively assess the accuracy of ultrasonography.

### 5. Conclusions

In conclusion, we found a positive and significant correlation between GV determined by GUSG and GRV determined by drainage method, without any difference between different feeding protocols in neuro-ICU patients. The ability to predict gastric volume without interrupting EN with USG is a significant advantage, as it allows continuous nutritional support while addressing potential issues like aspiration. Also, it is important to note that further research is necessary to validate these findings across different patient populations and clinical settings. This approach aligns with the current trend in ICU nutrition to minimise interruption of EN as it is crucial for the recovery and well-being of critically ill patients.

#### **AVAILABILITY OF DATA AND MATERIALS**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **AUTHOR CONTRIBUTIONS**

İC and SE—designed the study. SE, İK and HAK—performed the research. İC, SE and İK—wrote the manuscript. HAK—made the editorial changes. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was initiated with the approval of the Ethics Committee of University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital (2011-KAEK-25 2022/01-19; 12 January 2022) (ClinicalTrials.gov Identifier: NCT06700759). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patients or their legal heirs.

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#### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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