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Intestinal motility in extremely preterm infants Introducing: Ultra high-frequency ultrasound

Degree Project in Medicine

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1. Abstract

Background

Extremely preterm birth is defined as any birth before week 28+0 and equals greater risk to suffer from postnatal complications. Development of vital functions such as intestinal motility and permeability are immature.

Aim

The aim was to introduce a brand new examining method using abdominal ultra high-frequency ultrasound (UHFUS), and to determine its usefulness regarding intestinal motility and inflammation.

Method

UHFUS was introduced as a brand new examining method. Four preterm infants were enrolled in a pilot study to test the method.

Result

The intestinal motility in extremely preterm infants is not fully developed at birth. UHFUS can image intestinal villi and signs of intestinal inflammation. The thickness of the peritoneum and intestinal wall can be measured with great precision. It was possible to discern intestinal motility, but the numbers of contractions were not easily measured.

Conclusion

Intestinal motility is immature in extremely preterm infants.

Keywords: extremely preterm, intestinal motility, ultra high-frequency ultrasound, electrogastrography, necrotizing enterocolitis,

2. Background

Every year, worldwide, approximately 15 million babies are born preterm and the amount is increasing. These 15 million preterm births account for 11.1 % of the total amount of live births worldwide. According to the World Health Organization (WHO), only three countries (Croatia, Ecuador and Estonia) had reduced preterm birth rates between 1990 and 2010.

Preterm birth is defined by the WHO as any birth before 37 weeks of gestation. It can then be divided into several subgroups depending on the gestational age: extremely preterm (<28 weeks), very preterm (28–<32 weeks) and moderate preterm (32–<37 weeks). Extremely preterm birth equals greater risk to suffer from different kinds of complications, such as infections or disabilities compared to the other subgroups (1).

The preterm birth percentage varies considerably depending on the country of birth. Several European countries have a preterm birth rate around 5 %, while Asian and African countries may have a preterm birth percentage up to three times greater (1).

2.1 Embryogenesis

The gastrointestinal tract begins to develop early during embryogenesis, around week 4.

There are three types of germ layers from which all the body's future organs and structures originate. The ectoderm forms our skin and nervous system, the mesoderm forms our skeletal and muscular systems, and the endoderm forms the gastrointestinal tract and its epithelial lining. During embryogenesis, the endodermal gut tube is divided into three different sections: the foregut, midgut and hindgut (2).

The foregut forms the pharynx, esophagus, stomach and half of the duodenum. It also forms several other organs in the gut, including the liver, gallbladder and pancreas. At the end of week 4, the foregut will elongate and form the stomach (2).

The midgut forms the second half of the duodenum, jejunum, ileum, cecum, appendix, ascending colon and two thirds of the transverse colon (2).

The hindgut forms one third of the transverse colon, descending colon, sigmoid colon and rectum (2).

Simultaneously, as the different regions of the endodermal gut tube develop, so does the lumen. The gut tube consists of endodermal cells forming the epithelial lining, while the mesoderm surrounds the endoderm and forms the muscular layers and connective tissue.

2.2 Cell differentiation

The epithelial lining is initially composed of pseudostratified columnar epithelia; however, they later turn into simple columnar epithelia. During the 9th week, the surrounding mesoderm proliferates and projects towards the lumen, creating the villi together with the endodermal epithelia (2).

The endodermal epithelia consist of several different types of cells with different objectives. The turnover rate is incredibly fast, and it takes 2–3 days for the epithelium to be completely replaced by the stem cells differentiation (2). The stem cells differentiate into enterocytes, M cells, enteroendocrine cells, goblet cells and Paneth cells. The enterocytes are covered by microvilli making the cell excellent at absorbing nutrients and transporting them. The M cells are found above Peyer's patches and their objective is to present luminal antigens to lymphocytes, macrophages and dendritic cells. The enteroendocrine cells produce and secrete hormones as a response to different kinds of stimuli. The goblet cells function as a one-cell exocrine gland, which produces and secretes mucus with protective properties. Paneth cells produce and secrete a vast mixture of antimicrobial peptides as a response to being exposed to bacteria and other harmful antigens (3).

2.3 Slow waves

The intestinal wall consists of a longitudinal and circular muscle layer made up from smooth muscle fibres. These fibres are unitary in the sense that they are bundled together in the hundreds up to the thousands, and contract together as a single unit. When receiving electrical impulses, the signals can travel from one fibre to another through gap junctions. These gap junctions allow the signal to propagate to neighbouring cells, allowing rhythmic contractions. The smooth muscle bundles are connected at several different areas, which create a branching network of smooth muscle bundles that works as a syncytium. Firing an action potential within the muscle will make it propagate in all different directions. Inducing muscular movement in either of the gastrointestinal muscle layers will generally induce a response in the other due to these connections (4).

The contraction frequency in the gastrointestinal tract varies. In adults, it ranges from about three cycles per minute (cpm) in the stomach, eight to nine in the terminal ileum, and up to twelve in the duodenum. The frequency is determined by something referred to as slow waves. Slow waves are the constant change in the membrane potential among the smooth muscle fibres. It usually varies between -5 and -15 millivolts (mV) and they are not true action potentials. The resting membrane potential for smooth muscle fibres ranges from -50 to -60 mV. When the resting membrane potential exceeds -40 mV, an action potential called a spike potential will occur. Spike potentials are usually the ones inducing muscle contractions, not the slow waves. The slow waves are the ones causing the spike potentials, which then induce muscle contractions (4). In healthy adults and children, >70 % of the gastric myoelectrical activity consists of a slow wave frequency of 3 cpm (5). The emergence of slow waves is complex and not entirely understood; it is, however, believed that the smooth muscle cells interact with a special type of cell called the interstitial cells of Cajal. These cells are

referred to as “pacemaker cells” and they are located between the intestinal wall’s muscle layers, forming a network (4).

2.4 Enteric nervous system

The innervation of the gastrointestinal tract is managed by the enteric nervous system (ENS). The enteric nervous system is located in the wall of the gut and consists of two different nerve plexuses: the myenteric plexus located between the longitudinal and circular muscle layer, and the submucosal plexus located in the submucosa. The myenteric plexus covers the gut wall all the way around, and the location between the two muscle layers enables it to control muscle tone and rhythm of contractions. The submucosal plexus is able to control local secretion and absorption with the help of sensory signals from the intestinal epithelium. The ENS is a part of the autonomic nervous system (ANS) consisting of a sympathetic (SNS) and parasympathetic (PNS) division. The SNS and PNS communicate with the ENS, controlling gut motility, blood flow, absorption and secretion by using different transmitter substances. The ENS is capable of functioning independently without signals from the SNS and PNS, but the SNS and PNS greatly enhance and inhibit the effects of the ENS. Generally, the PNS increases the activity in the ENS, while the SNS inhibits activity in the ENS (4).

