ADMINISTRATION OF EXTRACELLULAR VESICLES FROM RAT AMNIOTIC FLUID STEM CELLS RESCUES BRANCHING MORPHOGENESIS AND INFLAMMATION LEVELS IN FETAL MICE WITH CONGENITAL DIAPHRAGMATIC HERNIA

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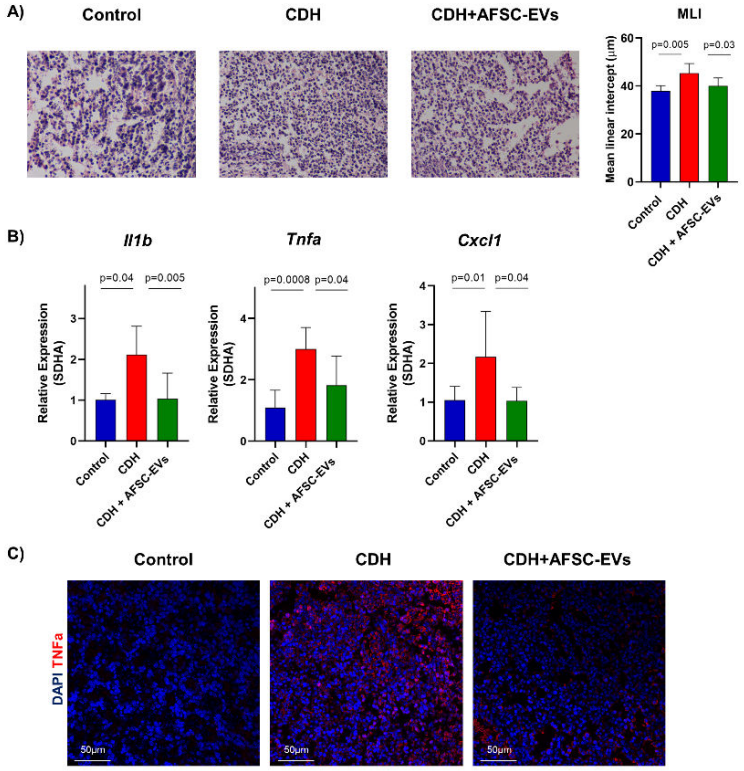
PURPOSE: Congenital diaphragmatic hernia (CDH) is a birth defect with high neonatal mortality mainly related to pulmonary hypoplasia. Hypoplastic fetal lungs secondary to CDH are inflamed and enriched with macrophages. Antenatal administration of amniotic fluid stem cell extracellular vesicles (AFSC-EVs) has shown promising regenerative and anti-inflammatory properties in the CDH rat model. Herein, we investigated whether 1) lungs of fetal mice with CDH also are inflamed and enriched with macrophages; 2) AFSC-EVs exert anti-inflammatory effects in this model.

METHODS: *Animals:* C57BL/6J dams (AUP#64247) were either fed with nitrofen+bisdiamine (CDH) or olive oil only (control) at embryonic day (E) 8.5. On E18.5, fetal lungs were dissected and grown *ex vivo* for 72h (medium alone or medium and AFSC-EVs). Only fetuses with a left-sided diaphragmatic defect were included. *Rat AFSC-EVs:* were collected via ultracentrifugation and characterized for size (nanoparticle tracking analysis), shape (electron microscopy) and canonical EV markers (Western Blot). *Outcome measures:* Pulmonary hypoplasia was assessed via mean linear intercept (MLI;H&E). Gene expression changes for Interleukin-1beta (*Il1b*), Tumor-necrosis factor alpha (*Tnfa*) and C-X-C Motif Chemokine Ligand 1 (*Cxcl1*) were measured via RT-qPCR. *Genotyping:* Pups were genotyped for SRY *Statistics:* Normality was assessed via Shapiro-Wilk or D'Agostino-Pearson. Fisher-Exact test, ANOVA or Kruskal-Wallis with post-hoc Tukey or Dunn's tests were used to determine statistical significance ($p<0.05$).

Figure 1



RESULTS: No difference was detected for occurrence of CDH and sex (RR: 2; 95%CI: 0.7-7.3; p=0.36). Compared to control, fetal CDH lungs had impaired lung growth with decreased airspace density (**Figure 1A**). Fetal CDH lungs were inflamed with increased gene expression of *Il1b*, *Tnfa* and *Cxcl1* compared to control (**Figure 1B-C**). Antenatal administration of AFSC-EVs *ex vivo* rescued fetal airspace density and gene expression of *Il1b*, *Tnfa* and *Cxcl1* (**Figure 1A-C**).

CONCLUSION: This study shows that the C57BL/6J mouse model of CDH has impaired lung growth with associated fetal lung inflammation. Antenatal administration of EVs from rat AFSCs modulates branching morphogenesis and inflammation in fetal mouse lungs. This highlights that the AFSC-EV cargo contains mediators that are not species specific.

NATIONAL CENTRALIZATION OF HIRSCHSPRUNG'S DISEASE IN SWEDEN: A COMPARISON OF POSTOPERATIVE OUTCOME

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PURPOSE: In Sweden, surgical treatment of Hirschsprung's disease (HSCR) was centralized from four to two pediatric surgery centers 1st of July 2018. In adults, centralization of surgical care for complex or rare diseases seems to improve quality of care. There is little evidence supporting centralization of pediatric surgical care. The aim of this study was to assess surgical management and postoperative outcome in HSCR patients following centralization of care.
METHODS: This study retrospectively analyzed data of patients with HSCR that had undergone pull-through at a pediatric surgery center in Sweden from 1st of July 2013 to 30th of June 2023. Patients managed from 1st of July 2013 to 30th of June 2018 (before centralization) were compared with patients managed from 1st of July 2018 to 30th of June 2023 (after centralization) regarding surgical treatment, unplanned procedures under general anesthesia or readmissions up to 90 days after pull-through as well as complications classified according to Clavien Madadi up to 30 days after pull-through.
RESULTS: In the five-year period prior to centralization 114 individuals from four treating centers were included and compared to 83 patients from two treating centers in the second period. There was no difference regarding age at pull-through or proportion of patients with a stoma prior to pull-through. An increase of laparoscopically assisted endorectal pull-through (8.8% to 39.8%) and endorectal pull through with a sub-umbilical incision (25.4% to 39.8%) was observed ($p < 0.001$). No significant differences were seen in postoperative hospital stay, unplanned procedures under general anesthesia or readmissions up to 90 days after pull-through. Also, complications classified according to Clavien Madadi up to 30 days after pull-through did not differ between the two time periods. HAEC treated with antibiotics increased following centralization (10.5% to 16.9%; $p=0.018$).
CONCLUSION: Centralization of care for HSCR does not seem to delay time to pull-through nor reduce unplanned procedures under general anesthesia or readmissions up to 90 days after pull-through, although the incidence rate of postoperative HAEC treated with antibiotics increased after centralization.

GLUCAGON-LIKE PEPTIDE PROMOTES MATURATION OF INTESTINAL ORGANOIDS DERIVED FROM NEONATES WITH NECROTIZING ENTEROCOLITIS

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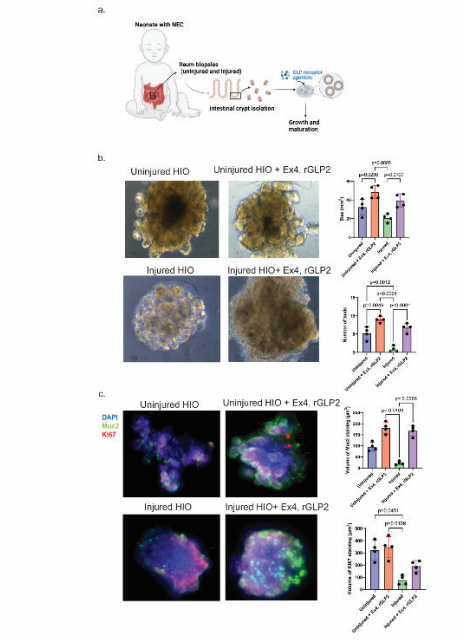
PURPOSE: Necrotizing enterocolitis (NEC) majorly affects premature infants, causing not only necrosis and inflammation but also feeding intolerance and gastrointestinal dysmotility, hinting at gut hormone secretion impairment. Particularly critical is the gestation period before 26 weeks where intestinal hormonal activity is partially developed, rendering preterm neonates highly susceptible to NEC. Emerging evidence suggests a role of gut hormones, especially glucagon-like peptides (GLP) in ileum development. Herein, the aim of this study was to uncover the role of GLP signaling in intestinal development in human NEC.
METHODS: To determine the role GLP signaling plays in intestinal development and during intestinal injury, we employed a human intestinal organoid (HIO) model derived from ileum tissue. After ethical approval, we obtained ileal biopsies from NEC patients in uninjured (distant from site of NEC injury) and injured intestine (site of injury). After collection, crypt isolation was performed, and HIOs were cultured for 2-3 days before glucagon peptide agonists (Exendin-4 [GLP1 receptor] and rGLP2 [GLP2 receptor]) were added daily in culture media (**Figure 1a**). Organoids were harvested on day 10 and were analyzed for morphological measures of maturation including organoid size and budding. Protein expression of differentiation marker (Mucin 2) and proliferation marker (Ki67) (IF) were also measured.
RESULTS: Within the same patient, injured HIOs had a decreased budding compared to uninjured HIOs. Treatment with GLP agonists increased organoid size number and budding compared to the untreated organoid in both uninjured and injured HIOs (**Figure 1b**). Treatment with GLP agonists significantly increased Mucin 2 expression in injured HIOs. Furthermore, uninjured HIOs had increased proliferation marker Ki67 compared to injured HIOs. Treatment with GLP agonists did not induce changes in HIOs proliferation (**Figure 1c**).
CONCLUSION: Patient-derived organoids provide a suitable *ex vivo* model to study NEC pathogenesis. Increasing GLP signaling in HIOs enhanced maturation of organoids derived from uninjured and injured neonatal human intestine. Further studies are underway to assess *in vivo*, the efficacy of GLP agonist administration in NEC.



STAT TRIAL: STOMA OR INTESTINAL ANASTOMOSIS FOR NECROTIZING ENTEROCOLITIS: SECONDARY OUTCOMES FROM A MULTICENTRE RANDOMIZED CONTROLLED TRIAL

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Purpose: The STAT trial is a multicentre randomised controlled trial aimed to demonstrate which is the most effective operation for neonates with necrotizing enterocolitis (NEC): intestinal resection with stoma formation (ST) or intestinal resection with primary anastomosis (PA). It has been shown that NEC patients having PA require a shorter duration of parenteral nutrition (PN) than those having ST and do not need a second operation for stoma closure. The aim of this study was to report secondary outcomes of the STAT trial including episodes of sepsis, days of antibiotics, requirement for ventilation and oxygen, and enteral calorie intake at one month.
Methods: A randomized controlled trial was conducted in 10 centres worldwide. Infants having a primary laparotomy for NEC were randomized online intraoperatively, using weighted minimization, to PA or ST if the operating surgeon thought that both were viable treatment options for that patient (no disease distal to stoma or anastomosis). A power calculation suggested that 40 patients in each arm would enable a difference in primary outcome (duration of parenteral nutrition [PN]) to be detected. Data are given as median (range) and compared using Fisher's exact test or the Mann-Whitney U test.
Results: A total of 80 patients were recruited from 2010-2019. As previously reported, infants undergoing anastomosis finished PN significantly earlier than patients undergoing stoma (**Fig.a**, p=0.011) with no disadvantage in terms of mortality or unplanned reoperation. We report for the first time that at one-month post-randomization, 4/16 PA patients had blood culture positive sepsis compared with 5/16 ST patients (p=1.0), with a similar duration of antibiotic use (16 [7-28] vs. 17 [7-36], p=0.77). There were no differences in ventilation (9/18 vs. 7/16 p=0.78) or oxygen requirement (10/18 vs. 7/16 p=0.73) at one month. Enteral intake was significantly greater in the ST group (p=0.043, **Fig.b**) reflecting a high stoma output (8/16) in this group of patients. The parenteral intake was similar between the groups.
Conclusion: Primary anastomosis should be considered at laparotomy for NEC when there is no disease distal to resected intestine, as it does not increase the risk of adverse outcomes and recovery from NEC.

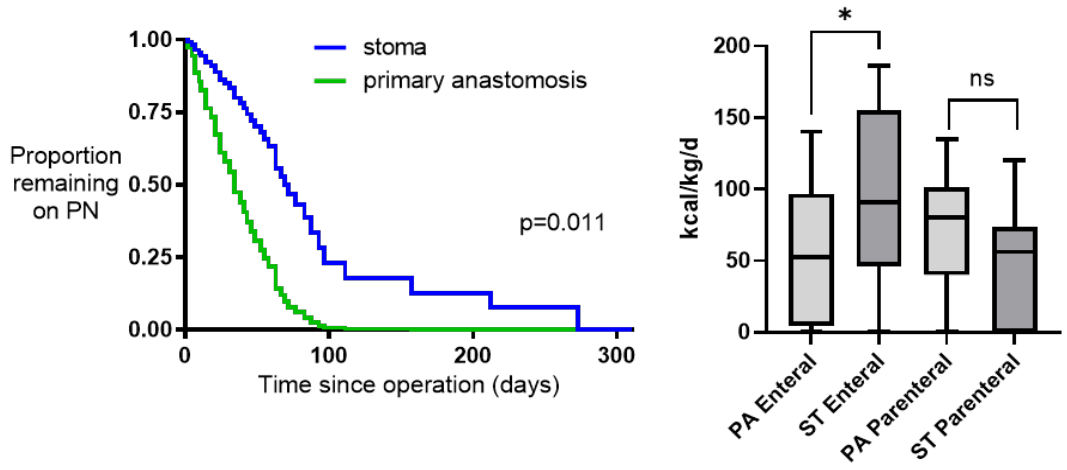

Figure. (a) Cox-regression analysis of time on PN (*EUPSA 2023*); **(b)** Enteral and parenteral intake (kcal/kg/d) in ST and PA groups.

DEFINING THE EFFECTS OF AMNIOTIC FLUID STEM CELL EXTRACELLULAR VESICLE ADMINISTRATION ON THE LIVER OF FETUSES WITH CONGENITAL DIAPHRAGMATIC HERNIA

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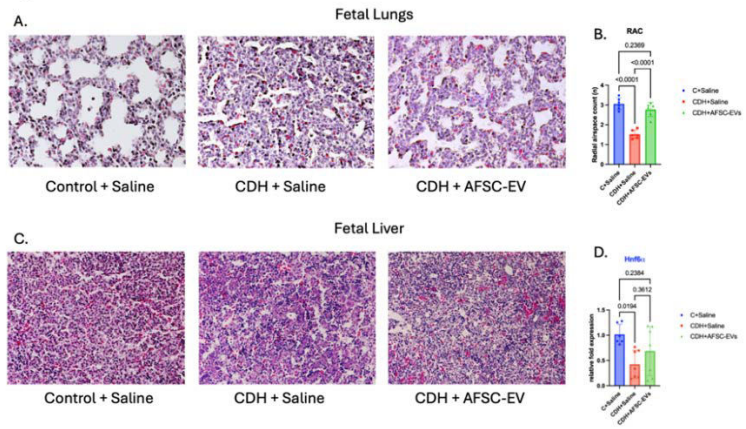
PURPOSE: It was previously reported that administration of amniotic fluid stem cell extracellular vesicles (AFSC-EVs) restores lung development in fetuses with pulmonary hypoplasia secondary to congenital diaphragmatic hernia (CDH). Despite promising findings in the fetal lung, tracking studies in experimental models of CDH indicated wide distribution of AFSC-EVs throughout the fetal body, raising questions about the effects of this treatment on off-target organs. The prominent role of the liver in metabolizing drugs and exogenous compounds remarks the importance of understanding the safety of AFSC-EV treatment in fetuses with CDH. Herein, we investigate the effects of intra-amniotically administered AFSC-EVs on the fetal liver of rat fetuses with CDH.

METHODS: EVs were isolated from rat AFSC conditioned medium by ultracentrifugation. On embryonic (E) day 9.5, rat dams were gavaged with olive oil (control) or nitrofen (CDH). At E18.5, fetuses received an intra-amniotic injection of saline (control+saline, n=6; CDH+saline, n=6) or AFSC-EVs (CDH+AFSC-EVs, n=7). At E21.5 fetal lungs and matched livers were harvested. Fetal lungs were histologically assessed for radial alveolar counting (RAC). Fetal livers were assessed for: 1) presence of morphological changes (hepatic cells hypertrophy, hepatic vacuolation, capillary sinusoidal congestion by H&E); 2) gene expression of hepatic growth (*Cyp1a1/Hnf4α/Hnf6α*), apoptosis (*Bcl2/Bak/Atf6/Casp3*) and cell differentiation (*Adrp/Epo*) markers (RT-qPCR).

