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Diagnostic tools and therapeutic interventions in the management of early onset neonatal sepsis

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PhD Thesis

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Introduction

Early-onset neonatal sepsis (EOS) is a rare, but life-threatening condition. Clinical signs are non-specific and infection can progress rapidly, leading to organ dysfunction, organ damage and potentially death.

In many cases supportive treatment of septic neonates involves non-invasive respiratory support or invasive ventilation techniques. Up until the mid 1980s, neonates underwent invasive procedures like lumbar puncture or endotracheal intubation without any medication for pain. This was based on the false assumption that infants and young children do not experience painful stimuli**.** Today, infants and neonates routinely receive premedication and analgesia during surgery. However, protocols for premedication of painful procedures, including neonatal intubation, still vary substantially.

Endotracheal intubation is a life-saving procedure, but it carries the risk of potentially significant adverse events. The goal of premedication is to eliminate pain and discomfort and to decrease the risk of bradycardia, hypotension or hypertension, increased intracranial pressure and decreased saturation, all of which can develop during neonatal intubation.

Data regarding pain management practice for neonatal intubation have been limited to single country analyses. Studies reported that in some countries only a minority of infants received premedication before endotracheal intubation, a French prospective study showed that the utilization of premedication prior to intubation, was either inconsistent or it did not follow current recommendations. Similar results were reported in a Saudi Arabian study. In contrast, a British survey performed in 2009 and repeated in 2015 reflected increased awareness among neonatal health care providers. The majority (90%) of the units in the United Kingdom routinely administered premedication and this meant that 93% of infants received premedication before elective intubation. A study from Australia and New Zealand similarly reported that 93% of patients received premedication for intubation in neonatal intensive care units (NICUs). Survey-based questionnaires completed by neonatal practitioners in the United States showed that most respondents believed that premedication should be used, but only approximately 35% reported using it regularly. Furthermore, an international registry study published in 2019 by Foglia et al. confirmed that a relatively high proportion (36%) of infants did not receive premedication during non-emergency intubations performed in the United States.

Using premedication is recommended by the American Academy of Paediatrics for all non-emergency neonatal endotracheal intubations. However, there is still little consensus as to which type of drug or drugs should be recommended, despite the fact that there is ample data available.

Bacterial meningitis is more common in the first month of life than at any other point in time. As part of sepsis, meningitis occurs in as many as 15 percent of neonates with bacteraemia. In prospective surveillance studies of neonates with sepsis or meningitis within 72 hours of birth, approximately 65 to 75 percent had infection caused by GBS and E. coli.

Lumbar puncture (LP) in neonates is most frequently carried out for the exclusion of meningitis, as it carries a risk of high mortality and long term disability. To avoid accidental nerve injury, LP is performed distal to the spinal cord. This space is somewhat lower in neonates compared with adults; thus, performing LP above the L2–L3 interspace is contraindicated.

Thus, the advised puncture site in neonates is the third (L3–4) and fourth (L4–L5) lumbar intervertebral space.

According to the literature, up to half of neonatal LPs result in an unsuccessful tap. The consequences of unsuccessful LP include increased patient discomfort and hospital time, delayed diagnostic and therapeutic interventions, and prolonged use of antibiotics. The most common causes of unsuccessful LP include excessive advancement of the spinal needle into an epidural venous plexus, no use of local anesthetic, excessive movement of the patient, and dehydration.

Thus far, few studies have assessed the spinal anatomy using ultrasound (US) to determine the optimal site for LP in neonates and clarify the reasons for LP failure. Moreover, even fewer investigations focused on the amount of cerebral spinal fluid (CSF) present in the spinal canal and its relation to ventricular size or demographic and clinical data.

Clinical signs of EOS are non-specific and infection can progress rapidly, leading to organ dysfunction, organ damage and can potentially be fatal. Appropriate and prompt initiation of antibiotics can be lifesaving. In consequence, antibiotics are started empirically when infection is suspected. Current guidelines and local protocols lead to substantial overtreatment of neonates with suspected sepsis, roughly 20-400 newborns are treated worldwide with intravenous antibiotics for one case of proven infection. Unnecessary antibiotic treatment in the early neonatal period might pose multiple short and long term risks. Use of antibiotics is associated with longer duration of hospital stay, mother-infant separation, and negatively impact breastfeeding rates, and increase healthcare costs. Antibiotic exposure early in life disrupts the developing microbiome, possibly contributing to numerous diseases later in life, including diabetes, obesity, inflammatory bowel disease, asthma, allergy, rheumatoid arthritis.

Multiple international studies have shown that the currently used guidelines lead to overuse of antibiotics in EOS. In 2017 Hungarian Neonatal Society issued a national guideline with the aim to rationalize and standardize the antibiotic use in early onset neonatal sepsis.

Aims

Premedication practice for neonatal intubation in Hungary was presumed to have high variability. To obtain objective data, clinical practice (including the utilised drugs) for non-emergency neonatal intubation was thoroughly assessed through an international questionnaire. The aim was to identify variations in premedication protocols for intubation in tertiary neonatal units in different countries.