2.5 Peristalsis and mixing movements

Peristalsis and mixing movements are the two different types of movements that occur in the gastrointestinal tract. Peristalsis occurs due to the syncytial properties of the smooth muscle cells. The cells can be stimulated in several different ways, including by distension, by chemicals or via the PNS. The waves have a velocity of 0.5–2.0 cm/sec and usually travel about 3–5 cm. When stimulated, the smooth muscle cells contract a few centimetres above the initial stimulus point, forming a ring-like structure. The contractile ring appears in the circular

muscle layer of the gut and its objective is to propagate in the intestinal wall, moving food forward towards the anus. In the stomach, mixing waves occur due to the slow wave potential of the interstitial cells of Cajal. Since the pacemaker cells determine the frequency of contractions, the number of mixing waves is the same, about three per minute in the stomach. They begin in the upper portion of the stomach and propagate towards the pyloric antrum. As the contraction reaches the lower portion of the stomach, the stomach content is pressed against the pylorus. The pylorus contracts as the stomach content is pressed towards it, forcing most of the content to travel back towards the stomach's body, letting only a small portion pass into the duodenum. This action is called retropulsion, and it mixes the stomach content together with digestive juices, forming the paste-like substance referred to as chyme. The chyme then passes into the small intestine, distending the intestinal wall. When distended it causes segmental contractions along the intestinal wall, mashing the chyme with the secretions of the small intestine (4).

2.6 Permeability

The anatomical conformation of the gastrointestinal tract is more or less fully developed at week 20 (6). However, it is far from fully developed when it comes to permeability and digestive enzymes (6, 7). The gastrointestinal tract poses as a barrier towards potentially harmful pathogens, and as a recipient and transporter of nutrients. In the preterm neonate, this could be seen as a double-edged sword. The preterm neonate needs nourishing nutrients to grow and become stronger, which means that it is beneficial for the gastrointestinal tract to have an increased permeability to promote nutritional uptake. However, increased permeability also increases the risk of penetration of pathogens and bacteria, which may cause infection or inflammation (7–9). Decreased intestinal permeability is associated with gut maturation (9). There are several different suggested factors that affect gut maturation,

including postnatal age, gestational age and feeding method (8, 10).

2.7 Necrotizing Enterocolitis

Extremely preterm neonates are not accustomed to the disadvantageous environment outside the womb and are in need of neonatal intensive care to survive. Even with advanced neonatal intensive care, about 7–12 % of the infants with a birth weight between 500–1500 g will develop necrotizing enterocolitis (NEC) (11–13). NEC is one of the most common and dreaded diseases in neonates, with a mortality rate around 20–30 % (11–15). The pathogenesis of the disease is poorly understood and seems to be multifactorial. Suggested factors include intestinal immaturity, feeding regimen, ischemic injury, microbial colonization and inflammation (11, 14). There are several signs and symptoms associated with NEC, including abdominal distension, feeding intolerance, increased gastric residuals and bloody stools (14). There are other symptoms associated with NEC in addition to the ones mentioned above, making the diagnosis nonspecific and difficult to identify. In order for the infant to receive proper treatment as fast as possible, it is important that nurses are experienced and have strong assessment skills. The diagnosis is often determined as a combination of clinical signs and abdominal radiography (12).

The type of treatment depends on the severity of the condition. It can either be treated medically with bowel rest, abdominal decompression, antibiotics and correction of blood pressure, perfusion and electrolytes. It can also be treated surgically in infants with intestinal perforation, or if the medical effort above has little or no effect and the clinical status deteriorates (11, 12).

2.8 Feeding Intolerance

The gastrointestinal tract is not fully functional at birth in extremely preterm infants. During the last trimester, normally the gastrointestinal tract elongates and increases its number of microvilli. In addition to this, there are several mechanical and biochemical features that may be underdeveloped, affecting the ability to assimilate enteral nutrition. One of the greatest challenges for the neonatologist is approaching this problem.

It is essential to feed the infant to prevent postnatal growth retardation and stimulate gut maturation, while avoiding NEC and feeding intolerance (FI). There is no set definition of FI; the definition may vary depending on research centre and author. According to Moore and Wilson, who conducted a review of all existing definitions, the most fitting definition for FI is: “the inability to digest enteral feedings presented as gastric residual volume (GRV) of more than 50 %, abdominal distension or emesis or both, and the disruption of the patient’s feeding plan” (16). While defining FI, it does not provide any tools to distinguish FI from NEC (16). FI is common among preterm infants and affects 16–29 % of the patients in the Neonatal intensive care unit (NICU) (17). The most common signs and clinical symptoms for FI include excessive gastric residuals, abdominal distension, apnoea, bradycardia and emesis. There are several different strategies regarding the prevention and treatment of FI; however, there has yet to be a final consensus on how to deal with the issue in the best possible way. Different strategies include choice of food, when to start enteral feeding, the advancement pace of enteral feeds and whether it should be continuous or intermittent (16).

2.9 Electrogastrogram

Intestinal motility in infants can be measured with different methods. Ultrasound is a non-invasive procedure and will be discussed in the next section. Manometry is an invasive

procedure and is not suitable for preterm infants due to their frailty. Electrogastrogram (EGG) however, is non-invasive, convenient and records gastric myoelectrical activity through electrodes placed on the subject's stomach (5). Alvarez performed the first recording of gastric myoelectrical activity in 1922, but the EGG was demonstrated for the first time in 1975 by Brown et al. (5). EGG measures slow waves originating from so-called pacemaker cells. Slow waves regulate spike potentials, which induce muscle contractions. The slow wave frequency in the stomach is approximately 3 cpm, but 2–4 cpm is considered normal. Bradygastria equals 1–2 cpm and tachygastria 4–10 cpm. The EGG records the percentage of the different slow wave frequencies mentioned above. In healthy adults and children, normal slow wave frequency is recorded in >70 % of the EGG. Preterm infants' percentage of normal slow wave frequency is lower compared to older subjects (18). EGG as a method has several problems. There are no standardized protocols regarding equipment, software, electrode placement etc., making it difficult to compare different studies. The subject also has to be quiet and still to prevent artefacts. Artefacts aggravate the interpretation and compromise the analysis (5).

2.10 Ultrasound

Ultrasound is a non-invasive procedure that is both convenient and readily available.