RESULTS: CDH fetal lungs had lower airspace numbers compared to control+saline and CDH+AFSC-EVs (Fig.1A-B). No morphological changes were observed in fetal livers from CDH+saline and CDH+AFSC-EV groups compared to control+saline (Fig.1C). Compared to control+saline, CDH+saline fetal livers had decreased expression of *Hnf6α*, a transcription factor that regulates hepatocyte proliferation (Fig. 1D). Conversely, CDH+AFSC-EV fetuses had *Hnf6α* expression levels similar to control+saline. Gene expression of other hepatic growth factors, apoptotic and differentiation markers were similar between control+saline and CDH+AFSC-EV fetal livers.

CONCLUSIONS: AFSC-EVs administered antenatally reach the fetal liver but do not affect the organ morphology, homeostasis, and cell differentiation. Further studies are needed to address AFSC-EV safety on off-target organs for the fetus and the mother.

Figure 1.



circRNA EXPRESSION PROFILE TO IDENTIFY NOVEL MARKERS FOR ABNORMAL LUNG DEVELOPMENT IN THE NITROFEN MODEL FOR CONGENITAL DIAPHRAGMATIC HERNIA

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PURPOSE: Circular RNAs (circRNAs) are highly stable non-coding RNAs and have a tissue- and developmental stage-specific expression; making them potential biomarkers for congenital anomalies. Previously, we showed a specific circRNA profile in human congenital diaphragmatic hernia (CDH) lungs. Hence, we aimed to investigate circRNAs during abnormal lung development in the nitrofen rat model.
METHODS: circRNA profiles of nitrofen-induced and control lungs of three fetuses were compared at embryonic day (E)15 and E21 using an Arraystar microarray. The results were validated with conventional PCR, amplicon sequencing, RT-qPCR and BaseScope™ *in situ* hybridization. The CircRNA Function prediction Tool (CRAFT) (), was used to predict differentially expressed circRNA interactions with mircroRNAs (miRNAs) and RNA-binding proteins, along with their coding potential.
RESULTS: The microarray revealed a circRNA biosignature specific for CDH, the developmental stage and sex. These results and evidence from the literature guided our selection of candidates for further validation of circRNAs derived from the parental gene Anp32e, Ppp3ca and TIAL1. Expression of circAnp32e was increased in nitrofen-induced lungs at E21 ($p=0.004$) in both qPCR and in situ hybridization. At E15, the circAnp32e expression in total lungs did not differ between CDH and control lungs ($p=0.22$); but sex-disaggregated analysis revealed an overexpression in male pups ($p=0.034$). Further, *in situ* hybridization showed a distinct spatio-temporal expression pattern in early development with an increased signal for circAnp32e in the epithelium at E15. circTIAL1 showed a nonsignificant trend to overexpression in CDH lungs ($p=0.07$); and a statistically significant increase only in male pups ($p=0.0167$). Bioinformatic analyses of this circRNA biosignature will allow prediction of their function by interacting with miRNA and RNA-binding proteins in abnormal lung development. Furthermore, the alignment of human and rat mature circAnp32e sequences showed a 90% overlap between the two species which warrants further investigation in the pathophysiology of human CDH.
CONCLUSION: The circRNA profile is dysregulated in nitrofen-induced fetal lungs and is specific for early and late stages of abnormal lung development. Furthermore, certain circRNA candidates show a sex- and cell-type specific expression pattern in nitrofen-induced CDH lungs. This warrants further investigation as a biomarker for prognostication in CDH.

ENHANCEMENT OF ENTERIC NEURAL STEM CELL NEUROGENESIS BY GLIAL CELL-DERIVED NEUROTROPHIC FACTOR IN EXPERIMENTAL HIRSCHSPRUNG'S DISEASE

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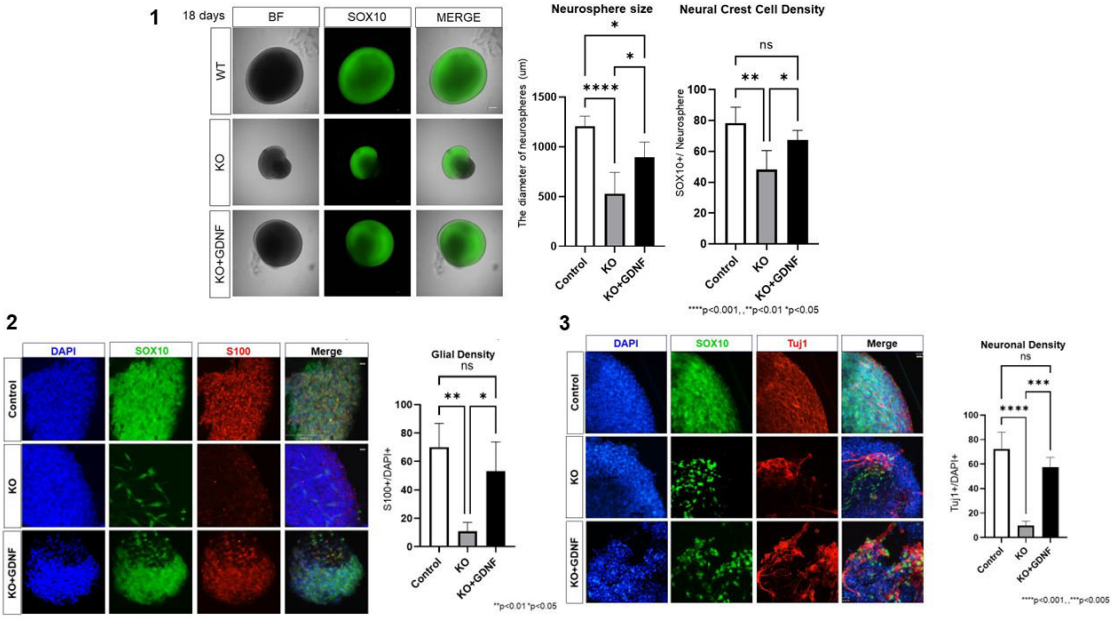
PURPOSE: Advanced medical applications, like stem cell therapy, offer treatment options for various conditions, including enteric neuropathies such as Hirschsprung's disease (HD). To promote regeneration, researchers have focused on optimizing stem cell efficacy by modifying the stem cells and their environment. Further exploration and improvement of *in vitro* stem cell culture conditions are required. During the development of the enteric nervous system, glial cell derived neurotrophic factor (GDNF) plays a critical role in neural survival, proliferation, migration and differentiation. It has been shown that enteric neural crest cells (ENCCs) derived from control gut increase in number and size when exposed to GDNF. This study aimed to examine the impact of GDNF administration on ENCCs using the endothelin receptor B (*Ednrb*) knockout mouse model, a known animal model of HD. The aim of this study is to design a novel cell therapy in experimental HD by evaluating the effects of GDNF on neurospheres formation.

METHODS: *Ednrb*$^{+/+}$ (Control) and *Ednrb*$^{-/-}$ (KO) embryos with *Sox10*-GFP transgenic expression were used. ENCCs were isolated from the fetal guts dissected on embryonic day 13.5 (E13.5) and dissociated. These cells were cultured for 7 days under non-adherent conditions to generate neurospheres. Neurospheres from controls were cultured in control media and neurospheres from KO were cultured with either control media or media supplemented with GDNF (100ng/ml). ENCCs differentiation was assessed by immunofluorescence staining after 18 days of culture, with Tuj1 as a marker for neuron and S100 as a marker for glia cells.

RESULTS: *Ednrb* KO neurospheres cultured in the presence of GDNF were larger compared to both KO without GDNF and control neurospheres. KO neurospheres with GDNF contained significantly more SOX10 positive ENCCs compared to KO neurospheres without GDNF(Fig. 1). Administration of GDNF enhanced the distribution of both Tuj1-positive and S100-positive cells in the KO neurospheres (Fig. 2,3).

CONCLUSION: GDNF is efficacious in enhancing the neurogenic potential of gut derived neural stem cells (neurospheres) derived from gastrointestinal tract in HD. This finding is crucial for the development of cell therapy in HD.



OMENTAL PRE-VASCULARIZATION OF DECELLULARIZED BLADDER MATRIX FOR MUCUS FREE BLADDER AUGMENTATION

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Purpose: Experimental studies aiming mucus free bladder augmentation showed promising integration of small acellular bladder matrix (ABM) stamps into the bladder. However, failed to demonstrate good results with larger, clinically relevant sized patches therefore translation this technology to clinical practice remained elusive,
We hypothesized omental pre -vascularisation will allow us to use clinically relevant sized large ABM patches for bowel free bladder augmentation.
Method: This study was carried out over three stages in 8 domestic pigs after ethical approval. Stage 1 - porcine ABM was sutured around a tissue expanding device -either a sponge covered in a silicone sheet (83mls), or a gastric balloon (100mls) or a sponge inside a surgical glove (50mls) - wrapped in omentum and sutured to the anterior abdominal wall. Stage 2 - After four weeks, hemicystectomy and bladder augmentation was performed with the pre-vascularized PABM. Stage 3 - After a further eight weeks, neo-bladders were analysed using cystoscopy, laser speckle contrast imaging and histology. Data is presented as median (IQR).
Results: Macroscopically all bladder patches at stage 2 were friable and vascularised. At stage 3 all patches were compliant and had integrated with the native bladder and were 83% (48-100%) of the original patch size.
Laser speckle contrast imaging analysis showed poor vascularity of the omentum & patch (stage 1), moderate vascularity of patch (stage 2) and good vascularity of patch (stage 3).
Histology demonstrated acute inflammatory response with fibroblast infiltration at stage 2, full coverage by urothelium was seen with positive staining for uroplakin 3 and CK7 antibodies at stage 3.
There were three complications - one abdominal wall hernia and two enteric fistulae, the latter thought to be due to the rectangular shape of the initial tissue expander which was subsequently changed.
Conclusion: Omental pre-vascularization allowed large, clinically relevant ABM patches to integrate into the bladder maintain their size and compliance. This may be a key step towards translation into clinical practice.

SURVIVAL RATE CHANGES IN CHILDREN WITH CONGENITAL DIAPHRAGMATIC HERNIA OVER THE PAST TREE DECADES: A NATIONWIDE, POPULATION-BASED PROSPECTIVE NESTED CASE-CONTROL STUDY

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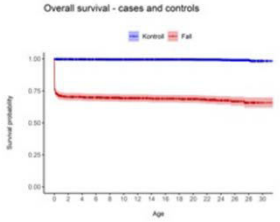
PURPOSE: The survival rate (SR) for neonates born with congenital diaphragmatic hernia (CDH) still remains high. Our aim was to investigate how changed the SR in children with CDH over the past decades.
METHODS: The study was a nationwide, population-based prospective nested case-control study within a cohort of newborn children who was born in Sweden during the observational period from 1st January 1982 until 31st December 2015. The study exposure was CDH and outcome was survival. Data were assessed through linkages between registries administrated by the Swedish National Board of Health and Welfare. The regional ethical committee approved the study, Dnr 2013/1550-31/3.
RESULTS: Out of 4672 control cases, 4654 (99.6%) children, while out of 972 CDH cases, 684 (70.4%, p<0.001) patients were survival at 2 years of age (Figure1). Any patients who were born with CDH has overall 5,8 times higher chance for not survive 2 years of age (p<0.001) than children without CDH (Figure1). The most event happened in the first 3 months of age in the CDH group. There was no significant difference in the SR after 2 years of age between controls and CDH. While the SR was not changed significantly in the past 3 decades in controls, it was significantly improved in CDH (p<0.01)(Figure2). Significantly higher number of patients were premature born in the CDH group than in the controls (227, 23.3% vs 284, 6.1%, p<0.001). The SR of premature born CDH patients increased over the past 3 decades (Figure2).
CONCLUSION: The SR of CDH patients were significantly increased in the past 3 decades. Although, the 2 years survival still remains 5,8 times higher than those who were not born with CDH. These changes were mainly attributed to the improved SR with premature born CDH patients.

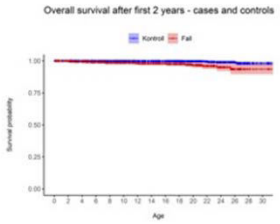


Estimated survival at 3 months:

	time	n	n.risk	n.event	n.censor	surv	lower	upper
Control	0.25	4672	4648	14	10	0.997	0.995	0.999
CDH	0.25	972	709	261	2	0.731	0.704	0.760

Estimated survival at 1 year:

	time	n	n.risk	n.event	n.censor	surv	lower	upper
Control	1	4672	4519	18	135	0.996	0.994	0.998
CDH	1	972	666	281	25	0.711	0.683	0.740



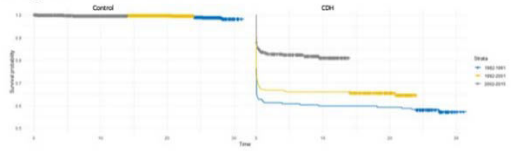
Estimated survival at 2 years:

	time	n	n.risk	n.event	n.censor	surv	lower	upper
Control	2	4672	4420	18	234	0.996	0.994	0.998
CDH	2	972	641	288	43	0.703	0.675	0.732

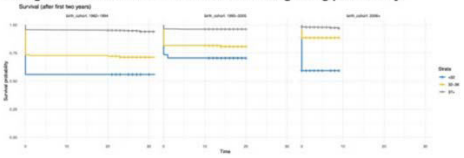
Overall survival cases compared to controls (given survived first two years):

	Estimate	HR	se	z-value	p-value	CI-lower	CI-upper
CDH	1.766	5.845	0.376	4.696	0	2.798	12.213

Changes in survival over birth cohort:



Changes in survival of CDH over birth cohort regarding prematurity:



FOLLOW-UP AND TRANSITION PRACTICES IN ESOPHAGEAL ATRESIA: A REVIEW OF EUROPEAN REFERENCE NETWORK ON RARE INHERITED AND CONGENITAL ANOMALIES (ERNICA) CENTRES AND AFFILIATES

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PURPOSE: Esophageal atresia (EA) is associated with respiratory, nutritional and gastrointestinal sequelae requiring surveillance into adulthood. Evidence-based consensus statements (ERNICA/ESPGHAN) provide uniform EA FU protocols. Despite this, FU and transition practices of EA patients across Europe remain largely unknown and patient organisations have reported concerns about variability of delivery. The purpose of this study was to understand the provision and distribution of EA FU and transition services across ERNICA member and affiliate centres.
METHODS: A questionnaire was sent via REDCap to clinical leads of 18 ERNICA member and 14 affiliate partner centers. Statistical tests include Fisher's Exact/Chi-square test; $p<0.05$ considered statistically significant.
RESULTS: 29 of 32 centres responded (91%), the majority of which were highly specialized; 79% performed >5 EA cases/year, all managed long-gap, and >90% performed re-do and replacement surgery. Two-thirds of centres had a dedicated EA clinic with a specialist multidisciplinary team (MDT), 90% led by paediatric surgeons. In 40%, this was offered to selected/complex patients only. ERNICA centres were more likely to offer an MDT FU clinic than affiliates (78 vs 55%, p=ns), with lack of resources most cited as a barrier to uptake (67%). Delivery of routine investigations in asymptomatic EA patients was heterogeneous (Fig 1). ERNICA centres were more likely to offer at least one endoscopy during FU (94% vs. 55%; p=0.01), but delivery of the recommended three endoscopies over the course of FU was universally poor (24%).
Only 55% of all centres had a dedicated transition pathway (18 (16-19) years), more prevalent in ERNICA centres (81% vs. 30%; $p<0.01$). 63% ran joint transition clinics with adult specialists with self-declared interest in EA (88%); 65% gastroenterology, 35% surgery. An education program for transitioning EA adolescents existed in two centres (7%). Overall, self-reported awareness of ERNICA and ESPGHAN guidance for FU and transition was poor (28%).
CONCLUSIONS: The majority of centres have specialist MDT clinics and dedicated EA transition pathways. Despite the existence of European follow-up and transition guidelines, their delivery is not uniform and may be limited by lack of awareness of the guidelines and a lack of resources.