Up to until now very few studies have assessed the spinal anatomy using ultrasound to determine the optimal site for LP in neonates and clarify the reasons for LP failure. The aim of our study was to investigate the amount of CSF present at the spinal sites at which LP would be routinely performed in neonates using cross-sectional lumbar US images. We planned to produce an index value, namely the ratio of CSF-to-spinal canal (CSF%), that would represent the relative amount of CSF in the spinal canal. Therefore CSF% would be independent of neonate size compared with absolute volume of CSF.

According to the available literature, dehydration is one of the risk factors associated with unsuccessful LP. We hypothesized that a low relative amount of CSF at the spinal levels may be a cause of the challenging dry or traumatic taps. Therefore, we sought to determine the

variation of CSF% present in neonates at the recommended LP sites, namely the L3–4 and L4–5 intervertebral spaces. Additionally, we aimed to identify a possible relationship between the amount of CSF present in the lateral ventricles represented by the ventricular index (VI) and quantity of CSF at the particular lumbar spinal levels. This study also investigated whether different clinical parameters exhibited a correlation with the quantity of CSF present at LP sites.

In accordance with the endeavours of reducing antibiotic exposure we have assessed our clinical practice during the years of 2014-2018, collecting data on term and late preterm (>34 gestational weeks) neonates admitted to Neonatal Intensive Care Unit, Department of Paediatrics and the Postnatal Ward, Department of Obstetrics and Gynaecology of University of Szeged. The time period involved 2017, when the Hungarian Neonatal Society issued a national guideline to rationalize the use of antibiotics in neonatal sepsis. Our aim was to retrospectively determine the frequency of prescribed antibiotics before and after the introduction of national guideline and also to assess signs of sepsis, number of neonates treated with antibiotics, incidence of early-onset neonatal sepsis, sepsis-related mortality in our cohort and compare our results with international data.

Methods

The cross-sectional study about neonatal intubation was performed between December 2018 and February 2019. Our aim was to focus practitioners working in NICUs with experience of neonatal intubation. We had initially intended to concentrate on Europe, but the international responses received meant that we expanded the focus of the study. During January 2019 we posted details of the survey on 99nicu.org, a website that is dedicated to neonatal staff around the world. Email invitations were also sent to all the European neonatology professionals whose e-mail addresses were accessible as correspondent authors for neonatology based research articles in the last five years. Only professionals who had performed at least five non-emergency endotracheal intubations in the last five years were able to complete the questionnaire. The survey was anonymous: no personal data were collected and no connections between particular invitations and responses were made.

To ensure that the content and language of the survey were valid, the questionnaire was developed by two investigators with expertise in neonatal intubation and verified by the statistical team. We pilot tested the online survey for clarity, readability and functionality and feedback was incorporated into the final version of the questionnaire.

The term non-emergency endotracheal intubation covered all elective and semi-elective endotracheal intubation procedures performed outside the delivery room.

The survey contained 205 questions on five screens and these covered: the respondent's status, premedication protocols, premedication practice, complications that had been experienced and the availability of documentation. The questions included the existence of any protocols, the number of drugs used and whether premedication was similar for term and preterm babies. The key items, such as the existence of a protocol, were mandatory and they could not progress to the next screen without answering them. The questions were presented in a fixed order and the respondents were asked to be careful that they did not submit the same survey response more than once. We did not limit the responses to just one per unit, because premedication practices may have varied from person to person if there was no unit protocol.

A convenience sample of answers was collected, and these represented a large group of neonatal providers with different levels of experience in intubation and varied approaches to the use of premedication.

Our prospective observational study on ultrasound imaging of the subarachnoid space to assess the CSF amount in neonates was performed between May 2019 and September 2020, involving term and preterm neonates aged ≤7 days who were admitted to the neonatal intensive care unit of the Department of Pediatrics of our hospital.

This study was approved by the institutional ethics board. Written informed consent was provided by the legal guardian of each patient prior to the examination using US.

Imaging was performed with a point-of-care GE Vivid US machine, for spinal examination a linear (12 MHz), for cranial imaging a microconvex (8 MHz) transducer was used. Coronal and sagittal cranial images were captured through the anterior fontanelle. Cross-sectional images of the third (L3–L4) and fourth (L4–L5) intervertebral spaces were taken with a linear transducer in the left lateral recumbent position of the newborn with knees flexed and without flexing the neck. The appropriate intervertebral space for examination was determined, similarly to a routinely performed LP, by using a line connecting the iliac crests.

All US examinations were performed by the same investigator, pre-trained and qualified for this study by Paediatric Radiologists. Intraobserver reliability of the US measurement was calculated with concordance correlation coefficient (CCC) and was found to be very good (>90%). US images were saved and analyzed in Digital Imaging and Communications in Medicine (DICOM) format by a second investigator who was blinded to the patient data. The following clinical parameters were recorded for each patient from the electronic patient recording system (IntelliSpace Critical Care and Anesthesia; Philips): gender, gestational age (weeks), postnatal age (days), birthweight (g), height (cm), and enteral fluid intake (%) on the day of US examination representing the ratio of enteral-to-total intake.