Regular ultrasound is often used in the NICU for cranial, lung, heart and bowel imaging.

Intestinal immaturity is thought to be involved in the pathogenesis of NEC and ultrasound is used as a complement to radiology, which is the primary imaging method for diagnostic purposes together with clinical symptoms (19). In addition to regular ultrasound, ultra high-frequency ultrasound (UHFUS) now exists. UHFUS is a novel imaging method and was released for use on the clinical market in early 2016. Clinical applications include vascular and musculoskeletal imaging as well as clinical uses in dermatology, neonatology and

pediatrics (20). Regular ultrasound uses frequencies ranging from approximately 2–15 MHz depending on the tissue examined, while UHFUS has transducers up to 70 MHz, making it ideal when imaging small superficial structures such as blood vessels – or in this case, the bowels of preterm infants. UHFUS yields a much greater resolution compared to regular ultrasound, with the drawback being a loss of depth penetration. Due to UHFUS being a relatively new imaging method that is not available at every hospital, its clinical uses have not yet been fully discovered. This study aims to introduce UHFUS as a new examining method on intestinal motility and intestinal inflammation. To the best of our knowledge, there are no studies using UHFUS for examination of intestinal motility in preterm infants.

2.11 Extended background

The intestinal motility in preterm infants is immature at birth (7, 18, 21–25).

Few EGG studies included infants with a gestational age of 28+0 while none included extremely preterm infants born before 28+0. Several studies have found a maturation pattern regarding the development of slow waves, and that the percentage of 3 cpm normogastric rhythm increases with gestational age (18, 21–24). A summary of the articles is found in table 1.

Cucchiara et al. (22) found peaks of normal electrical activity (2–4 cpm) in all gestational ages ranging from 29 to 42 weeks, but the percentages of normogastric rhythm and tachygastria started to resemble the full-term infant at 35 weeks of gestational age.

Statistically significant differences regarding the percentage of normal slow waves were found in preterm infants aged 29 and 32 weeks respectively compared to full-term infants.

According to Riezzo et al. (24), there is a difference concerning dominant frequency ranges in very preterm infants compared to full-term infants. Only full-term infants demonstrated a

dominant frequency range between 2 and 4 cpm. The percentage of 3 cpm activity was higher and the percentage of tachygastria was lower in full-terms compared to very preterm infants.

Fasting antral area and half-emptying time are parameters for gastric emptying. These parameters were measured using ultrasound. Preterm infants showed a wider fasting antral area and longer half-emptying time compared to full-terms. Preterm infants with an abnormal EGG showed longer half-emptying time compared to those with a normal EGG (24).

Lange et al. (26) recorded EGG on 57 healthy infants with a gestational age ranging from 29 to 42 weeks and did not find any difference in gastric slow wave activity between preterm and full-term infants. The distribution of normogastric slow waves, bradygastria and tachygastria were similar among both preterm and full-term infants.

Riezzo et al. (7) found that the intestinal motility in moderately preterm is quite stable and resembles that of the full-term infant. However, the intestinal barrier improves during the first postnatal week of life. This is shown by measuring the ratio of lactulose and mannitol in the urine after oral administration of the substances.

The percentage of 2-4 cpm normogastric rhythm increases with gestational age. Studies use different equipment, software, electrode placement and more, which yields a difference in the recorded percentage of normogastric rhythm. There is a difference when comparing studies regarding the time with normal slow wave percentage between 2 and 4 cpm. Patterson et al. (23) found the percentage to be 38.1 % in preterm infants 34 weeks of gestational age, while Riezzo et al. (24) found it to be 59.3 % in preterm infants 28–32 weeks of gestational age.

The gastric electrical activity in preterm and full-term infants is immature compared to older neonates and children, but there are no significant differences in the EGG pattern between preterm and full-term infants during the first two weeks of life (25).

Intestinal transit time is prolonged in the preterm infant due to an immature peristaltic motor pattern. The intestinal transit in the full-term neonate takes around 4 hours compared to a very preterm infant where the time is more than doubled (27).

Richburg and Kim (28) examined infants with a gestational age of 25–36 weeks using regular ultrasound. Ultrasounds were recorded the first five days of life (DOL) while examining the amount of contractions in each of the abdomen's four quadrants. Contractions in each quadrant were added together forming the cumulative motility (CM). CM became statistically significant during DOL 4 and 5 with an increase in the amount of intestinal contractions compared to DOL 1.

3. Aim

The aim was to introduce a novel examining method, UHFUS, and to determine its usefulness regarding intestinal maturation, motility and inflammation. In order to study the overall aim, a hypothesis was created which states that intestinal motility and inflammation can be recorded in extremely preterm infants by using an ultra high-frequency abdominal ultrasound.

4. Material and Methods

EGG was originally planned as the method of choice to study intestinal motility, but after having discussed with local experts in the area, we decided to test the feasibility of using UHFUS for examination of the intestines in extremely preterm infants.

Recording UHFUS tested the hypothesis. Four preterm infants were enrolled in a pilot study to test the method. The gestational age of the included participants ranged from 27–35 weeks. Infants were excluded if they were born full-term or deemed too fragile to examine. One of the participants had clinical signs of intestinal inflammation, while the three others did not.

The study patients were all born at Queen Silvia's Children's Hospital in Gothenburg. Since the objective was to assess the feasibility of using the method and its usefulness regarding determination of intestinal maturation, motility and inflammation, no controls were used in the pilot study. Abdominal UHFUS (Vevo® MD, Fujifilm VisualSonics, Canada) with a linear array transducer (UHF 48, Fujifilm VisualSonics, mean beam frequency range between 20 and 48 MHz) with a peak frequency of 48 MHz was used. Scans were performed using a frequency of 48 MHz and with an imaging depth ranging from 7 to 11 mm. Due to the infants' fragility and being in an incubator, the scans were made as swiftly as possible with respect towards the infants' wellbeing. The aim was to scan all four quadrants to obtain acceptable images, but this was not always possible. In the images of acceptable quality, visual assessments of intestinal motility as well as measurements of the intestinal wall and peritoneum were performed. The intestinal wall was measured from the leading edge of the external mucosa to the leading edge of the internal lumen (Figure C), the parietal peritoneum was measured from the leading edge of the peritoneum to the peritoneal/gastric cavity interface (Figures A and B).

4.1 Analysis procedure

The ultrasounds were analysed regarding its anatomical appearance, intestinal motility and signs of inflammation. Due to the small number of enrolled participants, and since the abdominal UHFUS is an entirely new method introduced by this pilot study with the purpose being to mainly evaluate its feasibility, a statistical analysis of the findings was not performed. Further studies with larger populations and comparisons to regular ultrasounds are needed to fully validate the method.