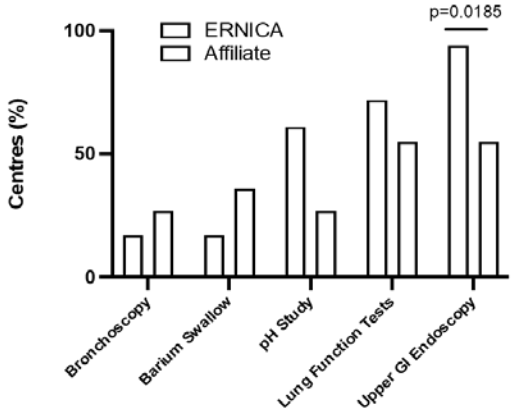

Figure 1: Routine use of investigations during the course of FU in asymptomatic patients.

UNVEILING HYPOXIC ADAPTATION AND PRO-ANGIOGENIC PHENOTYPE IN BIOREACTOR-MATURED ESOPHAGEAL GRAFTS

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(London, United Kingdom)

PURPOSE: Esophageal replacement, necessitated by conditions like esophageal atresia, caustic injuries, or cancer, is challenging. Tissue engineering presents a new solution, eliminating the need to sacrifice another organ. We've developed recellularized esophageal grafts, using autologous myogenic precursors injected in decellularized porcine esophageal scaffolds. These grafts matured in a bioreactor before transplantation into a minipig model. Bioreactor flow perfusion, known to promote cell survival, distribution, and tissue maturation, has an unknown specific effect on cellular function and adaptation. For the first time, we've used single-nucleus RNA sequencing (snRNAseq) to study transcriptional differences in cells pre and post-bioreactor incubation, enhancing our understanding of *in vitro* bioreactor maturation in tissue engineering.
METHODS: Decellularized porcine esophagi were repopulated with autologous minipig mesoangioblasts (MABs) and fibroblasts (FBs) from rectus abdominis biopsies, using a 7:3 MABs:FBs ratio and $3x10^6$ cells/injection. The recellularized scaffolds were cultured in proliferation media for seven days at 37°C in custom bioreactors. The cell mix and a graft ring were frozen pre and post-bioreactor incubation. Single nuclei were extracted and snRNAseq was performed on both populations (n=4 animals). After standard QC, normalization, and dimensionality reduction, gene set enrichment analysis (GSEA) was conducted between the pre- and post-bioreactor nuclei using the Hallmark gene set. All animal procedures were ethically approved (PPL:P43EF9FB6).
RESULTS: Different levels of marker gene expression suggest the post-bioreactor nuclei differ from the pre-bioreactor. GSEA suggests a metabolic switch and an adaptation to reduced oxygen condition in post-bioreactor nuclei, enabling cell survival to reduced oxygen concentration without evidence of senescence or cell death. Hypoxic adaptation underlines pro-angiogenic phenotype (i.e. downregulation of anti-angiogenic genes and upregulation of pro-angiogenic genes in post-bioreactor nuclei).
CONCLUSION: This preliminary analysis indicates that bioreactor incubation prepares scaffold cells for survival in hypoxic conditions, potentially enhancing graft preservation post-transplantation, especially in non-vascularised tissue. Future research will extend the snRNAseq analysis to cells from post-implantation grafts from corresponding animals.

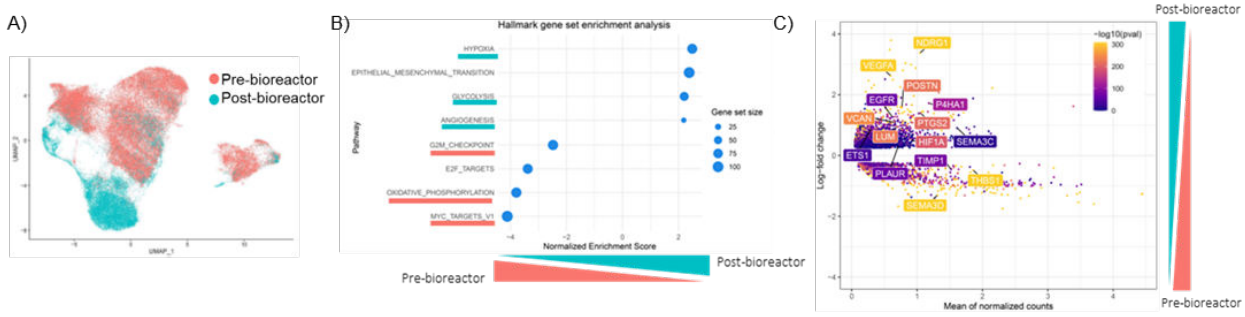

Figure: (A) UMAP suggests post-bioreactor nuclei have a different transcriptomic profile. (B) GSEA shows these nuclei upregulate gene sets related to hypoxia response, glycolysis, and angiogenesis, while downregulating oxidative phosphorylation, cell division, and transcription-related sets, (C) enabling pro-angiogenic phenotype.

HUMAN ENTERIC GLIA DIVERSITY IN HEALTH AND DISEASE: NEW AVENUES FOR THE TREATMENT OF HIRSCHSPRUNG DISEASE

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PURPOSE: The enteric nervous system (ENS) consists of an interconnected network of neurons and glia, which plays a crucial role in controlling intestinal motility. Defects in ENS development lead to enteric neuropathies, such as Hirschsprung disease (HSCR). While neuronal aspects have been extensively explored in HSCR pathogenesis, enteric glia have been largely disregarded. This study, aimed to explore the diversity of enteric glia in health and disease.
METHODS: Full-thickness intestinal resection material from pediatric controls and HSCR patients was collected, dissociated and enriched for the ENS population through fluorescence-activated cell sorting. Single-cell RNA sequencing was performed to uncover the transcriptomic diversity of the ENS in HSCR patients and controls, as well as in wildtype and *ret* mutant zebrafish. Immunofluorescence and fluorescence *in situ* hybridization confirmed the presence of distinct subtypes.
RESULTS: Our findings revealed two major enteric glial classes: Schwann-like enteric glia 1-6, resembling Schwann cells, and Enteric glia 1-2, expressing classical glial markers. Comparative analysis with previously published datasets confirmed our classification and revealed that whilst classical enteric glia are predominant prenatally, Schwann-like enteric glia become more abundant postnatally. In HSCR, ganglionic segments resembled controls, while aganglionic segments only showed the presence of Schwann-like enteric glia. Due to the known regenerative ability of Schwann cells, we further explored the therapeutic potential of Schwann-like enteric glia, using the zebrafish as a HSCR model. We observed that prucalopride, a serotonin-receptor agonist, induced enteric neurogenesis and partially rescued the HSCR phenotype in *ret*$^{+/-}$ mutants.
CONCLUSION: Our study unveils enteric glia diversity in the pediatric intestine on a transcriptomic level and shows that Schwann-like enteric glia remain unaffected in aganglionic segments of HSCR patients. Furthermore, we were able to induce neurogenesis upon prucalopride treatment in ret mutant zebrafish, demonstrating a potential novel therapy for HSCR.

ACTIVATED INFLAMMASOME IS NEEDED FOR SUCCESSFUL TREATMENT WITH AMNIOTIC FLUID STEM CELL DERIVED EXTRACELLULAR VESICLES IN A FETAL EXPLANT MODEL OF GASTROSCHISIS

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PURPOSE: The high morbidity of gastroschisis (GS) babies is mainly due to exposure of the bowel to amniotic fluid, leading to inflammation. Inflammasomes are multi-protein complexes that play a crucial role in inflammatory bowel conditions. We previously demonstrated that inflammasomes are activated also in fetal GS and that antenatal administration of extracellular vesicles derived from amniotic-fluid stem cells (AFSC-EVs) reduce intestinal injury by diminishing inflammasome activity. To confirm this mechanism of action, we herein investigated whether blockage of the inflammasome cascade in a knockout model of GS could lead to a suppressed inflammatory milieu preventing AFSC-EV beneficial effects.

METHODS: EVs were isolated from rat AFSC conditioned medium by ultracentrifugation and characterized for size, morphology, and canonical EV protein markers. Following ethical approval (AUP#39168), we established an *in vitro* explant model of GS by harvesting the intestine of Sprague-Dawley wildtype (WT) or Ninjurin 1 knockout (Ninj1$^{-/-}$) fetal rats at E19.5. Explants were grown for 24h and treated with: a) medium alone (control group), b) medium+interleukin-6 (IL-6 group), or c) medium+IL-6+AFSC-EVs (IL-6+AFSC-EVs group). RT-qPCR was performed to assess gene expression of key players of the inflammasome cascade.

RESULTS: Compared to the intestine of WT rats that had increased inflammasome marker (TNF-alpha and Caspase-3) expression levels in the IL-6 group, Ninj1$^{-/-}$ fetal intestine showed no inflammasome response to IL-6 stimulation (Fig. 1). Similarly, AFSC-EVs rescued TNF-alpha and Caspase-3 levels back to control WT, but had no effects on Ninj1$^{-/-}$ fetal intestine (Fig. 1). Furthermore, Ninj1$^{-/-}$ fetal intestine showed the same non-responsiveness to IL-6 stimulation and subsequent AFSC-EV treatment for other key players of the inflammasome cascade such as NLRP-3, IL-1beta, Gasdermin D, and Caspase-1 (Fig. 2).

CONCLUSION: This study confirms that activation of inflammasome cascade in the fetal intestine depends on Ninjurin 1, a key factor for pyroptosis. In absence of such activation, AFSC-EV administration can no longer exert beneficial effects on the fetal intestine.

Fig. 1

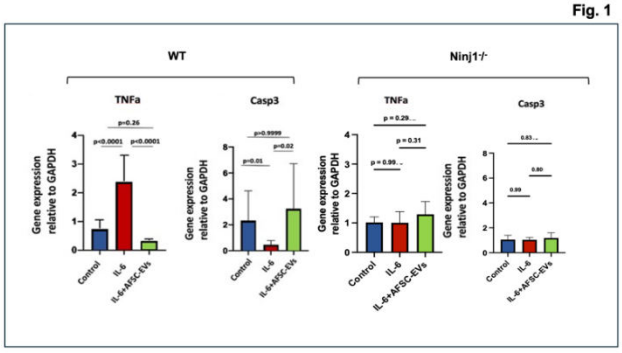
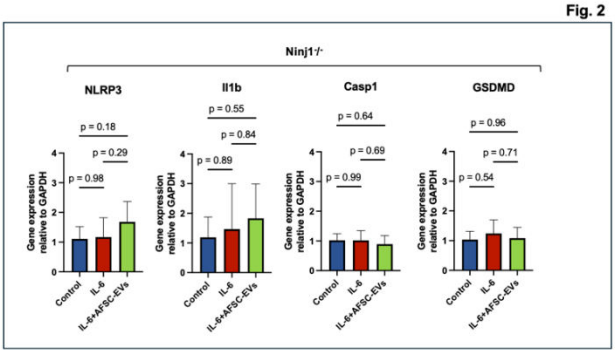


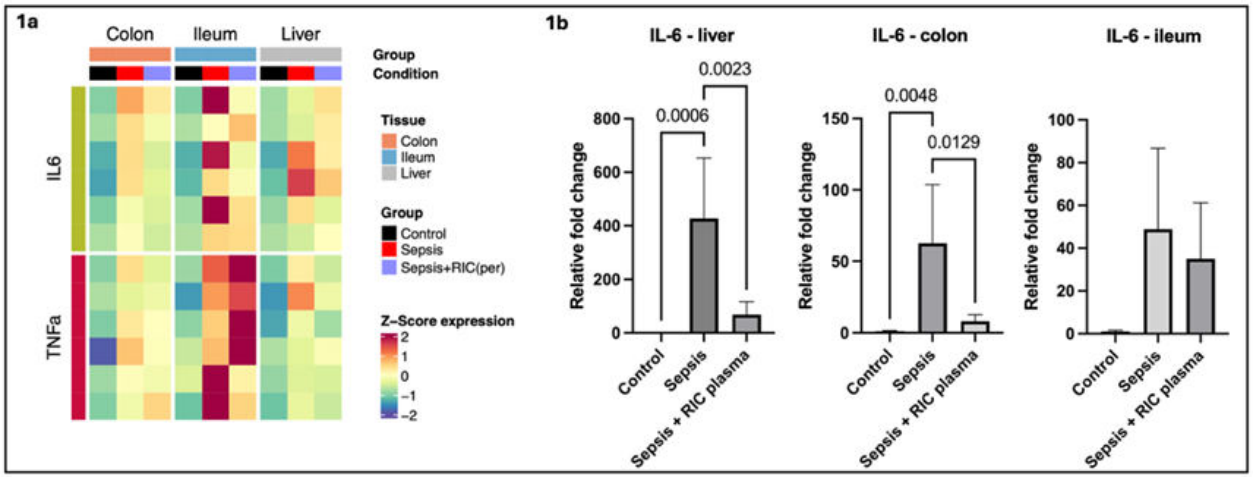
Fig. 2



REMOTE ISCHEMIC CONDITIONING IS ORGAN PROTECTIVE DURING NEONATAL SEPSIS VIA HUMORAL SIGNALING

P. Grassi, B. Li, G. Biouss, A. Zito, F. Balsamo, A. Pierro
(Toronto, Canada)

PURPOSE: Neonatal sepsis is a systemic infection, involving multiple organs. It has been shown that remote ischemic conditioning (RIC), a maneuver involving brief cycles of ischemia and reperfusion in a remote limb reduces mortality in experimental sepsis. The aim of the study are (I) to investigate the organ specific effects of RIC and (II) to evaluate whether the beneficial effect of RIC is transferred via plasma.
METHODS: Sepsis was induced by intraperitoneal injection of lipopolysaccharide (LPS 20 mg/kg) on post-natal day 9 (p9) mice which were sacrificed after 6 hours. Liver, colon, and ileum were harvested. There were two experiments: (I) RIC was administered in septic mice by applying 5-minute cycles of ischemia and reperfusion using a tourniquet. Three groups of mice were assessed including control, sepsis, sepsis with RIC for gene expression of pro-inflammatory cytokines Il-6 and TNFα. (II) In the second experiment, the effect of RIC plasma was evaluated. First, RIC was administered in healthy p9 pups, and then blood was harvested and plasma isolated. RIC plasma was injected intraperitoneally after sepsis induction, at a dose of 10 μl/g. Three groups including control, sepsis, sepsis with RIC plasma administration were assessed for gene expression of IL-6 and TNFα in the liver, colon, and ileum.
RESULTS: Experiment I: Compared to control, septic mice had an increase in the expression of IL-6 and TNFα in the evaluated organs, and RIC administration reduced the expression compared to the sepsis alone (figure 1a). Experiment II: Septic mice receiving RIC plasma had a significant decrease in IL-6 in the colon and liver compared to septic mice alone (figure 1b). There were no differences in the ileum.
CONCLUSION: During experimental neonatal sepsis, RIC decreases inflammation in liver, colon and small intestine. The effects of RIC are mediated humorally as plasma from RIC mice reduces inflammation in liver and colon. This study elucidates the mechanism of action of RIC during neonatal sepsis and highlight a novel future therapeutic strategy.



LIVER MITOCHONDRIAL MORPHOLOGY AND GENE EXPRESSION AS MARKERS OF LIVER RESERVE: PROGNOSTIC IMPLICATIONS FOR NATIVE LIVER SURVIVAL IN BILLIARY ATRESIA

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(Tokyo, Japan)

PURPOSE: Hepatocyte mitochondrial morphology and gene expression in biliary atresia (BA) were compared with infantile cholestasis (IC) and normal liver (NL) as predictors of prognosis.
METHODS: Liver specimens at portoenterostomy (PE) for BA, from intrahepatic bile duct paucity patients for IC, and from choledochal cyst or hepatoblastoma patients for NL were collected prospectively beginning in 2021 (P-BA=11, P-IC=9, P-NL=7) or retrospectively from paraffin-embedded tissue going back to 1981 (R-BA=25, R-IC=9, R-NL=4). The P cohort had transmission electron microscopy (TEM) to image mitochondria, immunoblotting for heat shock protein 60 (HSP60), and quantitative PCR (qPCR) for HSP60 and mitochondrial functional genes. Both cohorts had immunofluorescence for HSP60 quantified as a ratio to albumin-positive hepatocytes (ALB) using ImageJ with HSP60/ALB<1.0 as the cutoff limit (Fig. A).
RESULTS: HSP60 was significantly lower in BA/IC than NL on qPCR (p<0.01) and lower in BA than IC/NL on immunoblotting (p<0.05). HSP60/ALB was significantly higher in NL/IC than BA (p<0.001) (Fig. B). HSP60/ALB did not correlate with jaundice clearance (JC; TBil<1.2 mg/dL) but was significantly higher in native liver survivors (NLS) after PE compared with liver transplant (LTx) cases (p<0.05) (Fig. C) and significantly lower in LTx cases achieving JC than NLS achieving JC (p<0.05) (Fig. D). TEM (Fig. E) showed BA had significantly more mitochondrial inclusion bodies (p<0.05) and significantly larger cristae (p<0.01) (Fig. F) than IC/NL. qPCR showed significant repression of mitochondrial functional genes for mRNA stabilization and energy facilitation in BA (Fig. G).
CONCLUSION: HSP60/ALB appears to correlate with NLS after PE in BA.