Each US image was processed using the same gain setting. The spinal canal was manually delineated at the L3–L4 and L4–L5 spinal levels (Fig. 1a) using the ImageJ software. The area of the delineated section was determined by calculating the number of pixels present for all 255 gray-scale values within the delineated area (Fig. 1b). A ratio of the anechoic area to the whole spinal canal was necessary to determine the amount of CSF in the spinal canal. The GNU Image Manipulation Program software was used to decide which gray-scale values could be considered “anechoic.” Anechoic areas corresponded to gray-scale values ranging 0–25. Therefore, to obtain the CSF% in the spinal canal, we divided the ratio of the 0–25 gray-scale pixels by the total pixel count of the spinal canal (gray-scale values: 0–255). As a result of our calculation, CSF% indicates the amount of CSF present in the total area of the spinal canal (Fig. 1c).

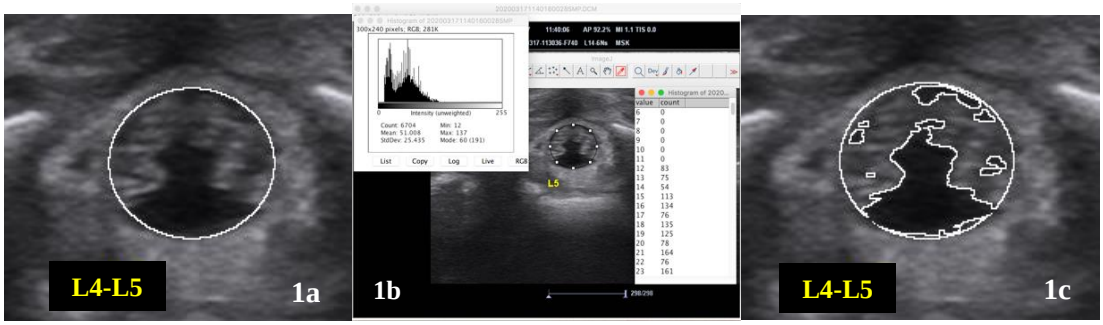


Figure 1. Manual delineation of the spinal canal (1a), The delineated area's gray-scale values with GIMP software (1b), Calculation of CSF% (1c)

The third research item retrospectively studied the use of antibiotics in term and late preterm neonates (>34 weeks of gestation) admitted to the Neonatal Intensive Care Unit, Department of Paediatrics and to the Postnatal Ward, Department of Obstetrics and Gynaecology of University of Szeged between 1st January 2014 and 31st December 2018. Investigation focused on the following data before and after introduction of the local protocol based on the national guideline published in 2017: gestational weeks, birthweight, gender, number of babies treated with antibiotics, timing and duration of antibiotic treatment, indication for antibiotics e.g. risk factors and clinical signs, positive blood culture, causative organism.

Criteria for EOS was a positive blood culture and/or CSF culture before the 7th day of life and the need for antibiotic treatment for more than 5 days.

Based on the number of positive blood cultures and the relevant clinical data the incidence of culture proven EOS was calculated. We analysed the contaminated and causative pathogens and their antibiotic susceptibility.

In addition to the antibiotic days, total antibiotic exposure and overtreatment index and mortality was also investigated on a yearly basis. Overtreatment index (OI) was also calculated showing the number of patients treated for one episode of culture proven sepsis and giving valuable information on unnecessarily administered antibiotics. Sepsis-related mortality was determined for the examined time period.

In all research R-studio software was used for statistical analysis. Pearson's rank correlation test was applied to detect correlations between variables. Student's t-test was used to determine the statistical significance ($p < 0.05$).

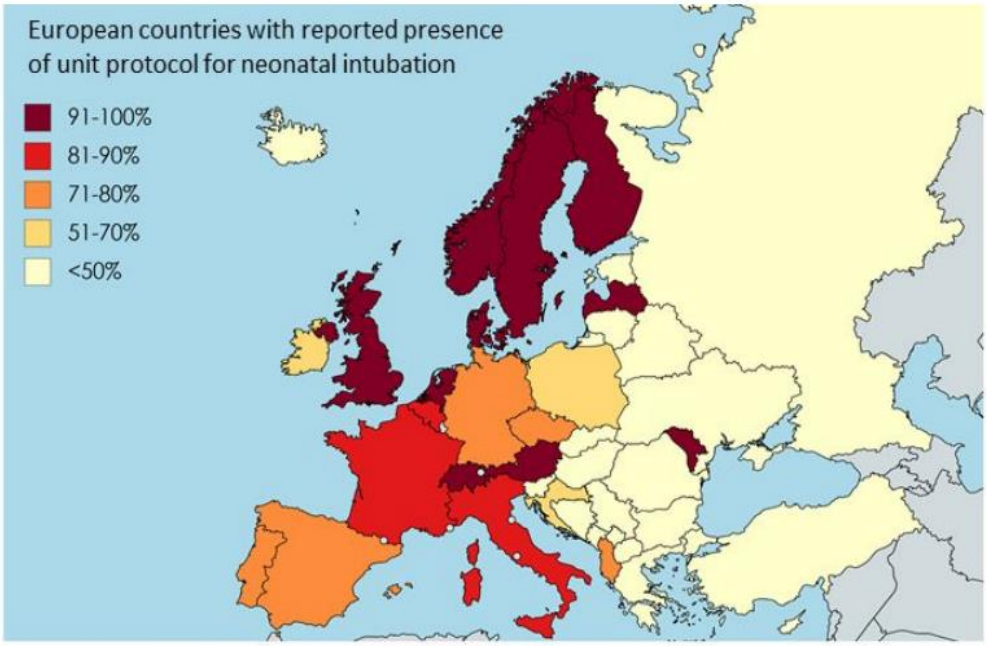
Results

The website of the intubation questionnaire was visited 1257 times and 718 questionnaires were submitted by 633 respondents from 40 European countries and 85 from 30 non-European countries. The responses came from 454 medical centres and the number of respondents from the same medical units varied from one to 11, with a mean of 1.58 and a median of one. We found that 457/718 (63.6%) questionnaires were fully completed. The distribution of the responses from European and non-European countries was similar. The majority of the responses (48.1%) were received from neonatologists working in level three units in Europe and more than half of the neonatal intubations were performed by neonatologists.