5. Ethics

The application form of ethical approval for the clinical ultrasounds was sent to the Regional Ethical Review Board in Gothenburg. The application, with registration number DNR-258-18, was approved on May 22, 2018. No children were examined without parental consent. Parents were given detailed information about the examination. There were no risks or side effects from the UHFUS, but the general frailty among the extremely preterm infants had to be considered before the examination. The examinations were painless and did not result in any exposure to radiation. There were no blood samples taken and all necessary information was gathered through the UHFUS. The pilot study was not immediately beneficial to the included children's health, but hopefully contributes to a greater understanding of gastrointestinal development and motility.

6. Results

6.1 Ultra high-frequency ultrasound

UHFUS was able to image intestinal villi and signs of intestinal inflammation. One participant with a gestational age of 28+4 had clinical signs of NEC and UHFUS was able to find intramural gas, free gas, bowel wall thickening and ascites (Figure D). The intestinal wall measured 2.0 mm and was more than twice as thick compared to a participant with no clinical signs of intestinal injury (Figure C). The thickness of the peritoneum and intestinal wall can be measured with great precision, as the resolution for the UHFUS allows measurements down to 0.02 mm. The participant with clinical signs of NEC had a thickening of the peritoneum measuring 0.4 mm as a sign of peritoneal irritation. This measurement is five times greater compared to a participant with no clinical signs of intestinal injury (Figure A and B). It was possible to discern intestinal motility with the propagation of bowel contents in

all four participants, but the number of contractions was not easily measured, partly due to difficulties in discerning between true contractions and movement caused by the infant or the transducer moving. The exact number of contractions could not be counted. The peritoneum, intestinal wall and bowel contents were seen in all four participants. Intestinal villi could be distinguished in two of four participants (Figure C and E). Measurements of the different structures varied depending on the placement of the transducer and its projection. Signs of intestinal inflammation were only seen in the participant with clinical signs of NEC. In addition to yielding a high resolution and accurately imaging the peritoneum and bowels, UHFUS is also able to image the skin followed by the subcutaneous fat and abdominal muscles in all four participants.

7. Discussion

Intestinal motility is immature in preterm infants. Gastric myoelectrical activity is not fully developed at birth and follows a maturation pattern with an increase in the percentage of normal slow wave frequency with gestational age. The amount of intestinal contractions increases during the first 5 DOL. UHFUS can image preterm infants' bowels with great precision, including intestinal villi, the peritoneum and signs of intestinal inflammation. Intestinal motility was seen but could not be measured objectively.

There are a very limited number of scientific publications studying intestinal motility in extremely preterm infants. In fact, only one of the studies included extremely preterm infants (28). Regarding the hypothesis, it was found that intestinal motility and inflammation can be recorded in extremely preterm infants using UHFUS.

The overall aim was fulfilled in this study. The literature search was helpful in determining whether intestinal motility is immature among extremely preterm infants. Most of the articles

used EGG as their main examining method. The only study we found using ultrasound as a mean to examine intestinal motility was Richburg and Kim (28), suggesting that the knowledge on the subject is sparse. The EGG studies on preterm infants were generally old. The most recent was from 2009, while the oldest dated back to 1998. There were even older studies, but they were excluded due to being too old.

UHFUS was successfully performed on all four participants, but there are some limitations to the study. Only four premature infants were enrolled to the pilot study using UHFUS. Not all participants were extremely preterm, only two of the four were born before week 28+0. The study did not have a control group, and no statistical analyses were made. There were several elements that hampered the selection. Some infants were too fragile. Some parents did not consent to the examination. It would have been optimal to enrol more children and adding a control group, but there was not enough time considering the fact that this study is a degree project in medicine.

The general consensus is that intestinal motility in preterm infants is immature at birth (7, 18, 21–25) and that a maturation pattern is seen regarding the increased percentage of 3 cpm rhythm with gestational age (18, 21–24). None of the EGG studies included extremely preterm infants. Some studies found that the slow waves matured and resembled the full-term infant somewhere between weeks 32–36 (7, 22, 24). However, the results were conflicting. Lange et al. (26) could not demonstrate a maturation pattern, and did not find any difference in gastric slow wave activity among preterm infants 29–42 weeks old. Precioso et al. (25) did not find any differences in the EGG pattern between preterm and full-term infants. There is a variance when comparing studies regarding the percentage of normal slow wave frequency because studies use different intervals as the normal EEG frequency range. This sometimes results in very preterm infants having higher percentages of normal slow waves compared to older subjects in other studies. The lack of standardized EGG protocols could also explain

this.

EKG studies are difficult to compare to one another because of several different reasons. They use different equipment and software. Electrode placement may differ. There are different ranges defining bradygastria and tachygastria. The recordings are made pre- and postprandial. The length of the recordings differs. Inclusion and exclusion criteria differ. The examined groups differ in age, size and range. The recordings are made at different times after birth. There are large individual differences in the individual groups. If it were possible to come up with a standardised protocol regarding equipment, electrode placement and more, it would simplify the interpretation.

EKG is a non-invasive procedure, which is preferable when examining preterm infants. It should theoretically be a fitting method to use on infants, due to the small amount of subcutaneous fat and tissue between the electrodes and the stomach. There is also no hair to reduce the impedance. However, there are drawbacks to be taken into consideration when analysing the recording. Motion and sound create artefacts, which compromises the analysis of the EKG. Excessive movement and sound can therefore render the recording useless.

The sample sizes in the EKG studies are generally small and the recordings seem to be difficult to interpret. Many of the studies are old. There are no new studies using EKG to measure slow waves in preterm infants since 2009. This is probably because there are issues that have to be addressed in order for the method to be reliable. A standardised protocol would be the most important step.

UHFUS is a novel method useful for imaging the bowels of extremely preterm infants. Intestinal motility was seen in all four participants, but quantification of the contractions was difficult due to problems in discerning between true contractions and movement caused by the infant or the transducer moving. Richburg and Kim (28) have used regular ultrasound and

measured the amount of contractions in each of the abdomen's four quadrants. Regular ultrasound suffers from the same difficulties as UHFUS. It is difficult to discern true intestinal contractions due to transducer pressure, which is greater in regular ultrasound. Movement of the infant and transducer further complicates the interpretation. A method where the transducer pressure is minimal, while the infant and transducer are completely still would increase the chances of successfully measuring true intestinal contractions. Regular ultrasound has lower resolution compared to UHFUS, which may facilitate counting the contractions. In this case, the high resolution of UHFUS might be disadvantageous since the overview is inferior compared to regular ultrasound. It is also possible that it will take extensive training until one can discern and count contractions as a measurement of intestinal motility. The experiences gained from the examinations are invaluable, not only regarding the gathered information, but on the technical execution of the UHFUS itself.