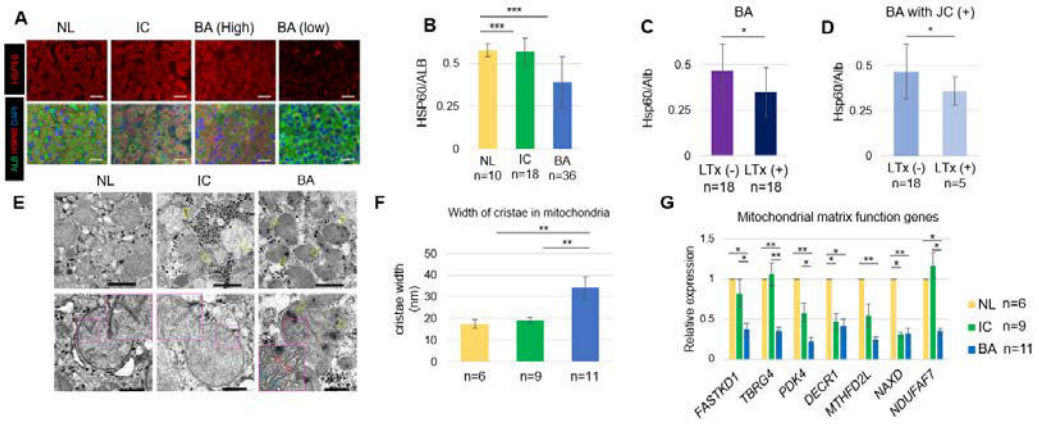

FIGURE LEGENDS
A) Typical immunofluorescence for HSP60/ALB. Scale bar: 25 μm. B) Significantly higher HSP60/ALB in NL/IC than BA (***p<0.001). C) Significantly higher HSP60/ALB in NLS than LTx recipients. D) Significantly lower HSP60/ALB in BA who achieved JC but required LTx (*p<0.05). E) In TEM, open yellow arrowheads indicate mitochondrial inclusion bodies. Electron hypodense regions (open red arrowheads) and enlarged cristae (open blue arrowheads) in BA. Scale bar; 1μm (top) and 0.5μm (bottom). F) Mean widest width of cristae in mitochondria for BA, IC/NL (**p<0.01). G) Significantly repressed Mitochondrial matrix function genes in BA versus IC/NL (*p<0.05, **p<0.01).

THE PLACENTA IN EXPERIMETNAL CONGENITAL DIAPHRAGMATIC HERNIA IS INFLAMED AND HAS DYSREGULATED ANGIOGENESIS

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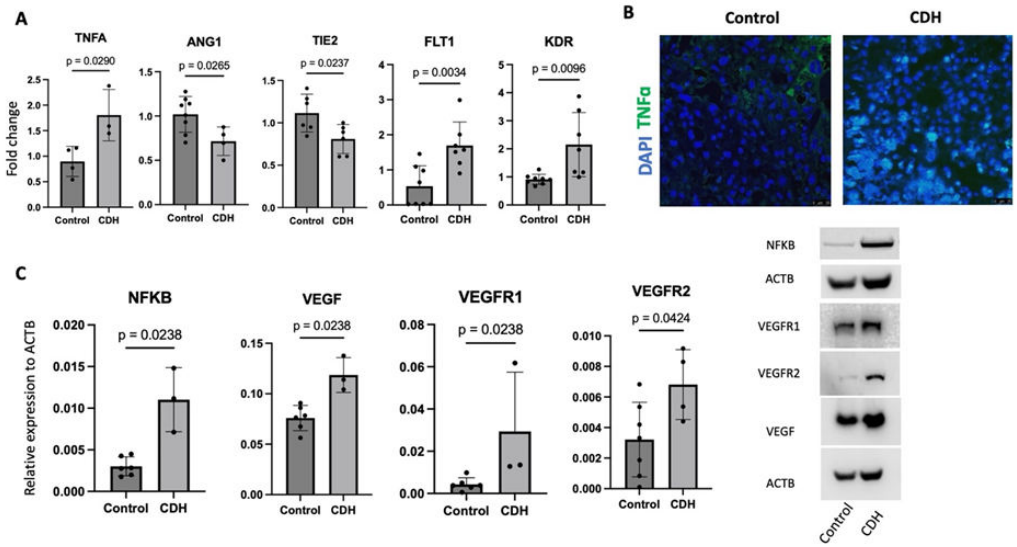
PURPOSE: It has previously been shown that lungs of fetuses with congenital diaphragmatic hernia (CDH) have an inflammatory profile with endothelial cell dysfunction. Moreover, we have recently demonstrated that CDH fetal lungs have an enrichment in macrophages, which are increased in number and are primary drivers of inflammation. The placenta is the major feto-maternal organ responsible for cellular, metabolic, and molecular homeostasis. Herein, we used a murine model of CDH and investigated whether the placenta: 1) presents an inflammatory profile; and 2) has normal angiogenesis.

METHODS: To assess inflammation in CDH, we utilized the MacGreen mouse strain, whose dams express EGFP tagged to the Csf1r (macrophage marker) gene. CDH was induced in the litter by maternal administration of Nitrofen/Bisdiamine on embryonic day (E) 8.5 (AUP#64247). On E18.5, CDH was confirmed via assessment of diaphragmatic defect, and placentas were harvested from both control and CDH fetal pups. To study inflammation and angiogenesis, we used gene (real-time qPCR) and protein expression (Western blotting and immunofluorescence) of canonical markers. Groups were compared using t-test or Mann-Whitney test.

RESULTS: We noted an upregulation of inflammatory markers Tumor necrosis factor alpha (TNFA) and Nuclear Factor kappa beta (NFKB) in CDH placentas compared to control, with marked nuclear uptake of TNF in CDH placentas compared to control (Fig1A-C). Moreover, CDH placentas had dysregulated angiogenesis with impaired Angiopoietin-1 (ANG1) and TEK receptor tyrosine kinase 2 (TIE2) mRNA (Fig1A), and upregulated Vascular endothelial growth factor (VEGF) signaling pathway member mRNAs (vascular endothelial growth factor receptor 1 gene; FLT1, and Kinase insert domain receptor; KDR) and proteins (VEGF, VEGFR1, VEGFR2) compared to controls (Fig1A&C).

CONCLUSIONS: This study demonstrates that CDH placentas have increased inflammation and dysregulated angiogenesis, with upregulation of angiogenesis pathway, suggesting a compensatory mechanism due to endothelial cell dysfunction in CDH fetal lungs. Further studies are underway to elucidate the downstream effects of these findings.



ESTABLISHMENT OF NEUROSPHERES FROM AMNIOTIC FLUID OF PREGNANCIES WITH DIAGNOSED SPINA BIFIDA

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Purpose: Spina bifida (SB) is a neural tube closure defect, that can cause spinal cord injuries and lead to a leakage of neural stem cells (NSCs) into the amniotic fluid (AF) during fetal life. Human NSCs are a valuable source for investigating neural development, and hold great therapeutic potential in neurodegenerative disorders. *In vitro* cultured NSCs can aggregate into free-floating organoids defined as neurospheres. The aim of this study is to establish neurospheres from AF-NSCs from patients with spina bifida, to offer a novel and promising *in vitro* model for SB.

Methods:

AF from SB patients undergoing fetal surgery at 25th gestational week was collected. AF cells were isolated and plated unsorted. Three samples of AF from patients without SB were seeded under the same conditions, as control. Through specific culture conditions and neural-inducing medium to promote NSCs proliferation, free-floating neurospheres were derived. The formed neurospheres were manually isolated to establish a cell line. The lines were subsequently characterized by immunofluorescence, Real-time PCR, and bulk RNA-sequencing.

Results: Neurosphere-like organoids were successfully grown in 5/5 unsorted samples from patients with SB. The first neurospheres formed after 14 days after seeding (range 8 – 21 days post seeding) on average. At immunofluorescence analysis, the SB-AF neurospheres exhibited neural stem cells marker expression, such as Sox2, Nestin, and Tuj1. RT-PCR and RNA sequencing characterization confirmed their spinal cord origin.

Conclusions: This is the first study reporting the successful *in vitro* derivation of human neurospheres from the AF of patients with SB. These preliminary findings underscore the potential of AF-derived neurospheres as a valuable model for studying the pathophysiology of SB at a cellular level. Moreover, this organoid model holds promise for future regenerative medicine applications.

BEHAVIOR OF SACRAL NEURAL CREST CELLS IN AGANGLIONIC MOUSE GUT: ARE THEY UNABLE TO MIGRATE OR SURVIVE?

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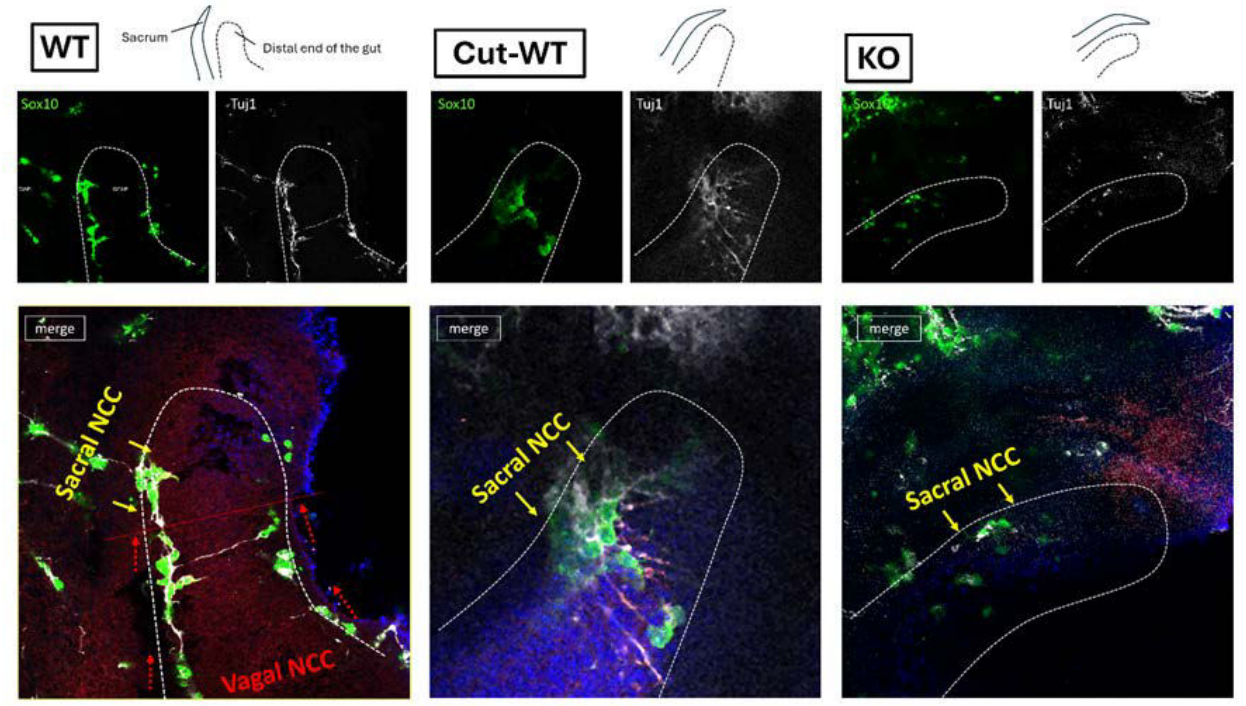
PURPOSE: Sacral neural crest cells (NCC) contribute to the development of the enteric nervous system, however, their behavior is not well understood. The aim of this study was to compare the behavior and differentiation of sacral NCC in the aganglionic region of a surgical model versus endothelin receptor B (*Ednrb*) knockout (KO) mice.
METHODS: Fetuses of *Sox10*-Venus (+) *Ednrb* KO or *Ednrb* wild type (WT) mice were harvested on embryonic day 10.5. To obtain aganglionic hindgut without the influence of caudally migrating vagal NCCs, the gut of WT mice was transected distal to the wavefront of the vagal NCC (cut-WT). The hindgut along with sacrum were cultured for 72 hours and the differentiation of sacral NCC was compared between KO, cut-WT and WT specimens (n =3 each) using wholemount immunohistochemistry with the neuronal marker, Tuj-1, and glial marker, GFAP.
RESULTS: In all groups, Sox10-positive sacral NCCs migrated into the hindgut and Tuj1 positive cells were present, indicating that sacral NCC had differentiated into neurons (figure). Sacral NCC migration was most advanced and ganglia were evident in cut-WT. Although Tuj1 positive cells were present, ganglia were not seen in the KO gut.
CONCLUSIONS: The finding that sacral NCC succeeded in migrating and forming ganglia in cut-WT may offer the potential for the effective use of sacral NCCs. Considering the fact that ganglion cells are absent in aganglionic gut in this model, the presence of Tuj1 positive cells in the KO gut indicates that sacral NCC can migrate and differentiate into neuron at this early developmental stage, but fail to mature or form ganglia during development.



IMPACT OF EPITHELIAL CLAUDIN-4 AND LEUKOTRIENE B4 RECEPTOR 2 IN NORMOGANGLIONIC HIRSCHSPRUNG DISEASE COLON ON POST PULL-THROUGH ENTEROCOLITIS

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(Tokyo, Japan)

PURPOSE: Leukotriene B4 receptor 2 (BLT-2), an upstream regulator of tight junction protein (TJP) Claudin-4, and TJPs were investigated as etiologic factors in Hirschsprung disease (HD) patients developing Hirschsprung-associated enterocolitis (HAEC) after pull-through (PT).
METHODS: HD was rectal/rectosigmoid (R/RS) or descending/transverse (D/T). Technically inadequate or transitional zone PT were excluded. Normoganglionic colon (HD-N) and aganglionic rectum (HD-A) specimens obtained intraoperatively comprised the prospective cohort (n=17; R/RS=15 and D/T=2; 2021-2024); specimens from preserved tissue comprised the retrospective cohort (n=29; R/RS=20 and D/T=9; 2009-2021); controls were anorectal malformation patients having colostomy closure (n=42). Prospective cases had immunoblotting for Claudin-4 and quantitative polymerase chain reaction (qPCR) for Occludin, TJP-1/2, Junctional adhesion molecule (JAM)-1/2, Claudin-1/3/4, and BLT-2. All specimens showing an association between Claudin-4 and HAEC had immunohistochemistry for Claudin-4 quantified by ImageJ (Fig. A).
RESULTS: Mean ages at PT/colostomy closure (years) were R/RS: 2.7±2.9, D/T: 1.6±2.2, and controls: 1.4±0.7. There were 18 episodes of post-PT HAEC in 14 cases (grade I=5, grade II=13). Post-PT HAEC was significantly more frequent in D/T (50.0 versus 6.4%; $p<0.001$); Claudin-4 was significantly lower in HD-N from post-PT HAEC cases, especially D/T ($p<0.05$) (Fig. B). TJP-1/2, JAM-1/2, Claudin-4, and BLT-2 were significantly lower in HD-N/HD-A compared with controls on qPCR (Figs. C-D). Claudin-4 was significantly lower in HD-N/HD-A compared with controls on immunoblotting ($p<0.05$) and immunohistochemistry ($p<0.001$) (Fig. E).
CONCLUSION: Lower Claudin-4 and BLT2 in post-PT HAEC HD-N (especially D/T) suggests epithelial barrier derangement with possible etiologic implications for HAEC.