The ratio of European practitioners following a written protocol is illustrated in Figure 1. Worldwide, 31.6% of the practitioners from 145 of the 454 medical centres claimed that they worked in a unit without a written protocol for neonatal intubation. Of those, 37.0% of the respondents reported that they did not use any premedication for non-emergency intubation. This equated to 11.9% of all the 718 respondents. Of the practitioners that used premedication, 60.4% chose the drugs according to personal preference. Single drug use was higher in the no protocol group (42/227, 18.5%) than protocol group (3/388, 0.8%).

Most practitioners (78.5%) reported using the same drugs for term and preterm infants. The most frequently prescribed combination for premedication was fentanyl, atropine and succinylcholine, at 6.8%.

The highest level of drug use uniformity was observed in Sweden and the UK. In the Swedish centres, 23/27 (85.1%) of the neonatal providers reported using the same combination of (remi)fentanyl, thiopental, atropine and succinylcholine. In the UK, 69/79 (87.3%) reported using the same combination of fentanyl, atropine and succinylcholine.



Country	Respondents	Units	%	Country	Respondents	Units	%	Country	Respondents	Units	%
Albania	6	4	75	Germany	29	25	80	Macedonia	3	2	50
Austria	17	12	100	Greece	18	5	40	Moldova	2	1	100
Belarus	5	1	0	Hungary	33	18	39	Romania	15	9	11
Belgium	11	8	88	Iceland	1	1	0	Russia	2	2	50
Bosnia and Herzegovina	6	4	50	Ireland	9	6	67	Serbia	17	7	43
Bulgaria	5	5	0	Italy	22	21	81	Slovakia	7	4	50
Croatia	8	5	60	Kosovo	3	1	0	Slovenia	1	1	0
Cyprus	1	1	0	Latvia	5	1	100	Spain	19	13	77
Czech Republic	5	5	80	Lithuania	14	5	20	Sweden	35	13	100
Denmark	8	4	100	Montenegro	1	1	0	Switzerland	6	5	100
Estonia	5	4	25	Netherlands	27	12	92	Turkey	28	20	45
Finland	7	6	100	Norway	12	6	100	UK	100	58	97
France	21	16	88	Poland	100	50	56	Ukraine	11	9	22
				Portugal	8	4	75				

Figure 1. Proportion of respondents with unit protocol in Europe

Routine sedatives were reported by 43.3% of the respondents, with 21.6% saying they only used them in special cases. A further 15.3% said they did not use any sedatives. The most frequently reported reason for not prescribing sedatives was avoiding adverse events (42.6%).

The frequency of muscle relaxant and vagolytic agent use was higher when respondents who followed protocols in their unit were compared with those who did not: 40.5% versus 7.0% and 38.7% versus 4.0%, respectively. A number of concerns were reported about the use of muscle relaxants and these included loss of spontaneous respiratory drive or masking pain or seizures (15.0%). The main reasons for not using them was that it was deemed unnecessary (17.7%).

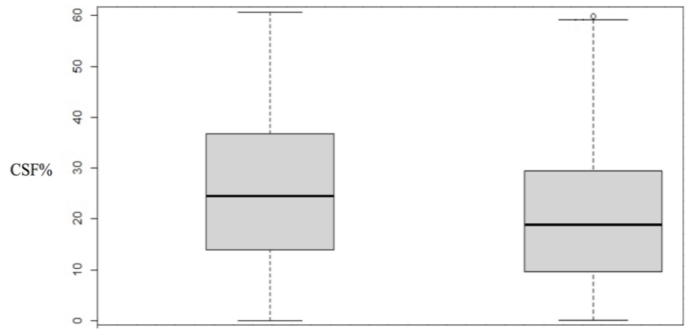
Respondents were asked about their satisfaction with the premedication they were using at the time of the survey. Nearly a quarter (24.8%) were completely content and didn't feel they needed to change. However, there was a higher level of satisfaction in the protocol group than those who did not follow a protocol (32.5% versus 11.9%).

Only 19.3% of the respondents kept records of personal intubations and only 11.4% said that their unit kept a register for intubations and any adverse events.

The prospective ultrasound study investigating the subarachnoidal spaces was conducted between May 2019 and September 2020. During this period, 386 neonates were admitted to the neonatal intensive care unit of the Department of Pediatrics of our hospital. Of those, 208 patients were finally included in the study.

All enrolled neonates were aged ≤7 days (gestational age, median: 35 weeks [mean ± SD: 34.61 ± 3.79 weeks]; birthweight, median: 2,200 g [mean ± SD: 2.413 ± 921 g]). US measurement was performed on the third day of life on average [mean ± SD: 3.21 ± 2.07].