Ultrasound as an imaging method has various advantages, but also a few drawbacks. The method is non-invasive, and the infant is not exposed to harmful radiation. The machine is portable, so the infant does not have to leave the NICU. Ultrasound images tissues in real time. The procedure is painless and does not require a peripheral intravenous catheter. It is also generally cheaper than other imaging methods. UHFUS has an increased resolution compared to other imaging methods such as MRI or CT. Artefacts are, however, a common problem. Furthermore, it requires extensive training to perform and interpret the findings making it user dependant.

Two of the infants were cared for in an incubator in the NICU, and the examinations were performed there. The two others had family rooms in the neonatal ward and used heated beds where the examinations were performed. It was more difficult to perform the UHFUS on the infants in the incubator due to space limitations and monitoring equipment. The images and projection of the intestines and peritoneum may vary depending on the placement and angle

of the transducer, making it important to determine the optimal settings and transducer placement for each scan.

Doppler ultrasound was not used to measure intestinal blood flow. No injuries or side effects have been reported when using UHFUS doppler on mice, but no previous studies have been performed in infants. As the energy levels are high, doppler was not used in this pilot study before accurately determining the risk for injury.

UHFUS uses transducers at much higher frequency ranges than regular ultrasound, which means a limited depth penetration, but greater resolution. UHFUS and regular ultrasound can therefore be used in combination to help facilitate the interpretation of findings. The extremely preterm infant can weigh less than 500 g and has very little subcutaneous body fat or other structures that interfere with the ultrasound imaging of the intestines. This together with the other advantages mentioned above makes ultrasound ideal to use on extremely preterm infants.

Due to the enhanced resolution when using a 48 MHz linear array transducer, the small anatomical structures can be measured with great precision compared to regular ultrasound. A structure like the peritoneum, which is very thin (Figure A and B), can sometimes be visualised with regular ultrasound, but when measured it is significantly overestimated due to the low resolution, whereas the measurement using UHFUS is more accurate (29). Intestinal villi were visualised by UHFUS in two of the four participants. To the best of our knowledge, this is the first time intestinal villi are imaged using ultrasound. The findings of villi are consistent with the embryological development where the number of microvilli increase during the start of the third trimester (16). It is possible that the two other participants had visible intestinal villi as well, but that the visualization of them failed. One reason could be that the UHFUS exams were made swiftly with respect towards the preterm infants'

wellbeing. Another possibility is that there is a difference in the development of intestinal villi depending on gestational age.

The participant with clinical signs of NEC had signs of inflammation when examined with UHFUS. The intestinal wall was more than twice as thick, while the thickness of the peritoneum was five times greater compared to a healthy preterm subject. There were also ascites and free gas. Bowel x-ray was performed before the UHFUS scan was initiated, and the result arrived during the UHFUS scan. The suspicion of NEC increased, and the infant was scheduled for surgical intervention, which confirmed the NEC diagnosis. In order to validate the method and determine if intestinal motility can be measured objectively, further studies with larger sample sizes and control groups are needed. This will help create reference material on different anatomical structures and intestinal inflammation, which may result in new possible diagnostic markers.

8. Conclusions

Intestinal motility is immature in extremely preterm infants. Gastric myoelectrical activity is not fully developed at birth and follows a maturation pattern with an increase in the percentage of normal slow wave frequency with gestational age.

UHFUS is a feasible method for visualisation of the small anatomical structures in extremely preterm infants, as it yields a higher resolution compared to normal ultrasound. Intestinal motility was seen but could not be quantified. Distinct findings of inflammation in a patient with clinical signs of NEC, which were later confirmed via surgery, gives hope that the method can be used for early detection of the disease.

In order to validate the method and determine if intestinal motility can be measured objectively, further studies with larger sample sizes and control groups are needed. This will

help create reference material on different anatomical structures and intestinal inflammation, which may result in new possible diagnostic markers.

9. Populärvetenskaplig sammanfattning på svenska

Barn ligger normalt 40 veckor i livmodern innan de föds. De barn som föds före vecka 28 föds extremt för tidigt och kallas för extremprematurer. I Sverige föds det ca 300 extremprematurer årligen och de löper större risk att drabbas av sjukdomar av olika slag. Tarmarna är färdigutvecklade rent utseendemässigt vecka 20, men vissa funktioner är omogna, t.ex. tarmarnas rörlighet. Tarmarnas rörlighet är viktig för att kunna tillgodogöra sig mat och näring. En av de farligaste sjukdomarna extremprematurer kan drabbas av heter nekrotiserande enterokolit, och gör så att delar av tarmen dör, vilket är livsfarligt. Man vet inte exakt varför detta sker, men man tror att tarmarnas omogenhet, inflammation och dålig syretillförsel i tarmen kan spela roll.

För att ta reda på mer om tarmarnas rörlighet kan de undersökas med ultraljud. Det är samma typ av undersökning som används när man undersöker barn i mammans mage, fast i detta fall undersöker man istället extremprematurers mage och tittar på tarmarna. Ultraljud är ofarligt för barnet. Nyligen uppfanns en ny metod med ett högfrekvent ultraljud med bättre och tydligare bild, men den har aldrig använts för att undersöka extremprematurers tarmar. Vi har därför i denna studie valt att testa detta. Fyra barn undersöktes med högfrekvent ultraljud. Barnen föddes mellan graviditetsvecka 27 och 35 på Östra sjukhuset i Göteborg. Utöver ultraljudundersökning undersöktes kunskapsläget avseende extremprematurers tarmrörelser.

Extremprematurers tarmrörelser är omogna jämfört med barn som föds i vecka 40. Den elektriska aktiviteten som styr tarmrörelserna mognar efterhand barnet blir äldre. Det högfrekventa ultraljudet har väldigt hög upplösning och kan förstora väldigt små detaljer

jämfört med vanligt ultraljud. Tarmarna rörde sig, men det var svårt att objektivt mäta hur mycket. Det gick t.ex. inte att räkna ut ett visst antal tarmrörelser per minut. I ett av barnen såg man tecken till inflammation. Detta barns tarm- och bukhinna var förtjockad jämfört med de andra barnens, vilket är tecken på att tarmen kan vara skadad. Detta barn visade sig ha nekrotiserande enterokolit, vilket bekräftade ultraljudsfynden. Ultraljudet kunde också se tarmludd, vilket är små strukturer i tarmens vägg som har som uppgift att ta upp näring.