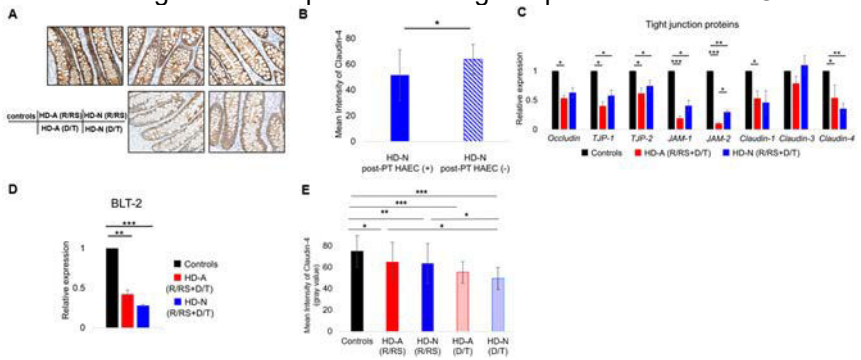

FIGURE LEGENDS
A: Representative images of Claudin-4 in controls, HD-A (R/RS) and (D/T), HD-N (R/RS) and (D/T). B: Significantly lower Claudin-4 in HD-N from post-PT-HAEC(+) (p<.05). C: Significantly lower expression of Occludin, TJP-1/2, JAM-1/2, and Claudin-1/4 in HD-A and HD-N than controls on qPCR (*p<.05, **p<.01, and ***p<.001). D: Significantly lower expression of BLT-2 in HD-A and HD-N than controls on qPCR (**p<.01, ***p<.001). D: Significantly lower Claudin-4 in HD-A (R/RS) and (D/T), HD-N (R/RS) and (D/T) than controls and significantly lower in HD-N (D/T) than HD-A (R/RS) and HD-N (R/RS) (*p<.05, **p<.01, and ***p<.001).

ABDOMINAL ULTRASOUND FOR THE DIAGNOSIS OF NECROTIZING ENTEROCOLITIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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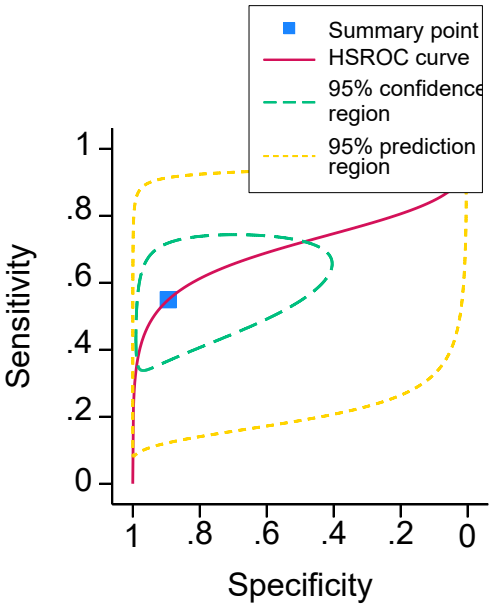
(Toronto, Canada)

PURPOSE: Necrotizing enterocolitis (NEC) is a severe gastrointestinal emergency in neonates, requiring prompt and accurate diagnosis for effective management. Abdominal ultrasound (AUS) has emerged as a more sensitive and comprehensive modality compared to traditional abdominal radiography (AXR). This systematic review and meta-analysis aimed to evaluate the diagnostic accuracy of AUS features for NEC.
METHODS: A systematic review was conducted including studies published in English full text between 1999-2024. Eligible studies were randomized controlled trials and prospective and retrospective cohort studies (cohort ≥ 25) involving preterm and/or term infants with suspected or confirmed NEC who underwent AUS evaluation. Diagnostic accuracy of AUS features was assessed using sensitivity and specificity. The meta-analysis utilized a bivariate random-effects model to generate hierarchical summary receiver operating characteristic curves (HSROC) with 95% confidence intervals (CI). Thresholds of 70% and 80% were considered for sensitivity and specificity respectively.
RESULTS: A total of 3105 studies were identified, of which 10 met the inclusion criteria, encompassing 1071 patients evaluated with AUS. The pooled sensitivity and specificity of AUS features are listed in **Table 1**. The combined diagnostic performance of moderately effective indicators of NEC including pneumatosis, focal fluid collection and diminished/absent peristalsis achieved a pooled sensitivity of 0.55 (95% CI: 0.41-0.68) and specificity of 0.89 (95% CI: 0.63-0.98) (**Figure 1**).
CONCLUSION: No single AUS feature achieved both high sensitivity and specificity thresholds. Highly specific indicators (specificity ≥ 90%) included bowel wall thinning, pneumatosis, anechoic free fluid, focal fluid collection, pneumoperitoneum, and portal venous gas. Moderately effective indicators of NEC (sensitivity ≥ 50% and specificity ≥ 80%) included pneumatosis, focal fluid collection and diminished/absent peristalsis. This study suggests that a combination of AUS features may enhance the reliability and accuracy of NEC diagnosis.

Table 1

AUS Feature	No. of studies	Sensitivity (95% CI)	Specificity (95% CI)
Bowel wall thickening	8	0.55 (0.22-0.85)	0.75 (0.59-0.86)
Bowel wall thinning	6	0.29 (0.11-0.58)	0.99 (0.82-0.99)
Pneumatosis	9	0.52 (0.33-0.70)	0.98 (0.76-0.99)
Intestinal Dilatation	5	0.54 (0.37-0.71)	0.61 (0.37-0.81)
Diminished/absent peristalsis	6	0.63 (0.45-0.77)	0.86 (0.48-0.98)
Anechoic free fluid	6	0.35 (0.27-0.44)	0.92 (0.51-0.99)
Focal fluid collection	5	0.55 (0.24-0.82)	0.99 (0.39-0.99)
Pneumoperitoneum	6	0.22 (0.13-0.34)	0.97 (0.89-0.99)
Portal venous gas (PVG)	10	0.32 (0.19-0.47)	0.97 (0.92-0.99)

Figure 1



PROTEOMIC SIGNATURE OF CONGENITAL DIAPHRAGMATIC HERNIA FROM HUMAN LUNG TISSUE SAMPLES

M. Jank, A.O. Aptekmann, D. Patel, M. Kraljevic, R. Balshaw, R. Zahedi, M. Boettcher, R. LeDuc, R. Keijzer
(Winnipeg, Canada)

PURPOSE: Congenital diaphragmatic hernia (CDH) is characterized by a diaphragmatic defect, cardiac hypoplasia, and abnormal lung development, the latter largely determining morbidity and mortality in patients. Despite extensive research, the underlying pathogenesis remains unknown. We compared the proteomic profile of lung tissue sections from human CDH and control infants.
METHODS: Formalin-fixed paraffin-embedded lung tissue samples from four deceased CDH infants were matched to five controls. Samples were analyzed by the data independent acquisition (DIA) method on a timsTOF Pro2. Proteins were identified by tandem mass spectroscopy. We implemented a hierarchical linear model to compare the protein expression profile of CDH and control lungs defining $p<0.05$ and log fold change >1.5 as statistically significant. Pathway and gene ontology enrichment analyses were performed with Panther, EnrichR and custom R-Software.
RESULTS: We included lung tissue from four CDH patients and five controls. After performing principal component analysis, one CDH sample was identified as an outlier and subsequently excluded from further analysis. Proteomics detected 4,545 unique proteins, 317 upregulated and 177 downregulated in CDH compared to control. Gene set enrichment analysis (GSEA) demonstrated activation of clusters associated with mRNA processing, splicing and surveillance pathway. According to Gene Ontology (GO) enrichment analysis, down-regulated proteins may be involved in mitochondrial electron transport and integrin cell surface interaction. Further analysis of these proteins using KEGG pathway revealed enrichment for retrograde endocannabinoid signaling.
CONCLUSIONS: Proteomic profiling of hypoplastic lungs in CDH suggests the involvement of pathways related to mRNA splicing and mitochondrial electron transport. These findings reveal new approaches in the pathogenesis of CDH and novel therapeutic targets.

ANGIOPOIETIN1 AMELIORATES LIPOPOLYSACCHARIDE-INDUCED ENDOTOXEMIA BY IMPROVING INTESTINAL VASCULAR PERMEABILITY IN A HIRSCHSPRUNG'S DISEASE MURINE MODEL

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(Tokyo, Japan)

PURPOSE: We previously reported that vascular permeability (VP) increased abnormally via upregulated vascular endothelial growth factors A/B (VEGFA/VEGFB), potentially predisposing normoganglionic ileum in an endothelin receptor-B null Hirschsprung's disease mouse model (KO) to a pro-inflammatory state (Suda et al., Pediatr Surg Int, 2022). Angiopoietine1 (Angpt1) is a proangiogenic growth factor known for improving VP and vascular remodeling in ischemic vasculature. Here, we investigated the anti-inflammatory effects of Angpt1 on lipopolysaccharide (LPS)-induced intestinal inflammation in KO.

METHODS: Endotoxemia was induced in KO (n=9) and wild-type (WT) mice (n=6) within 24 hours of birth by administering 5mg/kg of LPS intraperitoneally. Normoganglionic ileum was harvested from each mouse 6 hours later. VEGFA/VEGFB expression and CDH5, a gene associated with vascular endothelial adhesion, were analyzed by quantitative polymerase chain reaction (qPCR). VP was assessed using the Miles assay whereby Evans blue was injected intravenously after administering LPS and processed to quantify extravasated Evans blue. In 6 mice (KO: n=3 and WT: n=3), Angpt1 was injected intraperitoneally 2 hours before LPS to evaluate its vascular protective action using qPCR.

RESULTS: With endotoxemia, VEGFA/VEGFB were significantly upregulated (p=0.0162, 0.047, respectively), and CDH5 was slightly downregulated (p=0.42) (Figure A). VP was significantly increased in KO compared with WT (p<0.0001) (Figure B). LPS-induced inflammation was suppressed by Angpt1 with significantly downregulated VEGFA/VEGFB (p=0.01, 0.007, respectively), while CDH5 was moderately upregulated (p=0.322) in KO compared with non-Angpt1 (Figure C).

CONCLUSION: Angpt1 attenuated inflammation by improving VP possibly through tightening vascular endothelial cell junctions.

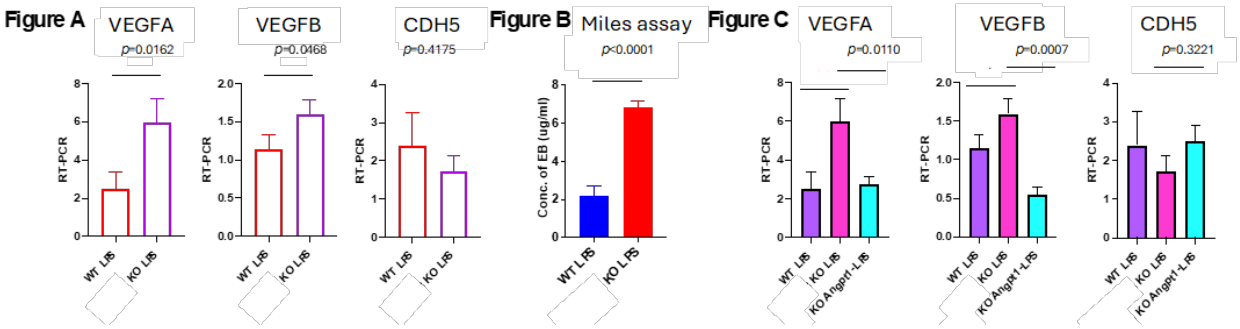

FIGURE LEGENDS: A: VEGFA/VEGFB and CDH5 after LPS; KO vs. WT (qPCR). Note significantly increased VEGFA/VEGFB and slightly reduced CDH5.
B: Extravasated Evans blue (Miles assay). Note significant increase in KO vs. WT.
C: Effect of Angiopoietin1 (qPCR). Note significantly decreased VEGFA/VEGFB and CDH5 upregulation.

DEVELOPMENT OF INDOCYANINE GREEN-LOADED NANOCAGES FOR IDENTIFICATION OF TUMOR MARGINS IN PEDIATRIC FLUORESCENCE-GUIDED SURGERY

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PURPOSE: Fluorescence-guided surgery (FGS) is rapidly advancing in pediatrics, equipping surgeons with powerful tools to enhance the visualization of normal and pathological structures. Detailed visualization of small anatomical structures in pediatric oncological surgery can reduce positive tumor margins, decrease procedure time, minimize surgical complications, and, ultimately, improve outcomes. This study aims to develop an Indocyanine green (ICG)-loaded nanocage as a nanotracer suitable for effective pediatric tumor margin identification during surgery, as previously demonstrated for breast cancer.
METHODS: We nanoformulated Indocyanine green (ICG) into a natural nanocage constituted by 24 human heavy-ferritin monomers, that self-assembled into a cave-sphere quaternary structure (HFn-ICG). Heavy ferritin (HFn) physiologically specifically recognizes the transferrin receptor 1 (TfR1), which is overexpressed in most tumors. This nanocage displays a natural tumor tropism since the HFn-TfR1 interaction triggers HFn internalization. To assess the suitability of HFn, as an ICG delivery vector in pediatric cancers, we assessed TfR1 expression in neuroblastoma (i.e. LA-N-1, Kelly, SK-N-BE(2)-C, and patient-derived mesenchymal stem cell) and rhabdomyosarcoma cells (i.e. SJCRH30 cells). We evaluated the binding affinity of fluorescently labeled HFn to tumor cells at two concentrations and different incubation times. The specificity of binding was confirmed through competition studies. We also investigated the intracellular localization of fluorescent signals at different time points.
RESULTS: Neuroblastoma and rhabdomyosarcoma cells exhibited high levels of TfR1 expression. HFn nanocages demonstrated strong binding affinity to these tumor cells, with higher fluorescence intensity at increased concentrations and longer incubation times. Competition studies confirmed the specificity of HFn binding to TfR1. Fluorescent signals were predominantly localized within the lysosomes and endosomes, indicating effective receptor-mediated internalization and intracellular trafficking of HFn. Quantitative analysis revealed a significant enhancement in fluorescence signal, which is a promising result for improved tumor delineation during surgery.
CONCLUSION: Thanks to the high specificity of HFn for TfR1 and the utility of ICG for FGS applications, this study highlights that HFn-ICG can also enhance the visualization of pediatric solid tumors during surgery, as has been already demonstrated in a murine model of breast cancer. *In vivo* studies are necessary to validate the enhanced fluorescence signal and specific tumor targeting observed with HFn-ICG nanocages, assessing their potential to improve surgical outcomes and treatment efficacy in pediatric surgical oncology.

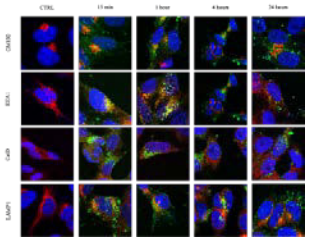

Figure. Confocal microscopy images obtained following the incubation of the SK-N-BE(2)-C neuroblastoma (NB) cells with H-Ferritin nanocages labeled with FITC at a concentration of 100 µg/ml for 15 minutes, 1 hour, 4 hours, 24 hours at 37°C. Cells were also stained with anti-GM130, anti-EEA1, anti-CatD, anti-LAMP1 antibodies.

EXTRINSIC FETAL PULMONARY COMPRESSION IN A SURGICAL CONGENITAL DIAPHRAGMATIC HERNIA RAT MODEL IS ASSOCIATED WITH THE OVEREXPRESSION OF PROTEINS RELATED TO THE APOPTOSIS AND SENESCENCE PATHWAYS

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PURPOSE: We conducted a study to evaluate the effect of pulmonary compression during the fetal lung development using a Congenital Diaphragmatic Hernia (CDH) surgical animal rat model. Our previous studies have shown that the compression of the fetal lung parenchyma causes local hypoperfusion, chronic hypoxia, and cellular energy failure and therefore may activate apoptosis and cellular senescence mechanisms. This study aims to study the most relevant proteins of the fetal lung's apoptosis and cellular senescence pathways in CDH fetuses.

METHODS: The study involved 22 rat fetuses, including 10 with small or moderate diaphragmatic defect (types A and B), 4 with large diaphragmatic defects (types C and D), 4 sham fetuses (fetuses that underwent intrauterine surgery but did not generate the diaphragmatic defect), and 4 control fetuses (fetuses without intrauterine surgery). In all groups, Western blot and Immunofluorescence were used to assess the expression of crucial proteins related to the intrinsic apoptosis pathway (p53 and Cleaved-Caspase 3) and those of the cellular senescence-related pathway (p16, p21 and non-phosphorylated Rb2) in fetal lung parenchyma.

RESULTS: The expression of apoptosis-related proteins (p53 and Caspase 3) (Fig.1), as well as senescence-related proteins (p16, p21, and active Rb2) (Fig 2), are increased in the subgroup of fetuses with large diaphragmatic defects compared to the control and Sham groups ($p<0.05$ in all groups). On the other hand, in fetuses with small and moderate diaphragmatic defects, there is a trend but no significant increase in the expression of the evaluated proteins compared to their controls or Sham. Interestingly, by immunofluorescence, this increased expression of proteins related to apoptosis and senescence is found following a geographic pattern, in the peripheral regions of the fetal lung parenchyma, where the compressive effect is greater compared to other lung areas. This finding was observed predominantly in fetuses with large diaphragmatic defects.