The cross-sectional US images revealed significant variation in the quantity of CSF among newborns. Some neonates had visibly more CSF at the same spinal level than others. Statistical analysis showed a wide variation of CSF% at both the L3–L4 (0%–61%) and L4–L5 (0.1%–70%) levels. The CSF% in L3–L4 was significantly higher than that in L4–L5 (25.75 ± 14.82 vs. 21.78 ± 15.13, respectively; p = 0.007) (Fig 2). There was a positive correlation between the CSF% at L3–L4 and L4–L5 (r = 0.67).



	n	Median	Mean ± SD	IQR	Range
L3–L4 (CSF%)	205	24.53	25.75 ± 14.82	22.7	0.00–60.66
L4–L5 (CSF%)	206	19.64	21.78 ± 15.13*	20.61	0.05–70.36

Figure 2. CSF% in lumbar third and fourth intervertebral spaces **p = 0.007 (p < 0.05)*

There was no significant correlation between the clinical parameters (gestational age, postnatal age, birthweight, enteral fluid intake and height) and CSF% measured at L4 and L5.

The analysis of the coronal cranial images revealed that the VI ranged 0.18–0.44. However, it is important to emphasize that the VI had a very narrow distribution range in the middle 50% of the population, with an interquartile range of 0.06 (Fig. 7). The VI was independent of gestational age, weight, length, postnatal age, and ratio of enteral feeding (correlation coefficient: 0.02–0.34).

There was no correlation found between the VI and the measured CSF% at the L4 and L5 spinal levels.

In regards of the antibiotic exposure of term and late preterm neonates the following result were found. In the year of 2014 21,10% (n=517), in 2015 16,63%-a (n=401), in 2016 17,84% (n=456) of the neonates received antibiotic treatment. In 2017 only 2,39% (n=61), in 2018 2,69%-a (n=64) of the newborn babies were administered antibiotics for presumed early onset sepsis. The number of babies given iv antibiotics has been significantly reduced since 2017. Between 2014 and 2016 clinical signs of possible EOS were recorded in 10,06% (n=52), 10,47% (n=42), 9,21% (n=42) of neonates treated with antibiotics, respectively. The ratio of babies with clinical signs has not changed significantly (p=0,285) in the following years [2017 75,41% (n=46), in 2018 89,06% (n=57)]. (Fig 3)

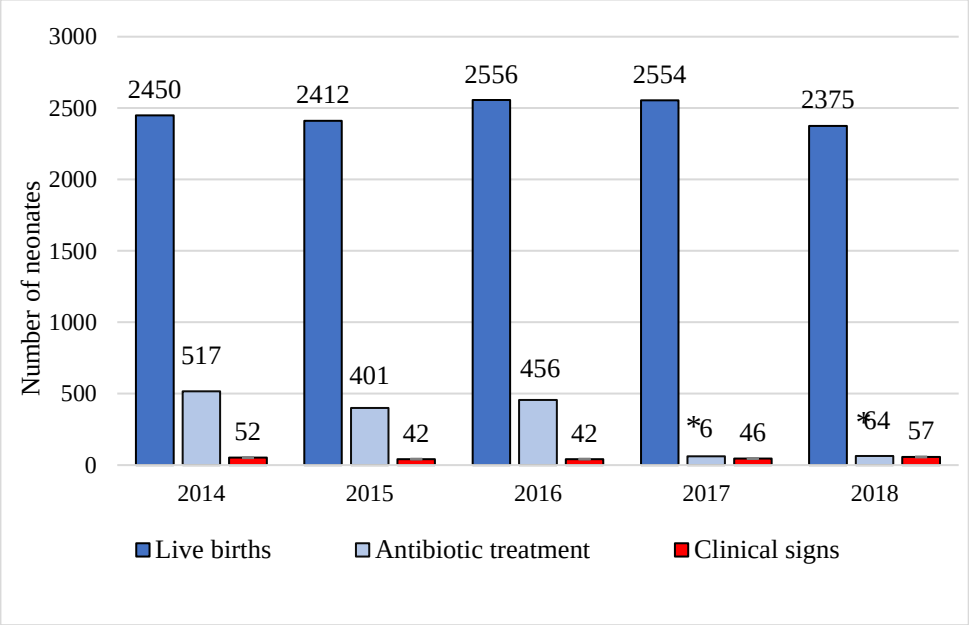


Figure 3. Total live birth, number of neonates given antibiotics and number of neonates with clinical signs on a yearly basis

Most frequent clinical indication (72,5%) for initiation of antibiotics was respiratory distress, namely tachypnoea, use of accessory muscles and grunting. In smaller proportion feeding difficulty, apnoea, low saturation level, temperature regulation instability, delivery room distress were the reasons for initiating treatment. Only rarely did we note seizure, poor perfusion, tachycardia, hypoglycaemia, irritability as indication for EOS treatment. Clinical

signs were present in all blood culture positive sepsis cases in our study: in three cases respiratory distress and one neonate were noted to have feeding difficulty.