Studiens slutsats är att tarmrörelser är omogna hos extremprematurer men att de utvecklas efterhand som barnet blir äldre. Högfrekvent ultraljud är en ny och lovande metod för att undersöka extremprematurers tarmar eftersom den ger mycket bättre och tydligare bilder än vanligt ultraljud. Man såg tarmrörelser men det var svårt att mäta antalet. Metoden upptäckte inflammation i ett av barnens tarmar vilket medför förhoppningar om att snabbare kunna hitta sjukdom i framtiden. Det behövs fler och större studier samtidigt som man jämför mot friska barn födda i vecka 40 för att kunna dra ytterligare slutsatser.

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11. Appendices

11.1 Figure A

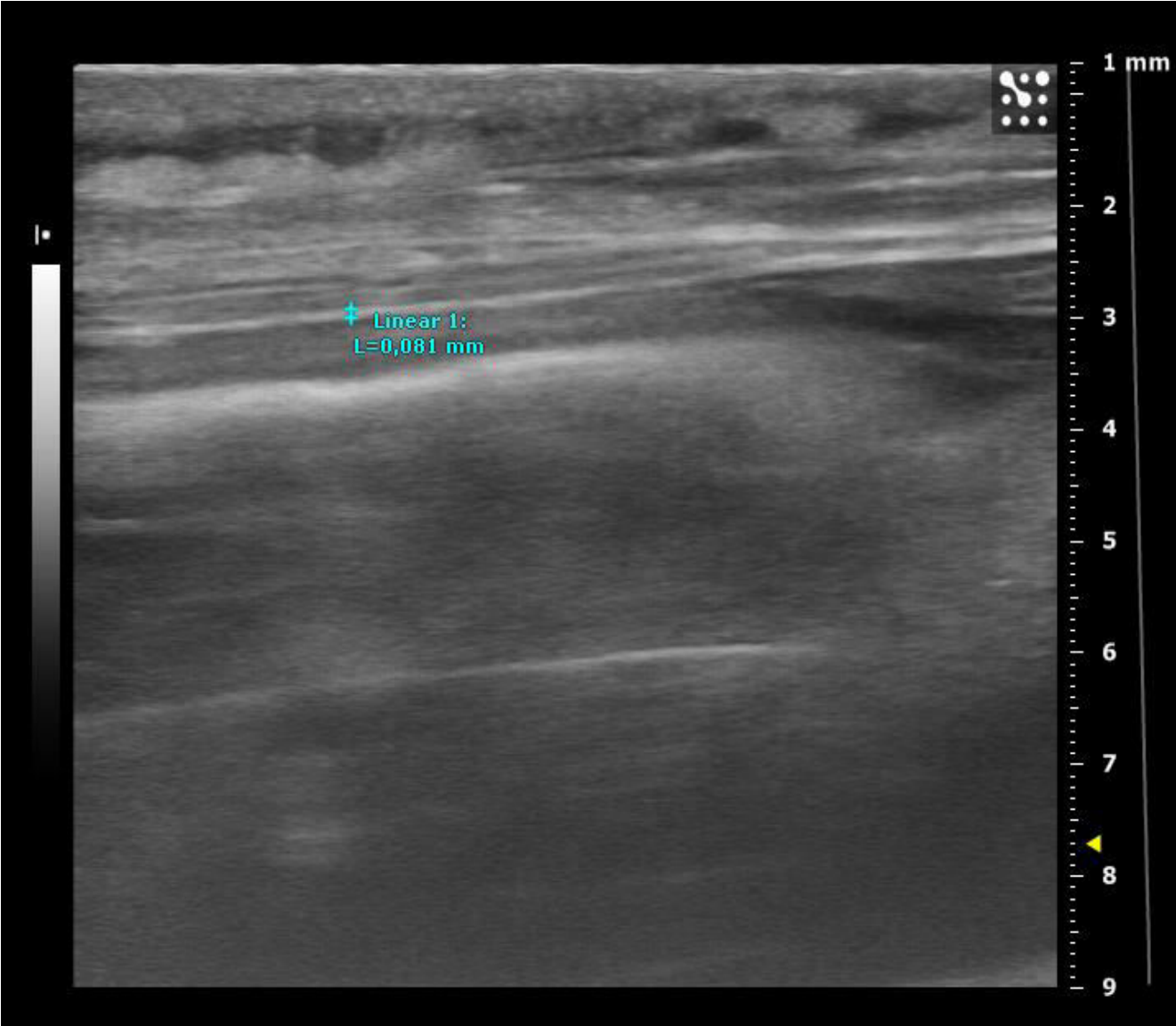


Fig. A – Peritoneum imaged with UHFUS at 48 MHz in patient with gestational age of 35 weeks with no clinical signs of intestinal injury.