CONCLUSION: This work demonstrates that extrinsic compression of lung parenchyma by herniated abdominal organs in CDH promotes overexpression of the intrinsic apoptosis and senescence-related pathways in fetal lung parenchyma that may partially explain the pulmonary hypoplasia found in the context of this congenital disease.

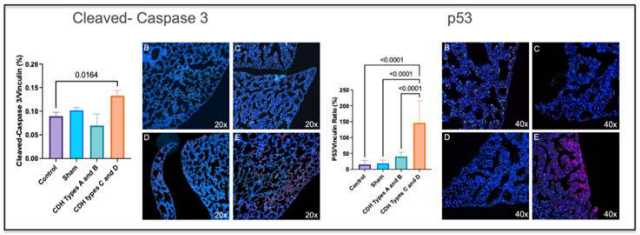

Fig. 1 Western blot analysis of the protein expression of each of the proteins related to the **intrinsic apoptosis pathway**. On immunofluorescence, fetuses with CDH show the highest proportion of p16, Rb2, and p21 protein expression in the peripheral regions of the lung. **(B)** Control, **(C)** Sham, **(D)** CDH types A or B, and **(E)** CDH types C or D. P21 (red), DAPI (blue), red blood cells (green) (20x) (n: 3-5). The differences among multiple groups were analyzed by one-way analysis of variances (ANOVA) using Tukey's post hoc test.

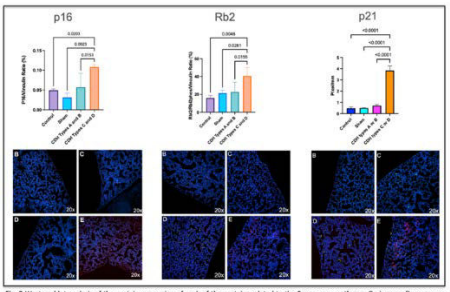

Fig. 2 Western blot analysis of the protein expression of each of the proteins related to the **Senescence pathway**. On immunofluorescence, fetuses with CDH show the highest proportion of p16, Rb2, and p21 protein expression in the peripheral regions of the lung. **(B)** Control, **(C)** Sham, **(D)** CDH types A or B, and **(E)** CDH types C or D. P21 (red), DAPI (blue), red blood cells (green) (20x) (n: 3-5). The differences among multiple groups were analyzed by one-way analysis of variances (ANOVA) using Tukey's post hoc test.

CANCELLED

PERITONEAL DRAINAGE VERSUS LAPAROTOMY AS PRIMARY SURGICAL INTERVENTION FOR SMALL INTESTINAL PERFORATION IN EXTREMELY LOW BIRTH WEIGHT INFANTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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PURPOSE: To investigate the primary reasons behind small intestinal perforations in extremely low birth weight infants (ELBW; <1000g) and to compare outcomes following peritoneal drainage (PPD) and laparotomy (LAP) as initial surgical interventions.
METHODS: A systematic review adhering to PRSIMA guidelines was conducted, encompassing a comprehensive search of PubMed, Embase, Web of Science, and Cochrane Library databases up until March 2022. Studies were categorized into PPD and LAP groups based on their primary surgical intervention. The primary outcome was mortality rate, and secondary outcomes included mortality rate on postoperative days 30, 90 and 180, reoperation rate, time to full enteral feeding, duration of hospital stay, and incidence of postoperative complications. Subgroup analysis was performed based on the etiology of perforation. Additionally, sensitivity analysis was employed for results with high heterogeneity. The quality assessment of randomized controlled trial (RCT) and non-RCT (nRCT) studies were performed using the Cochrane bias risk assessment tool and ROBINS-I respectively.
RESULTS: Three RCTs (n=466) and 10 nRCT (n=525) studies were included. 539 infants underwent PPD and 452 infants underwent LAP. Necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP) were diagnosed in 437 and 371 infants respectively. Mortality rates were compared between PPD and LAP groups for both NEC-infected infants and those with SIP; however, only SIP infants showed a statistically significant lower mortality rate following LAP [(RR=1.10; 95%CI 1.03, 5.00; P=0.04)]. The reoperation rate was significantly higher in infants managed with PPD compared to LAP [RCTs (RR=2.07; 95%CI 1.49, 2.87; P＜0.01); nRCTs (RR=10.34; 95%CI 3.69,29.00; P＜0.01)]. The overall risk of bias was "low" for RCTs and "moderate to severe" for nRCT studies.
CONCLUSION: NEC and SIP are key causes of small intestinal perforation in ELBW infants. Both PPD and LAP result in similar survival outcomes among ELBW infants. Yet, PPD might lead to higher reoperation rates in ELBW infants and potentially increase mortality in SIP infants based on nRCTs. Therefore, considering risk of complications and the specific condition of each infant, LAP is recommended as the primary surgical intervention for small bowel perforation in ELBW, unless there are absolute contraindications against surgery.

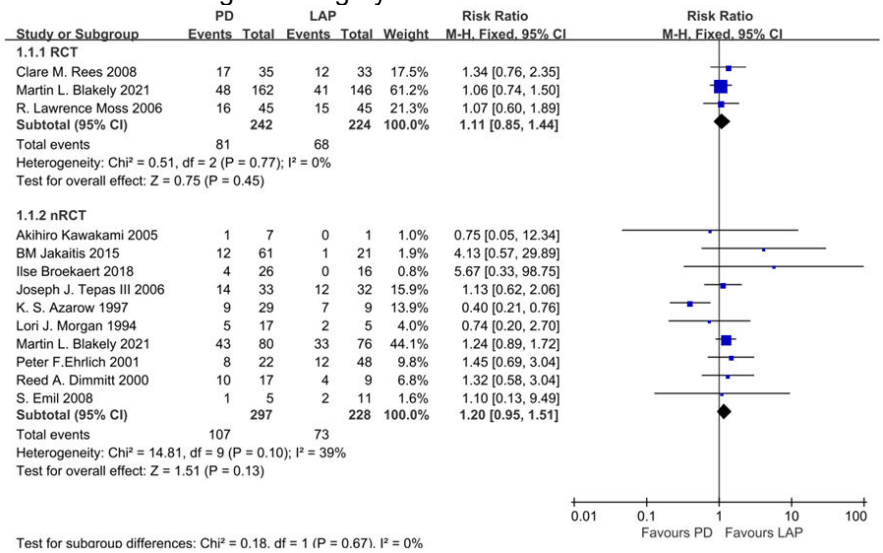

Figure Comparison of primary outcome (mortality rate) between PPD and LAP groups.

QUEST FOR ENDOGENOUS RENAL STEM CELLS AS A TARGET FOR RENAL PRESERVATION IN OBSTRUCTIVE RENOPATHY

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PURPOSE: Endogenous renal stem cells (RSC) may exist; with a plausible role in renal preservation in obstructive renopathy (OR). Experimental models of unilateral and bilateral OR were established complemented with a search for renal stem cells.
METHODS: Following Animal Ethics Committee approval, 45 mice weighing 40-50 grams (6-8 weeks) were divided into 5 groups (Group A -E) with 9 mice each. OR models were established by ligating the left ureter at upper (Group A), middle (Group B), lower (Group C) ureter, urethra around a sling for partial obstruction (Group D) and both lower ureters (Group E) with nonabsorbable suture. Mice were euthanized on days 3,7,10. Both kidneys were harvested. Histopathological examination (Haematoxylin and Eosin) was done on one longitudinal split half kidney. The other half was microscopically dissected into cortex, medulla and papilla with two curvilinear incisions parallel to corticomedullary differentiation plane. Staining with Ki67, CD150, CD117, CD34 and CD133 was followed by fluorescence-activated cell sorting (FACS) for Groups A-C. Findings of obstructed kidneys were compared to contralateral kidneys without obstruction (Control).
RESULTS: Group A-C models showed renal changes. Group E model survived only 3 days and was excluded. OR triggered decreased glomeruli number, delayed tubular maturation, decreased vascular tuft area, dilatation of Bowman's space, tubulo-glomerular disconnection, tubular necrosis, tubular basement membrane thickening, tubular apoptosis, tubular atrophy, interstitial inflammation, interstitial fibrosis and decreased peritubular capillary density. In Group A, haematopoitic (CD34) and RSC (CD117, CD133) were higher in contralateral side being maximum in medulla. The brunt of kidney injury (Ki 67) was maximum in papilla on ligated side compared to contralateral side. In Group B, ligated side medulla had Ki67 positive cells while CD133 positive cells were more in contralateral kidney. In Group C, CD133 cells were increased in cortex and papilla of ligated side while CD150 was higher on contralateral side. Bilateral lower ureteral obstruction was incompatible with life. Partial urethral obstruction was a better model for bilateral OR.
CONCLUSION: RSC were identified in OR. Increased activity of RSC in contralateral kidney in unilateral OR can explain the contralateral renal hypertrophy with non-functioning or obstructive kidney.

INTESTINAL ORGANOIDS: NEW INSIGHTS INTO HIRSCHSPRUNG'S DISEASE

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PURPOSE: Hirschsprung's Disease (HD) is a congenital intestinal disorder characterized by a variable length of distal colonic aganglionosis, leading to impaired intestinal motility. It is caused by aberrant processes of the enteric neural crest-derived cells that could include proliferation, migration, differentiation, and survival. In this study, we investigate the development and characteristics of patient derived intestinal organoids from different regions of the HD colon. We set up a workflow which allows us to investigate the mucosal cell organoid growth *in vitro* and its characterization in order to better understand the pathology of Hirschsprung Disease.
METHODS: Mucosal stem cells were isolated from HD patient tissue. Culturing the cells in Matrigel and organoid growth medium led to formation of organoids. The organoids were grown for multiple passages and frozen down for further experiments. Microscopic observations were carried out to compare the growth and differentiation rate of cells from aganglionic and ganglionic parts of patients' colon tissue.
RESULTS: Organoid growth was observed in all parts of the resected colon. However, a marked difference in growth of organoids from different segments of HD colon was detected. In the aganglionic parts a lower proliferation rate of mucosal stem cells was observed compared to the segments with histologically proven ganglionic cells. Dissimilar organoid differentiation was also observed in the different parts of the resected colon. In the aganglionic and transition zones we saw a higher number of differentiated organoids, growing budding-like structures, whereas in the ganglionic zone the organoids were growing much more fetal-like, cystic and fast proliferating spheres.
CONCLUSION: Our results demonstrate an evident difference in the growth and differentiation rate of intestinal organoids from ganglionic and aganglionic parts of the colon. This suggests that the absence of the enteric nervous system may lead to a decreased self-renewal capacity of the colonic mucosa of the affected colon segments. We can now further characterize the HD organoids, as well as prepare co-cultures with other cell types isolated from patient colon tissue. Intestinal organoids generated from Hirschsprung's diseased colon provide an excellent patient-derived models for this disease and could be used to better recapitulate and individualize therapeutic approaches for this challenging congenital disease.

A NEW IN-VITRO MODEL OF ALVEOLAR EPITHELIAL-ENDOTHELIAL CO-CULTURE

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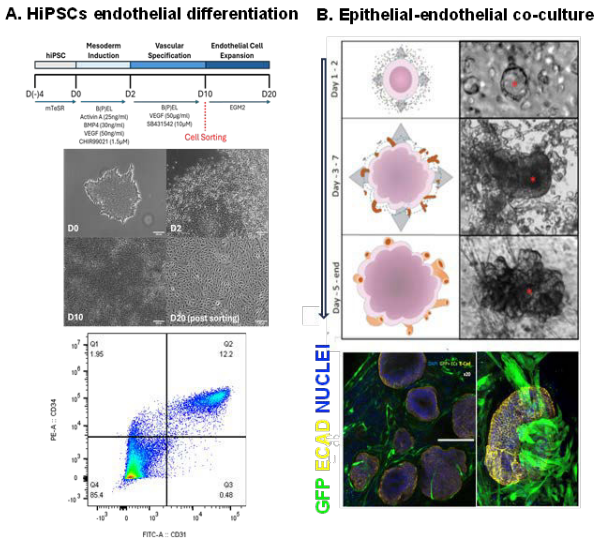
PURPOSE: The alveolus is the functional unit of the lung. It consists of flattened AT1 cells, responsible for the gas exchange, and cuboidal AT2 cells, which produce surfactants. Alongside AT1 and AT2 cells, endothelial cells (ECs) of the capillaries help form the air-blood barrier which enables gas exchange.
Disruption of alveolar development can lead to lifelong lung disease in newborns and children. In vitro models that recapitulate the endothelial-epithelial crosstalk are currently missing. The aim of this study is to develop a novel in-vitro model of alveolar development from amniotic fluid stem cells to better understand the pathogenesis of congenital lung diseases.
METHODS: We optimized an established differentiation protocol to derive ECs from an amniotic fluid stem cell-derived hiPSC line. Human alveolospheres (hAO) were generated from reporter hiPSCs (NKX2.1-GFP/SFTPC-TdTomato) according to established protocols.
As proof of concept for lung organoid and EC co-culture, we used HUVEC cells overexpressing ETV2 (RVECs), carrying an EGFP reporter to test media formulation that can support the co-culture and enable monitoring tubulogenesis.
Results of the differentiation and the co-culture were monitored through brightfield images, immunostainings, flow-cytometry, and qPCR.
RESULTS: HiPSC-ECs differentiation resulted in clear morphological changes with visible transition away from hiPSC colony phenotype. Flow cytometry of hiPSC-ECs at day 11 revealed variable differentiation efficiency ranging from 1% to 12% CD31/CD34 double-positive cells (Figure A). Supplementation of bFGF and Heparin to RVECs culture in lung epithelial expansion medium resulted in an increase of the area covered by cells-forming tubules. The co-culture of hAOs and RVECs showed a progressive integration of endothelial cells with 3D lung organoids as resulted by immunostaining analysis (Figure B) as well as in the upregulation of AQP5 (AT1 marker) and SFTPC (AT2 marker) gene expression.
CONCLUSION: We developed an efficient protocol for differentiating amniotic fluid stem cells-derived hiPSCs into hiPSC-ECs.
We also developed a proof-of-concept experiment for co-culturing endothelial and lung epithelial organoids in 3D. Our in-vitro model of distal lung development will be useful to model and better understand the pathogenesis of newborn children lung diseases in vitro.



A NEW INNOVATIVE DEVICE FOR SAFE AND SUCCESSFUL NASOGASTRIC TUBE INSERTION WITH A BIOLOGICALLY TRANSPARENT ILLUMINATION IN PEDIATRIC PATIENTS

R. Satake, H. Nakamura, H. Yamakawa, N. Aoki, R. Tanaka, S. Yoshimoto, T. Okunobou, T. Doi
(Osaka, Japan)

PURPOSE: Misplacement of a nasogastric (NG) tube in the respiratory tract or esophagus can result in severe morbidity and mortality. Although, X-ray examination is recognized as the gold standard for confirming the correct position of the NG tube insertion, this method also has disadvantages including radiation exposure in children. A new innovative device, biologically transparent light (BT light), is highly bio-permeable red light from a light-emitting diode. BT light emitted in the abdominal digestive tract can be visualized from outside the body. Identification of BT light emitted from the tip of an NG tube confirms the tip position. The aim of this study was to evaluate the usefulness of BT light device to place the tip of the NG tube in the epigastric area successfully in children.
METHODS: This prospective observational study included 106 patients aged 0-16 years who required the NG tube insertion because of bag-mask ventilation after induction of general anesthesia for general surgery. After the BT light catheter was set into the NG tube, the NG tube was gently inserted through the nasal or oral cavity and proceeded by confirming whether the BT light was transmitted in the cervical, thoracic, and epigastric regions. The position of the NG tube was confirmed by X-ray examination. This study was approved by the ethics committee of our institution.
RESULTS: The mean age was 3.8 years and male to female ratio was 72:34. Of 106 patients, BT light was identified in the epigastric area in 105. However, BT light was not clearly observed in one patient who was 9 years old boy. X-ray examination confirmed the presence of the NG tube in the stomach in all patients. Therefore, sensitivity of the BT light was 99%. The mean insertion time was 38 seconds, and the mean abdominal thickness of the patients was 9.8mm.
CONCLUSION: A new innovative BT light device proved to be a safe and highly effective technique with 100% placement of the tip of NG tubes under the condition of BT light detection in the epigastric area, reducing the risk of radiation exposure in children.