In 83,43% (n=287) of the cases the blood culture was taken on the first day of life. There were a total of six positive blood cultures during the examined 5 year period out of which we have considered 2 cases as contaminant. Therefore we had four cases of blood culture positive early onset sepsis cases. The isolated pathogens were identified as E. Coli, Streptococcus agalactiae, Staphylococcus haemolyticus and Staphylococcus epidermidis in one cases each, time to positivity ranged between 8 and 12 hours. There was no cases of concomitant meningitis. Of the contaminated blood cultures Micrococcus luteus and Staphylococcus warnerii have grown.

During the analysed period there were a total of eight deaths in the first week of life, none of them were related to EOS.

Based on the number of blood culture positive EOS cases during the investigated years in our hospital, the EOS incidence were determined 0,324 per 1000 live births.

In 2014 2450 neonates were born, the calculated antibiotic days for these babies were 1350, in 2015 for 2412 newborns 963, and in 2016 for 2556 infants 1077 antibiotic days were recorded. These numbers from 2017 are 249 and 269 for 2554 and 2375 newborns respectively. There was a significant decline (p=0,00001) in the total number of antibiotic days since 2017.

Overtreatment index (OI) shows the number of patients treated with antibiotics for one episode of culture proven sepsis. This shows the number of neonates that were given antibiotics for suspected EOS to treat one culture proven neonatal sepsis.

Between 2014 and 2018 total of 1502 neonates had antibiotics prescribed and we had four cases with culture proven sepsis. Therefore the overtreatment index for the 5 years is 375,5. In case we divide the period for pre-guideline and post-guideline period after 2017, we can see that the OI for the first three years is 1371 and it has radically decreased to 41,6 for the remaining two years after the guideline adaptation.

Discussion

The main goal of the described investigations was to determine the effectiveness of three key elements of the management of early onset sepsis in neonates:

The international survey of professionals in 70 countries showed great diversity of premedication protocols (or the lack of them), with high variability of drugs used during endotracheal intubation. The results can help shape future unified guidelines in premedication. Our US study of LP sites showed great variability of CSF ratio at both L3-L4 and L4-L5 levels, with the L3-L4 level being preferable to perform the procedure at.

The perinatal antibiotic study showed a great decrease in unnecessary utilisation of antibiotics if a nationwide guideline is implemented.

This was the first survey to report the use of premedication for neonatal intubation by combining data from different countries. We conducted an Internet-based survey across 40 European and 30 non-European countries. Data was collected on practitioners (grade, experience), premedication for intubation protocols, attitudes and practitioners' experiences regarding safety, side effects and efficiency. Just over half the respondents from 70 countries

reported following a protocol for premedication for neonatal intubation. The largest number of responses came from neonatologists followed by paediatric or neonatal trainees. More than third of the respondents who did not follow a protocol did not provide any type of premedication for neonatal intubation and this equated to just under 12% of the total responses. The most frequently reported combination of drugs was fentanyl, atropine and succinylcholine. The main reason for not providing pain and anxiety relief for endotracheal intubation were concerns about potential drug side effects.

When we developed the survey, we followed the Checklist for Reporting Results of Internet E-Surveys statement. However, we do acknowledge that using convenience samples in Internet-based open surveys can lead to considerable bias due to the self-selection of participants, also known as the volunteer effect. Furthermore, we invited individual participants to respond to the survey, which might have led to an unequal representation of units. We must also accept that, given the retrospective character of our study, all the data on the frequency of observed side effects were limited by human memory.

Considering the above, we need to be careful when interpreting our results. Survey data and actual practice are usually not the same. However, the low rate of premedication use reported in our survey was consistent with a prospective register study published by Foglia et al.

Repeated painful stimuli may lead to poor neurodevelopment outcomes. Despite this common knowledge, more than one-third of the respondents in the no protocol group still failed to provide adequate comfort for infants undergoing endotracheal intubation. Some of the reasons reported by our participants were concerns about adverse reactions, toxic drug effects, inadequate time to administer medication and the perception that the risks outweighed the benefits of premedication for intubation. However, a trial that evaluated the use of fentanyl, which was the most frequently used analgesic in our study, plus atropine and a paralytic agent showed no significant adverse effects in neonates. One of the primary concerns related to using fentanyl, which is chest wall rigidity, can be reduced by slow administration and reversed by naloxone.

Nearly 100% of the units in Australia, US and UK provide written protocols for neonatal intubation. Our study showed that a significant number of respondents practicing in Eastern Europe did not follow written guidelines for intubation premedication. This was most probably a result of false assumptions and low awareness of the possible consequences of repeated painful stimuli, leading to a lack of local unit policies or practitioners being unaware of a written protocol.

Our study included questions about the use of sedatives, analgesics and muscle relaxants. Fentanyl was the most popular analgesic agent, as it was in most other reported studies. However, another study published in 2012 stated that morphine remained the most popular analgesic in Australia and New Zealand. Fentanyl has a number of desirable characteristics when it is used as a premedication analgesic, including a more rapid onset and shorter duration of action than morphine. That is probably the reason for its popularity among physicians.