11.2 Figure B

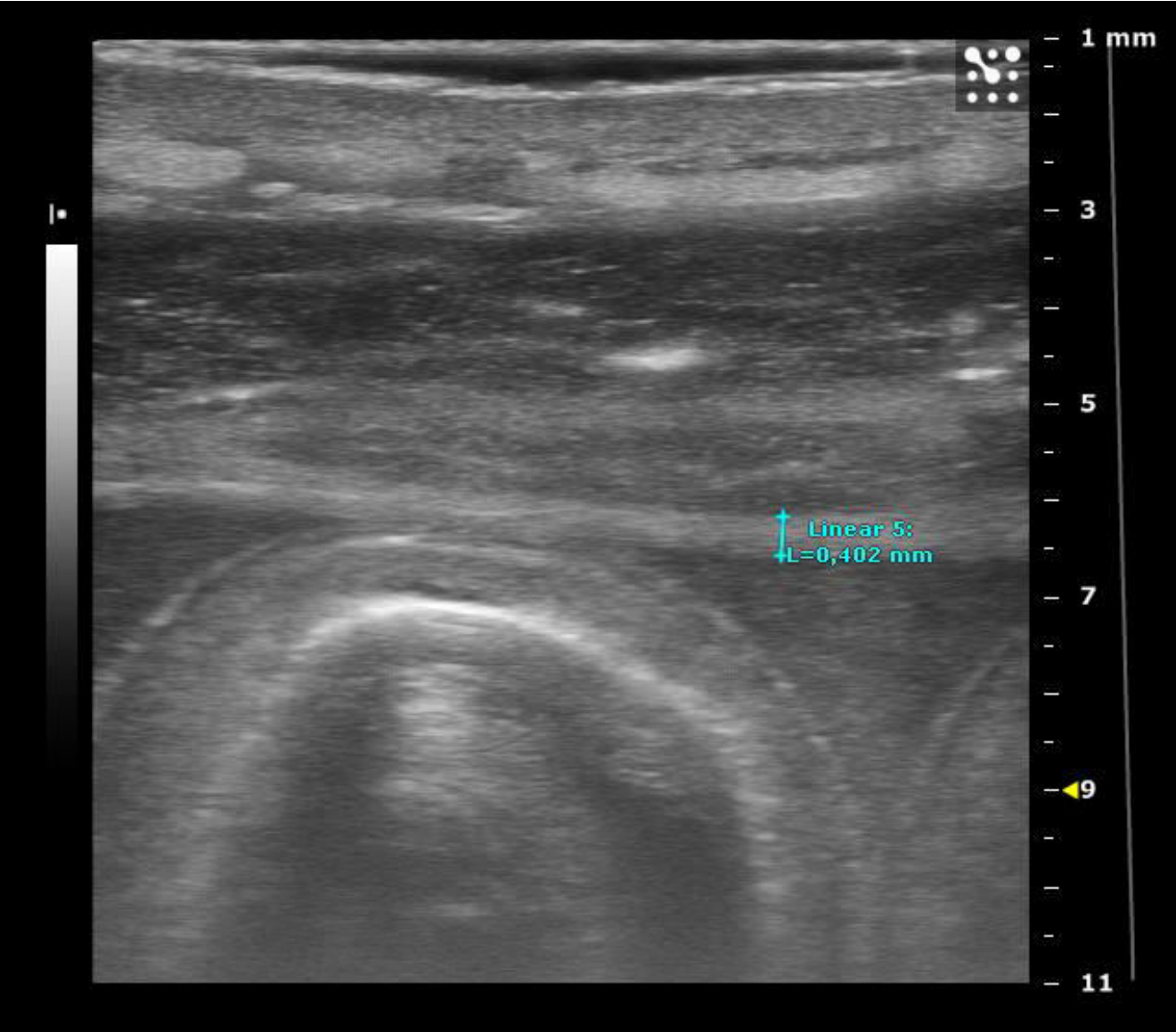


Fig. B – Peritoneum imaged with UHFUS at 48 MHz in a patient with gestational age of 28+4 with clinical signs of necrotizing enterocolitis. The image shows a thickening of the peritoneum five times greater compared to Fig. A

11.3 Figure C

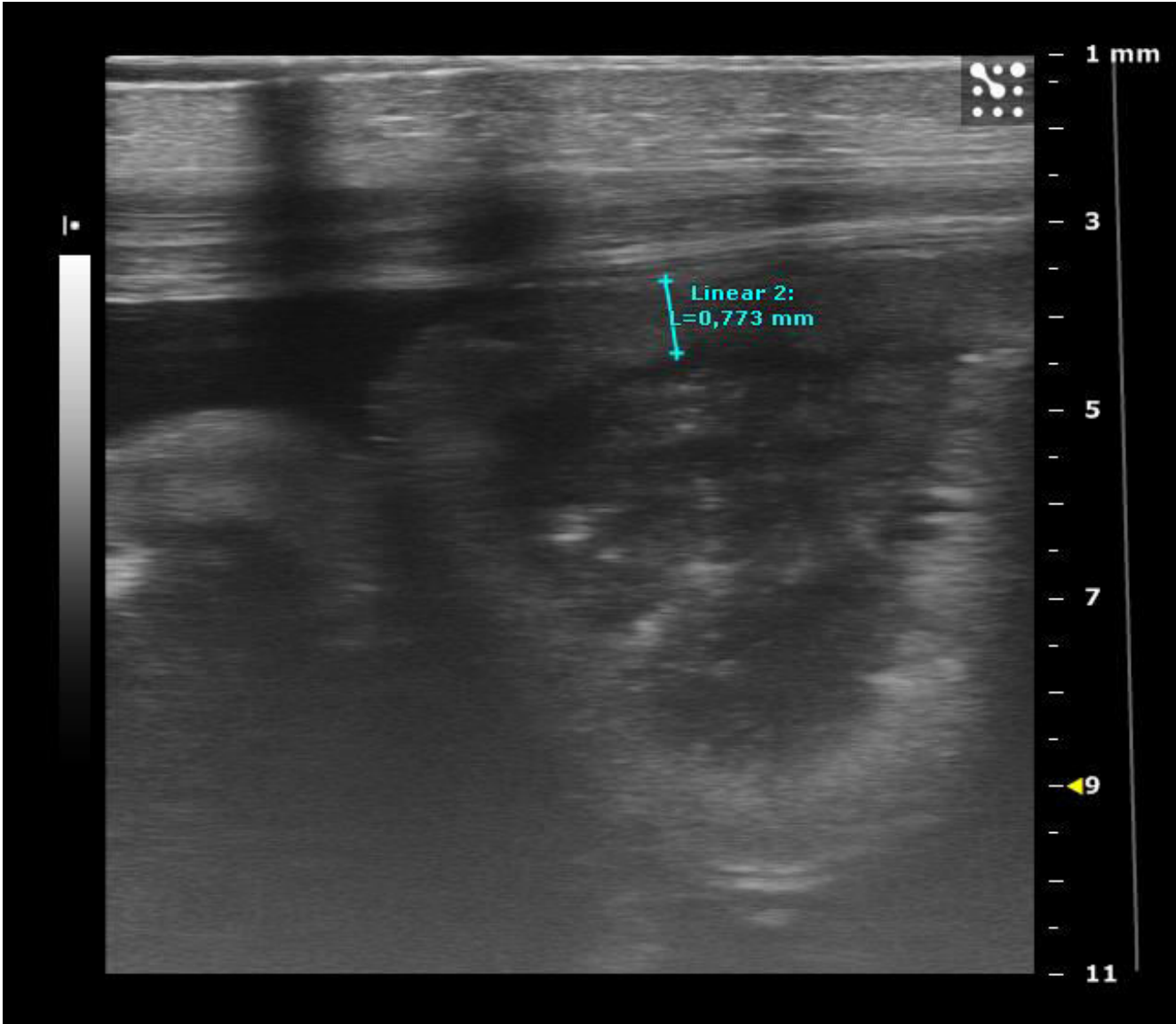


Fig. C – Intestinal wall, lumen and villi imaged with UHFUS at 48 MHz in a patient with gestational age of 35 weeks with no clinical signs of intestinal injury.