YES-ASSOCIATED PROTEIN IS DYSREGULATED IN HUMAN CONGENITAL DIAPHRAGMATIC HERNIA PATIENTS DURING MID AND END GESTATION

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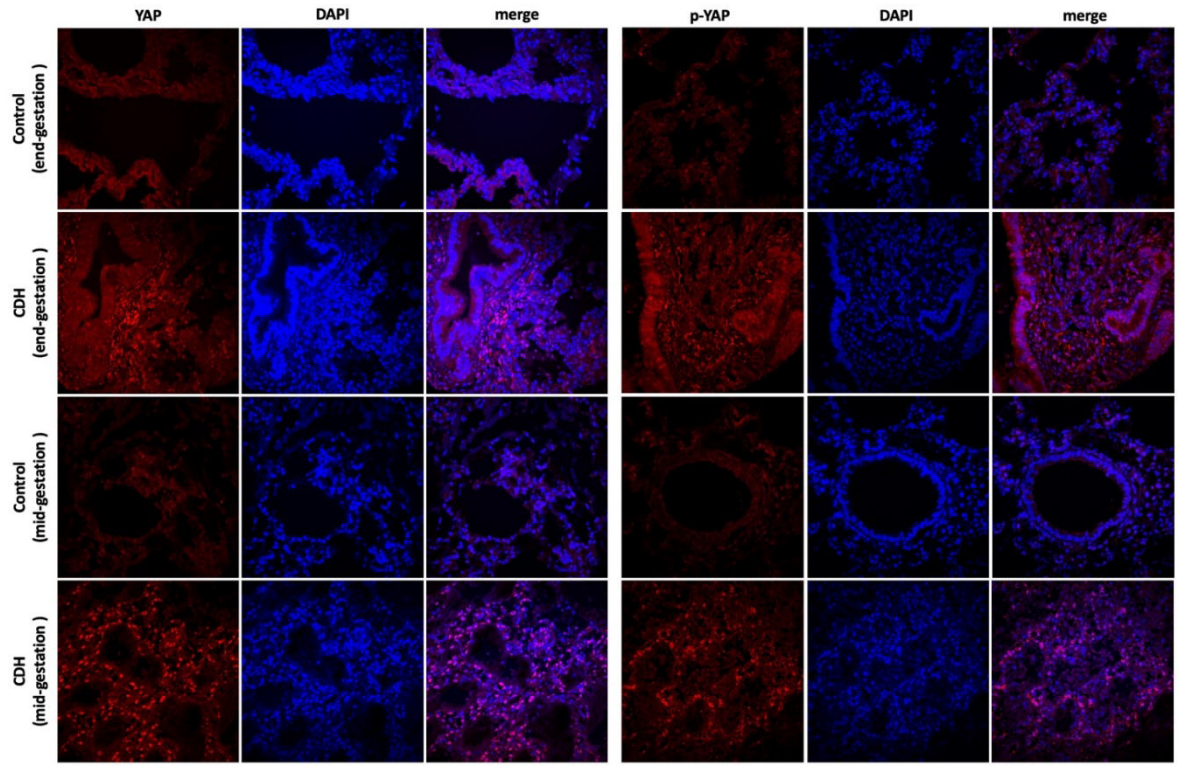
PURPOSE: Yes-associated protein (YAP) is implicated in congenital diaphragmatic hernia (CDH). Although previous studies have demonstrated the role of YAP in animal models, its abundance and function in human tissues remain unclear. This study aims to investigate the abundance of YAP and its inactive form, phosphorylated YAP (p-YAP), in fetal lung tissues from CDH cases compared to control cases at mid-gestation and end-gestation.
METHODS: Immunofluorescence was performed to assess the abundance of YAP and p-YAP in lung tissues from human fetuses with CDH and control fetuses who died from causes other than CDH. Comparisons were made between mid-gestation and end-gestation. Additionally, the impact on cellular differentiation was evaluated by assessing the expression of Homeodomain-only protein X (HOPX) as an alveolar type I cell marker and Surfactant Protein C (SPC) as an alveolar type II cell marker.
RESULTS: Immunostaining revealed higher abundance of YAP and p-YAP in both mid-gestation and end-gestation lung tissues. Notably, the abundance of p-YAP was significantly increased in CDH tissues compared to controls, indicating higher levels of the inactive form of YAP in CDH. HOPX and SPC staining showed CDH tissues had a higher number of SPC-positive cells and fewer HOPX-positive cells at end-gestation.
CONCLUSION: Our findings suggest that in CDH, YAP is predominantly present in its inactive form, p-YAP, potentially disrupting normal lung development and differentiation. This underscores the potential involvement of YAP dysregulation in the pathogenesis of CDH and highlights the importance of further research into therapeutic strategies targeting YAP signaling in CDH



THE EFFECT OF DNA APTAMER RAISED AGAINST RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE) IMPROVES LUNG INJURY IN SEPTIC MICE

Y. Koga, N. Hasizume, M. Daisuke, H. Yoshida, T. Aiko, T. Kurahachi, H. Nakahara, N. Higashidate, A. Sotokawauchi, T. Kaji
(Kurume, Japan)

OBJECTIVE: Acute respiratory distress syndrome (ARDS) with sepsis is a leading cause of neonatal mortality, but no effective therapy has yet been established to improve the survival rates. High mobility group box 1 (HMGB-1) is known to be involved in both the inflammatory reactions and organ damage in sepsis through the interaction with the receptor for advanced glycation end-products (RAGE). We recently discovered that DNA aptamers targeting RAGE (RAGE-aptamer) can inhibit sepsis in animal models. This study aimed to examine the effects of RAGE-aptamer on ARDS in mice.
METHODS: A sepsis model was established in 8-week-old male BALB/c mice through the intraperitoneal administration of lipopolysaccharides (LPS) at a dose of 20 μg/g body weight. Five minutes after LPS administration, the mice were injected intraperitoneally with either the control aptamers or RAGE aptamers at a dose of 40 pmol/g body weight. Various parameters, the including survival rate, blood chemistry, enzyme-linked immunosorbent assay (ELISA) results, and gene expression levels, were evaluated to assess the impact of the treatments.
RESULTS: The administration of RAGE aptamer significantly improved the survival rates in the LPS-injected mice compared to the control group (Fig.1). The extent of lung damage in the RAGE-aptamer group was significantly reduced compared to that in the Control-aptamer group (Fig.2). Furthermore, the gene and cytokine expression levels of Interleukin-6 (7.9±3.4 RAGE-aptamer group vs 60.7±34.1 Control-aptamer group, $p<0.05$)(Fig.3), were significantly lower in the RAGE-aptamer group compared to the control group (4.1±3.2 RAGE-aptamer group vs 20.6±5.62Control-aptamer group, $p<0.05$)(Fig.3).
CONCLUSION: The findings of the present study suggests that RAGE-aptamer can attenuate inflammatory reactions and lung damage in septic mice, thus leading to an improved survival. The blockade of the RAGE signaling pathway by RAGE-aptamer therefore represents a promising therapeutic target for ARDS associated with sepsis. This novel approach has the potential to modulate the inflammatory response and protect against organ damage.

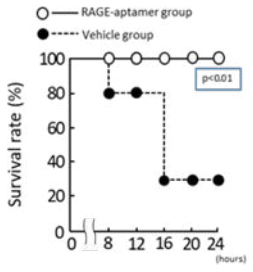


Fig.1 Survival rate

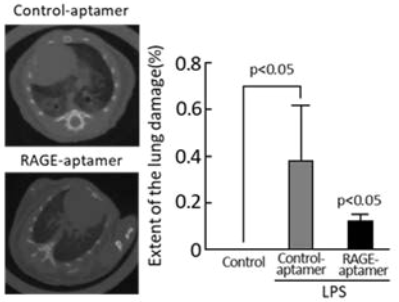


Fig.2 Extent of the lung damage

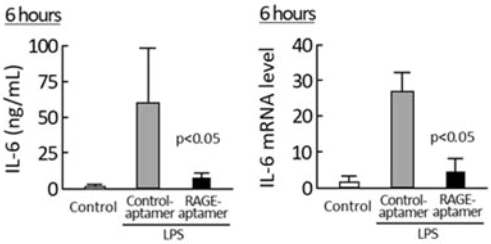


Fig.3 IL-6 ELISA and mRNA level(rt PCR)

ROLE OF REMOTE ISCHEMIC CONDITIONING IN MODULATING INFLAMMATORY AND ANGIOGENIC RESPONSES AFTER MASSIVE BOWEL RESECTION: A MOUSE MODEL STUDY

D. Ko, H. Lee, J.K. Youn, H.-Y. Kim
(Seoul, South Korea)

Purpose: Intestinal adaptation follows massive small bowel resection (MSBR) to improve nutrient absorption, yet MSBR may lead to short bowel syndrome (SBS) requiring nutritional therapy. Remote ischemic conditioning (RIC), involving ischemia-reperfusion cycles, enhances resistance to injury in distant organs. This study explores RIC's potential to enhance intestinal adaptation post-MSBR using a mouse model, focusing on clinical outcomes, histological changes, and early vs. late responses on days 3 and 7 post-MSBR.
Methods: Forty 10-week-old C57BL/6 mice were divided into control, MSBR, and MSBR-RIC groups. MSBR involved laparotomy, resection of 75% of the small intestine, and anastomosis. RIC was performed on the hind limb with three 5-minute cycles of ischemia-reperfusion. Histological assessments and mRNA/protein analyses for angiogenesis and cell proliferation were conducted on days 3 and 7 (3MSBR-RIC, 7MSBR-RIC).
Results: The 3MSBR-RIC group showed significantly less weight loss compared to MSBR alone. However, the 7MSBR-RIC group did not significantly reduce weight loss. Nonetheless, the 7MSBR-RIC group exhibited significantly higher survival rates than the 7-day MSBR group. Villi height in the ileum increased on days 3 and 7 in the MSBR-RIC group compared to MSBR alone. Additionally, 3MSBR-RIC demonstrated increased VEGF expression via immunochemical staining, validated by higher HIF and VEGF levels in qPCR, and increased PI3K/Akt pathway protein expression. Conversely, the 7MSBR-RIC group showed decreased NK-κB expression via immunochemical staining, reduced mRNA expression of TNF-α and IL-1β, and confirmed decreases in NF-κB pathway protein expression.
Conclusions: RIC following MSBR enhanced intestinal adaptation, reduced early weight loss, increased villi length, enhanced angiogenesis, and attenuated inflammation, thereby improving survival rates post-MSBR. The study underscores the roles of the PI3K/Akt and NF-κB pathways in these processes, suggesting RIC as a promising therapeutic strategy for SBS patients with implications for clinical practice.

SINGLE-STAGED IN VIVO CO-TRANSPLANTATION OF AUTOLOGOUS MUSCULAR AND MUCOSAL MICROGRAFTS IN A COMPOSITE TUBULAR URINARY SCAFFOLD

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PURPOSE: Tissue engineered cellularized collagen-based grafts for reconstructive purposes can be created perioperatively as a one-stage procedure using autologous micrografts. For urinary hollow organ reconstruction, a muscular graft layer can be desirable to resemble the architecture of the bladder wall. However, co-transplanting muscular micrografts with urothelial micrografts has previously shown to inhibit urothelial micrograft expansion. We hypothesize that this could be mitigated by transplanting the micrografts in two different compartments of a composite scaffold. The aim of this study was to examine the effects of adding muscular micrografts to a separate layer of a urinary collagen-based tubular scaffold.
METHODS: Autologous tissue from three full-grown minipig bladders was used to construct tubular urinary collagen-based grafts which were implanted subcutaneously as a single-staged procedure *in vivo*. Four tubular grafts were implanted in each animal. Two of the four grafts contained urothelial micrografts (uro-group) while the remaining two grafts contained co-transplanted urothelial and detrusor muscle micrografts (co-group). After six weeks, the excised grafts were assessed morphologically and by immunoassay for identification of epithelium (pancytokeratin) (10 grafts, 5/group), differentiated urothelium (uroplakin II), smooth muscle (α-SMA), inflammation (CD68 & TUNEL apoptosis assay), and vascularization ($CD31^+$ vessels) (12 grafts, 6/group).
RESULTS: Six weeks post-implantation, 6/10 (60%) grafts, 3 from each group, presented with luminally pancytokeratin-positive epithelium. One graft from each group was removed due to artefacts from tissue preparation. No significant difference was found between the uro-group and the co-group related to the number of uroplakin II-positive cells/mm^2 (842 ± 682 (SD) vs. 1036 ± 959 (SD), p=0.70), α-SMA-positive cells/mm^2 (3305 ± 1542 (SD) vs. 3495 ± 2160 (SD), p=0.87), vessels/mm^2 (19.2 ± 3.0 (SD) vs. 18.5 ± 7.0 (SD), p=0.83), $CD68^+$ cells/mm^2 (194 ± 136 (SD) vs. 179 ± 80 (SD), p=0.81), or rate of apoptotic cells/mm^2 (1086 ± 370 (SD) vs. 977 ± 670 (SD), p=0.78).
CONCLUSIONS: Our findings indicate that muscular micrografts can be co-transplanted in a separate layer of a collagen-based scaffold without inhibiting co-transplanted urothelial micrograft expansion. These results affirm our ambitions to construct more complex grafts that mimic native organ microanatomy for future. reconstructive purposes.

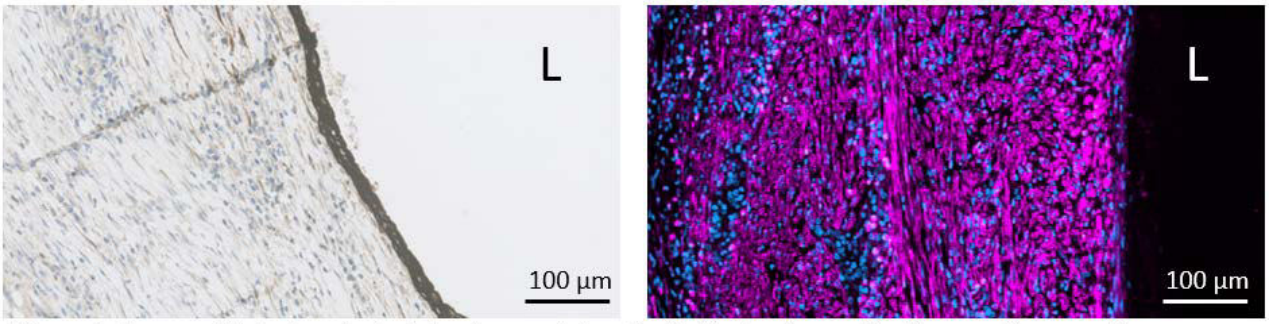

Figure 1: Immunohistochemical stain of pancytokeratin (left) showing epithelium and immunofluorescence imaging (right) of α-SMA (pink) with DAPI. Both images show a co-group graft. L indicates lumen.

ENGINEERING INTESTINAL ORGANOIDS

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PURPOSE: Tissue organoids hold great potential for a variety of applications, including disease modeling and drug testing. While they contain all the cell types of the native organ, they lack a perfusable vasculature and other supporting cells. The present study investigates the effects of endothelial cells and mesenchymal like stem cells on mouse intestinal organoids. We hypothesized that co-culturing intestinal organoids with endothelial and dental pulp stem cells enhances organoid growth.
METHODS: Following ethical approval, mouse intestinal organoids were established using ileal crypts from 9-day old pups. Organoids were either cultured (i) alone without other cell types, or co-cultured with (ii) Human Umbilical Vein Endothelial Cells (HUVEC), or (iii) Human Dental Pulp Stem Cells (DPSC), or (iv) both HUVEC and DPSC. Organoid growth was assessed daily. Immunofluorescence staining of epithelial cell marker (EpCAM) was performed.
RESULTS: Intestinal organoids were successfully formed from ileal tissues (Fig. 1A). Organoids co-cultured with HUVEC gave rise to budding organoids (Fig. 1B), while spherical organoids were observed when organoids were co-cultured with DPSC (Fig. 1C). Organoids co-cultured with both HUVEC and DPSC yielded mostly spherical organoids (Fig. 1D). The number of budding and spherical organoids were quantified. There were 70% of budding organoids when cultured alone, while 90% of organoids were budding when co-cultured with HUVEC (Fig. 2A). When organoids were co-cultured with DPSC or with both HUVEC and DPSC, 92% were spherical organoids (Fig. 2B). The diameter of the spherical organoids was largest when co-cultured with DPSC (Fig. 2C). All organoids stained positive for epithelial cell marker EpCAM indicating epithelial integrity (Fig. 3).
CONCLUSION: We demonstrated cell-cell interactions of organoids with both the endothelial and dental pulp stem cells. This study shows that endothelial cells promote organoid differentiation and villus formation, while dental pulp stem cells maintain organoids in a fetal-like state. Our co-culture system offers a novel modality of intestinal organoid engineering which is important for regenerative medicine and disease modelling.