In the UK and Australia, the most predominant muscle relaxant has been reported to be suxamethonium. In contrast, a French survey revealed that most practitioners preferred the

use of benzodiazepine, with or without opioid sedation or muscle relaxants. A cohort study of NICUs that participated in the National Emergency Airway Registry for Neonates, revealed that the use of neuromuscular blocking agents was associated with favourable intubation outcomes. However, potential disadvantages were cited as reasons for not including muscle relaxants in premedication protocols, such as cessation of breathing and de-recruitment of alveolar space during the procedure.

Propofol was used by 16.2% and 10.9% of the respondents in our study for term and preterm infants respectively. Practitioners also reported using propofol during INSURE (intubation-surfactant administration-extubation) or less invasive surfactant administration procedures. One of the reasons given for using propofol as a single agent was that it rarely causes apnoea at low doses, which enables physicians to avoid prolonged periods of ventilation. A randomised controlled trial showed that propofol at the currently recommended dose was effective in only 50% of neonates and that many infants did not actually recover quickly and needed prolonged periods of ventilation. On the contrary, a study published in 2018 showed quick recovery in the propofol group, but the risk of hypotension was higher among these infants, even though they did spontaneously recover.

Our survey showed that, despite national and unit-based recommendations for non-emergency intubation, a significant percentage of practitioners still avoided premedication. Educational initiatives and on-going educational programmes may improve compliance with guidelines, as shown in published studies.

According to our findings of the ultrasound measurements, there was a wide variation of the observed CSF% in both investigated intervertebral spaces in neonates aged ≤7 days. On average, we found significantly higher CSF% at L3–L4 versus L4–L5, suggesting the presence of a relatively greater amount of CSF at the higher LP site compared with the lower position. There was no correlation between the observed clinical parameters (gestational age, postnatal age, birthweight, height, and ratio of enteral intake) and the percentage of CSF at any spinal level. Furthermore, there was no correlation detected between the size of the lateral ventricles (represented by the VI) and the calculated CSF% in the lumbosacral regions of L3–L4 or L4–L5.

The collection of CSF from neonates with a potentially life-threatening condition is of great importance for both diagnostic and therapeutic purposes. Although this approach is one of the most commonly performed procedures in the pediatric emergency setting, only a limited number of studies have investigated the amount of CSF present in neonates and clinical factors that may affect the volume of CSF.

The observed high variability of CSF% might explain some of the unsuccessful LP attempts if we consider neonates with none observed or scant amount of CSF on cross-sectional views. According to Coley et al. absence of visible CSF or narrowing of the subarachnoid space by US complicates LP and limits its feasibility. Therefore, measuring the amount of CSF at the LP site is of great importance. Lo et al. and Oulego-Erroz et al. investigated the subarachnoid space by measuring the subarachnoid space width (SSW) at the lumbar spinal levels using longitudinal spinal images in different LP positions of the infants. Oulego-Erroz et al. concluded that gestational age exerted a significant effect on SSW; moreover, the sitting

position and body flexion increased the SSW measured posterior to the filum terminale. Lo et al. found a positive correlation between weight and CSF volume expressed as SSW in mms. However, contrary to the findings reported by Oulego-Erroz et al., they did not observe a significant change in the SSW between three different LP positions.

In contrast to both aforementioned investigations, the present study utilized transverse US views to measure the relative ratio of the CSF to the spinal canal, rather than an absolute value such as the SSW. Expressing the amount of CSF as a ratio may explain the absence of a positive correlation between gestational age, weight, and CSF volume in the spinal subarachnoid spaces. We think that this ratio is a more precise approach to comparing the CSF volume among newborns of different sizes. Moreover, we did not detect a positive correlation between the VI and relative CSF amount found at the level of the LP. One cannot predict the presence of a "puncturable" amount of CSF based on the cranial US measurements of the ventricular size.

The relatively greater amount of CSF at L3–4 versus L4–5 may be explained by the narrowing of the spinal canal when moving caudally from the L1 level. With this narrowing the cauda equina-to-CSF ratio increases, therefore CSF% decreases.

It has been hypothesized that severe dehydration affects the amount of CSF. Rankin et al. investigated whether rehydration of fluid-depleted infants through the administration of intravenous fluid boluses would increase the size of the subarachnoid space; their findings did not confirm this hypothesis. In contrast to the study conducted by Rankin et al., we investigated the possible effect of the hydration state based on the amount of enteral intake on the day of the measurement. Despite the limited data available on this matter, the present findings do not indicate a correlation between the ratio of enteral feeds and the quantity of CSF. Compared to our study, Rankin et al. measured the CSF within the spinal canal in terms of square millimeters rather than a proportion. Using a ratio instead of an absolute number is a more precise approach to comparing measurements in infants of varying sizes.

In this study, we were particularly interested in identifying the factors complicating LP during the neonatal period. Studies have shown that the success rate in the neonatal population can be as low as 50%. We determined an index number (CSF%) that would represent the amount of CSF in the spinal canal and be independent of neonate size. It is reasonable to assume that the quantity of CSF at sites in which LP is routinely performed is an important factor for LP success, similar to other factors discussed in the literature. To our knowledge, this study was the first to use an index number to describe the volume of CSF in neonates and investigate the correlation between the amount of CSF present in the lateral ventricles and quantity of CSF at the advised LP levels.

The present study included a large patient sample (n = 208), which allowed us to observe the variations of CSF% among neonates with a wide range of gestational age and birthweight. A single researcher captured the images, thereby standardizing the quality of the images. Also, all images were performed by using a standardized position of the neonates and a standardized DICOM format.