11.4 Figure D

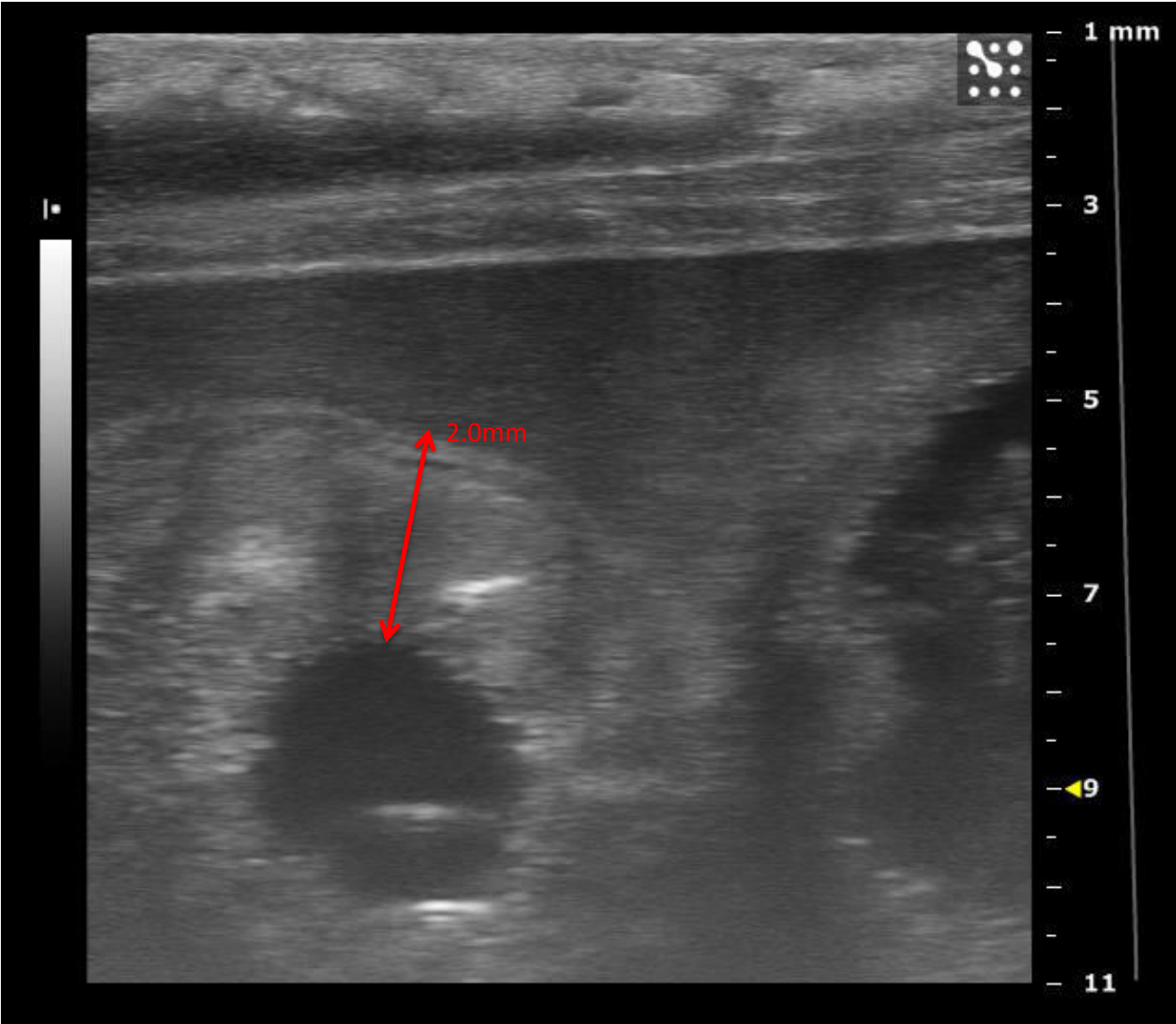


Fig. D – Intestinal wall, lumen and villi imaged with UHFUS at 48 MHz in a patient with gestational age of 28+4 with clinical signs of necrotizing enterocolitis. The image shows signs of intramural gas, bowel wall thickening and ascites. The intestinal wall measures 2 mm and is more than twice as thick compared to Fig. C.

11.5 Figure E



Fig. E – Intestinal villi imaged with UHFUS at 48 MHz in a patient with gestational age of 35 weeks.

11.6 Table 1

Author	Publication year	Examining method	Number of patients	Gestational age (weeks)	Normogastric range (cpm)	Normogastric (%)	Main findings
Cucchiara et al. (22)	1999	EKG	27	29–34	2.0–4.0	60	A maturation pattern of increased gastric slow wave activity with gestational age. 3 cpm rhythm becomes dominant around week 35.
Lange et al. (26)	2005	EKG	62	29–42	2.0–4.0	50	No difference in gastric slow wave activity between preterm and full-term infants. Finds EKG unsuitable for clinical use.
Liang et al. (21)	1998	EKG	19	29–34	2.0–4.0	37	A maturation pattern of increased gastric slow wave activity with gestational age was seen during the first 6 months.
Patterson et al. (23)	2000	EKG	9	34	2.6–3.7	38	A maturation pattern of increased gastric slow wave activity with gestational age was seen between birth, 6 months and 24 months.
Precioso et al. (25)	2003	EKG	45	28–41	2.4–3.7	44	Immature gastric slow wave activity at birth. EKG patterns between preterm and full-term infants were similar.
Richburg et al. (28)	2013	Ultrasound	20	25–36	-	-	Intestinal motility increases over the first 5 days of life.
Riezzo et al. (24)	2000	EKG/Ultrasound	33	28–40	2.0–4.0	59	A maturation pattern of increased gastric slow wave activity with gestational age. Immature EKG pattern and delayed gastric emptying in very preterm infants compared to full-term.
Riezzo et al. (7)	2009	EKG/Ultrasound	38	28–36	2.0–4.0	65	Gastric slow wave activity was quite stable in late preterm infants. Intestinal permeability matures the first 7 days of life.
Zhang et al. (18)	2006	EKG	57	28–41	2.0–4.0	48	A maturation pattern with increased gastric slow wave activity with gestational age. No significant difference in the percentage of normal 2–4 cpm slow waves between preterm and full-term infants.

Table 1 - A summary of the extended background regarding year of publication, examining method, number of patients, gestational age, normogastric range, percentage of normal gastric slow waves and main findings. The percentage of normogastric differs widely and only one study included extremely preterm infants.

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