The main limitation of this study was that LP was not actually performed in our patients. The delineation of the spinal canal using the ImageJ software and deciding which gray-scale values to include into our calculation as part of the anechoic CSF center were subjective and

relied on our observations. All patients were subjected to a normohydration plan, with different ratios of enteral-to-total intake (0%–100%). Therefore, extremes in hydration state could not be analyzed.

According to the latest international population based and multicentre research the incidence of EOS is 0,25-0,95/1000 among term and late preterm infants. There is a high variance in the number of newborns treated with antibiotics for suspected EOS depending on the applied national, local guidelines, protocols and the use of sepsis calculator. Sepsis calculators estimate the risk of EOS, based on maternal risk factors, clinical signs, the locally estimated incidence of EOS, and give recommendation on the management strategy. Based on reported data from Scandinavian, American, French, British and Italian authors, the proportion of neonates treated with antibiotics for EOS varies between 1,2-10% and also shows substantial disparity even within countries.

In relation of international data our observed 16-20% before the adaptation of the national guideline in 2017, antibiotic exposure was considered very high. On the contrary, the 2,3% and 2,7% for the following two years is acceptable even when compared to findings of international research.

In addition to get information about the possible antibiotic overuse, our investigation aimed to search for its reason. In years before the national guideline the unit used antibiotics with the aim of preventing infection when EOS was suspected, even without clinical signs present. In these cases the neonates received a two day course of antibiotic treatment on average. The indication for commencing antibiotics were mostly maternal risk factors e.g. positive vaginal swab, chorioamnionitis, maternal fever, premature rupture of membranes, unfollowed pregnancy, birth out of the hospital.

During the years of 2018-2019 most of the neonates (75-89%) had clinical signs at the time of commencement of the antibiotics. Respiratory distress was the clinical problem that prompted prescription of antibiotics in most of the cases. As the signs of delayed transition from intrauterine to extrauterine life are very similar to that of in respiratory signs in case of EOS, it is extremely difficult to distinguish between the self-limiting, benign adaptation problem and the early phase of the rapidly progressive disease, purely based on clinical signs and observations. Numerous international research emphasises the importance of close observation and repeated physical examination of the neonate, finding this approach a safe and reliable method of identifying infants with EOS and helps to avoid unnecessary antibiotic use. In a French multicentred study found that presence of clinical signs were more predictive of EOS when compared to maternal risk factors. The same study group investigated the role of serum procalcitonin (PCT) taken from the umbilical cord and concluded that values of PCT>0,6 had a strong positive predictive value of EOS.

The national guideline published in 2017 aimed to standardize the treatment of EOS, in hope that with the adaptation of specific criteria, the antibiotic exposure would decrease considerably. The above mentioned guideline was only published in the second half of 2017, but our unit had adapted a new local protocol in accordance of the content of the provisional version of the guideline in January 2017. We believe that the significant decrease in our hospital’s antibiotic use is the consequence of the national guideline.

During our data processing we noted that prior to the new protocol, antibiotic treatment was initiated within couple of hours of birth. After 2017 the indication of administration of antibiotics was mostly the presence of clinical signs. In exceptional cases there were few neonates without clinical signs whom were antibiotic prescribed on the basis of multiple maternal risk factors in addition to highly elevated infection markers suggestive of infection.

During the study period there was a significant reduction in antibiotic use for EOS from 2017 without an observed increase in the incidence of EOS or sepsis related mortality.

The present study did not investigate the short or long term consequence or the complications of antibiotics given in the early days of the neonatal period. Similarly we did not study the possible beneficial effect of the reduced antibiotic use. For future direction, it would be interesting to investigate that with the cessation of antibiotic use, based purely on risk factors would have an effect on the number of invasive procedures and on the finances of the neonatal and postnatal units.

Conclusion

Education about the potential harms and complications of intubation without analgesia and sedation should be enforced worldwide, as false assumptions and myths still prevail. Our survey of healthcare professionals in 70 European and non-European countries found wide-ranging policies and practices. This highlights the need for international consensus that is based on expertise and clinical trials.

Great variation of CSF% was observed among neonates, showing a significantly greater relative amount of CSF present at the higher LP site of L3–4. Based on our assumption that the volume of CSF influences the success rate of LPs, the higher recommended LP site may be preferable. One cannot estimate the relative amount of CSF and draw a clear conclusion on LP success based on the investigated clinical parameters or ventricular size. Imaging of the lumbar region using point-of-care US and calculation of the CSF% may increase the success rate of neonatal LP.

Our retrospective study showed that the introduction of the new EOS protocol adapted from the national guideline had a significant impact on antibiotic use in our hospital, we could safely decrease our antibiotic prescription for suspected EOS without increase in mortality. The observed antibiotic exposure in the years after the protocol change is comparable with internationally accepted levels.

More national and international research is needed on early onset sepsis management to enable us to precisely localise our practice in comparison to other units, other hospitals, and other countries, to keep us motivated to further optimize the management of EOS and to further reduce unnecessary antibiotic use.

The incidence of EOS derived from our data is in accordance with internationally published research, shows that early onset sepsis in late preterm and term neonates is a very rare but potentially fatal condition.

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