Figure 1

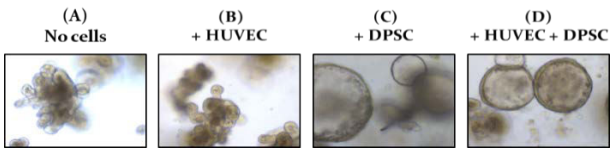


Figure 2

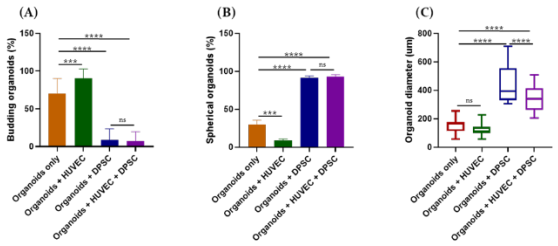
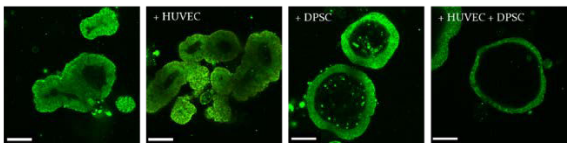


Figure 3



TARGETING OF HYPOXIA-INDUCIBLE ENZYME CARBONIC ANHYDRASE IX IN PATIENT-DERIVED TUMOR MODELS

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PURPOSE: Neuroblastoma is the most common solid childhood tumor outside the central nervous system, sarcoma the most common in adolescents, in adults the incidence of esophageal carcinoma is still increasing worldwide. All three tumor entities have in common that metastatic and relapsed disease have a poor prognosis. Development of new treatment methods to improve therapy and outcome of patient with advanced disease is slow despite being urgently needed. In children, the possibilities for clinical trials are limited. This increases the need to improve and advance preclinical tumor models to preselect favorable candidates for novel therapeutics. This study investigates the use of patient-derived static and perfused 2D and 3 D model for testing the inhibition of carbonic anhydrase IX (CAIX).
METHODS: We established perfused bioreactor-based 3D models using neuroblastoma and sarcoma cell lines and tissue slice-culture as well as 2D esophageal carcinoma experiments and evaluated treatment response. Several inhibitors against hypoxia-induced carbonic anhydrase IX as well as SLC-0111 against carbonic-anhydrase IX and XII. Isothermal microcalorimetry was used for measuring real time thermogenesis in 3D cell and tissue constructs.
RESULTS: 3D neuroblastoma cell constructs presented with similar morphological features compared to neuroblastoma cells in intact tumor tissue featuring small, round and blue cells. Perfused neuroblastoma slice-culture maintained its primary structure in the bioreactor for up to 10 days. Both 3D cell constructs and neuroblastoma and sarcoma tissue responded to inhibition with significant decrease of thermogenesis measured by microcalorimetry, if the correlating hypoxic factor (CAIX or CAXII) was expressed. The CAIX inhibitor SLC-0111 decrease heat production of 3D neuroblastoma cultures as well as esophageal carcinoma cells.
CONCLUSIONS: We demonstrate the use of preclinical 2D and 3D models to evaluate treatment response of CAIX inhibition of neuroblastoma, sarcoma and esophageal patient-derived tumors using viability testing as well as isothermal microcalorimetry. Monitoring times can be increased by use of the perfused bioreactor. Our models are an important first step to preclinically evaluate new drugs using patient-derived tumor tissue exemplified here by in targeting CAIX.

IMPACT OF VESICULAR NUCLEOTIDE TRANSPORTER INHIBITOR ON INTESTINAL FAILURE-ASSOCIATED LIVER DISEASE IN SHORT BOWEL SYNDROME MODEL RATS

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Purpose: Intestinal failure-associated liver disease (IFALD) is a life-threatening complication in patients with short bowel syndrome (SBS) who require long-term parenteral nutrition. Clodronate is a first-generation bisphosphonate used as an antiresorptive therapy for osteoporosis. Recently, clodronate was reported to be a selective and potent inhibitor of vesicular nucleotide transporter (VNUT), a transporter responsible for the vesicular storage of ATP. In addition, VNUT inhibitors have been reported to have anti-inflammatory effects. We herein report our evaluation of the effect of a VNUT inhibitor on IFALD in an SBS rat model.

Methods: SD rats underwent jugular vein catheterization for continuous total parenteral nutrition (TPN) and 90% small bowel resection. Animals were divided into three groups: TPN plus SBS (Control group), TPN plus SBS plus intravenous administration of a low-dose VNUT inhibitor (20 mg/kg twice per week; Low group), or TPN plus SBS plus intravenous administration of high-dose VNUT inhibitor (60 mg/kg twice per week; High group). On day 7, the rats were sacrificed, and lipogenesis and hepatocellular injury were evaluated. Liver weights were compared among the three groups. Histological findings were analyzed based on the non-alcoholic fatty liver disease (NAFLD) activity score. Gene expression associated with lipogenesis was evaluated by real-time polymerase chain reaction.

Results: The histological findings are shown in Figure 1. The liver weights were not significantly different among the three groups (Fig. 2a). Hepatic steatosis was observed in all three groups. While the groups did not differ significantly in steatosis score (Control group: 1.67±1.15, Low group: 1.00±1.41, High group: 0.25±0.50, p=0.28), the higher VNUT inhibitor dose group tended to show lower steatosis scores than the other groups (Fig. 2b). The relative SREBP1c expression was most suppressed in the High group (Control group: 0.83±0.49, Low group: 0.81±0.43, High group: 0.29±0.13, High group vs. Control group: p = 0.07, High group vs. Low group: p = 0.02) (Fig. 2c).

Conclusion: The anti-lipogenic effects of VNUT inhibitors improved IFALD in SBS model rats.

Fig.1 Histological findings of liver specimens

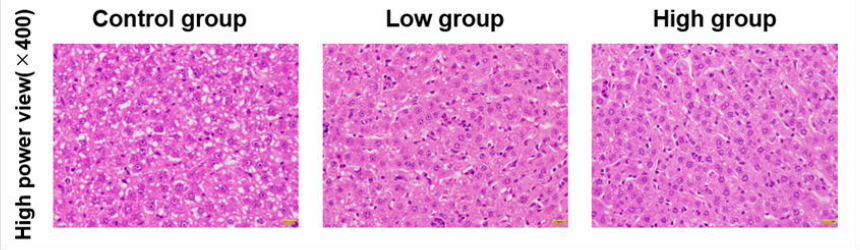


Fig. 2a

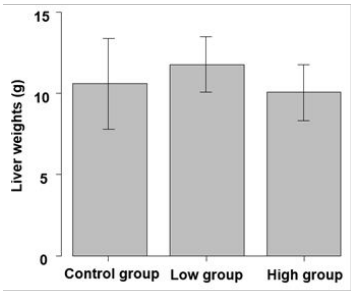


Fig. 2b

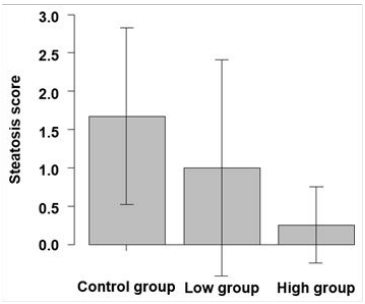
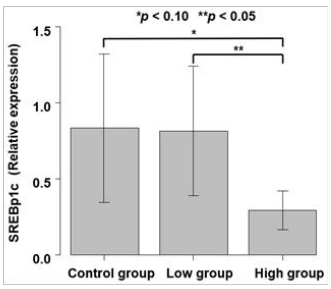


Fig. 2c



NEUROBLASTOMA-DERIVED NEUROSPHERES: A NOVEL DISEASE MODEL IN THE FIELD OF FLUORESCENCE-GUIDED SURGERY

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PURPOSE: Complete tumour resection is crucial in the management of neuroblastoma. Intraoperative precise delineation of tumour margins may be challenging. Targeted fluorescence-guided surgery (FGS) has emerged as a promising technique for enhancing tumour visualization during surgery by using fluorescent-labelled probes against specific cancer antigens. This study aims to establish patient-derived 3D neuroblastoma neurospheres as a new clinically relevant in vitro model to validate the use of targeted FGS in paediatric solid tumours.
METHODS: Four patient-derived neuroblastoma cell lines were cultured to form neurospheres. The neurospheres were characterized for neuroblastoma markers, including CD56, GD2, and B7H3, using flow cytometry, immunostaining, and RNA expression profiling. B7H3 expression was quantified using BD-QuantiBrite quantification beads. The neurospheres were finally used to test binding specificity and internalisation properties of an anti-B7H3 high-affinity monoclonal antibody conjugated with IRDye800 in vitro.
RESULTS: Flow cytometry and immunofluorescence have demonstrated ubiquitous expression of CD56, GD2, and PHOX2B compared to controls (Fig 1-A-C). All lines show high expression of B7H3, confirming its relevance as a target in FGS. The anti-B7H3 antibody conjugated to IRDye800 demonstrated binding specificity, with fluorescence emission observed up to 7 days post-staining (Fig 1-D). In immunofluorescence, the presence of intracellular antibody was observed 5 minutes after staining (Fig 1-E).
CONCLUSIONS: 3D culture of patient-derived neuroblastoma neurospheres represents a clinically-relevant in-vitro model to validate the use of targeted FGS for paediatric solid tumours. Future studies will focus on further characterizing these cells to explore the use of new antigens as targets for FGS.

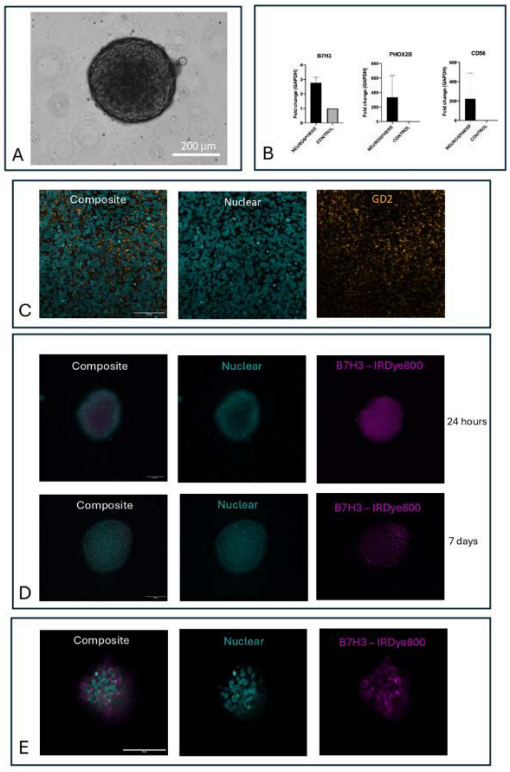

Figure1: A-Neurosphere; B-RT-PCR for B7H3, PHOX2B, CD56; C-Immunofluorescence anti-GD2; D- Immunofluorescence 24h and 7 day after staining; E-Internalization 5 minutes after the staining.

UNRAVELING THE POTENTIAL ROLE OF FBLL1, A PREDICTED RNA MODIFICATION ENZYME, IN HIRSCHSPRUNG'S DISEASE PATHOGENESIS

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PURPOSE: Widespread genomic analyses have been proven useful for understanding etiology and risk stratification in Hirschsprung's disease (HD), a developmental disorder of the enteric nervous system. However, genetic factors alone account for only about 60% of HD cases, suggesting that stochastic environmental factors and epigenetic modifications play crucial roles. This study aims to explore potential non-genetic contributors to HD risk by profiling tRNA modification enzymes in affected individuals.
METHODS: We performed transcriptomic analysis on gut tissue samples from ganglionic versus aganglionic segments of 4 infants with recto-sigmoid HD. The expression levels of 85 human tRNA modification enzymes and related protein factors were profiled using a human tRNA modification enzyme PCR array. This approach was utilized to identify potential non-genetic contributors to HD risk through the understanding of biochemical pathways involved in tRNA modifications.
RESULTS: The expression levels of 85 human tRNA modification enzymes and related protein factors were profiled **(Figure)**. FBLL1 (fibrillarin like 1), a predicted site-specific RNA 2'-O-methyltransferase located in the fibrillar center and nucleoplasm, was markedly upregulated in the aganglionic segment, which was consistent with subsequent qPCR array validation.
CONCLUSION: Profiling human RNA modification enzymes and related protein factors may provide new insights for further understanding Hirschsprung's disease. Further investigation is needed to verify FBLL1 function and its impact on enteric nervous system development, potentially revealing new therapeutic targets or avenues for intervention.

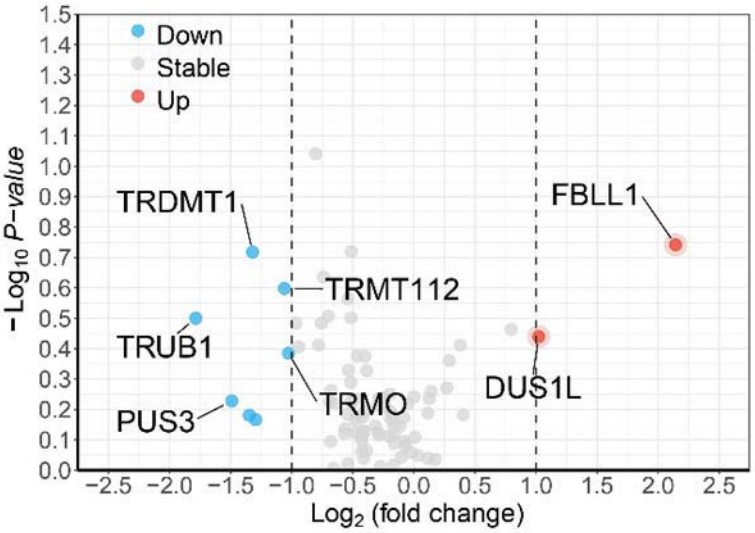

Figure Profile of human tRNA modification enzymes and related protein factors in gut tissue from patients with recto-sigmoid HD.

ASPARTATE AMINOTRANSFERASE TO PLATELET RATIO IN BILIARY ATRESIA: PROGNOSTIC VALUE AND RELATION TO LIVER HISTOPATHOLOGY

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Purpose: Biliary atresia (BA) is the leading cause of end-stage liver disease and the primary reason for liver transplantation (LT) in paediatric population. Clearance of jaundice (CoJ, postoperative decrease of serum bilirubin <20 µmol/L) after Kasai portoenterosotomy (KPE), remains the best clinical predictor of native liver survival (NLS) in BA. We studied the prognostic value of Aspartate aminotransferase to platelet ratio index (APRi) at the time of KPE in relation to liver histopathology.
Methods: Serum and liver specimens, collected from BA patients (N=109) at KPE, were included in this retrospective study. APRi was measured in an accredited hospital laboratory and the key components of histological ductular reaction (DR), biliary epithelium (BE), and parenchymal intermediate hepatocytes (PIH), were quantified with an image processing neural network of cytokeratin 7 stained slides. Liver fibrosis was quantified with computer-aided measurement of Sirius Red staining in addition to manual scoring using Metavir fibrosis staging.
Results: Following KPE, 54% achieved CoJ, and 60% underwent LT during a median follow-up time of 2.45 years. NLS was 56% at 2 years and 44% at 5 years. Median APRi was increased in patients who did not achieve CoJ (0.96 vs 0.78, p=0.019). Patients who underwent subsequent LT showed higher median APRi compared to native liver (NL) survivors (0.94 vs 0.77, p=0.043). In receiver operator curve (ROC) analysis, the optimal APRi cutoff for NLS in 5 years was 0.91 with the area under the ROC curve of 62.4. In Cox hazard modelling, higher APRi predicted inferior NLS in all patients (Figure 1A) and in patients with isolated BA (HR:1.21, p<0.001), without prognostic value in patients who achieved CoJ (HR:1.23, p=0.27). Although APRi correlated positively with DR (R=0.40, p<0.0001), PIH (R=0.36, p<0.001) and BE% (R=0.37, p<0.001), it showed no significant correlation with Metavir stage (R=0.17, p=0.08) or Sirius Red-quantified liver fibrosis (Figure 1B). However, in Cox hazard modelling, increased APRi (≥0.91) predicted inferior NLS when combined with Sirius Red-quantified liver fibrosis (HR:2.43, p=0.001) or with Metavir stage (HR:2.13, p<0.01).
Conclusion: Increased APRi at KPE predicted poor outcomes with limited accuracy, while associating with DR rather than liver fibrosis.

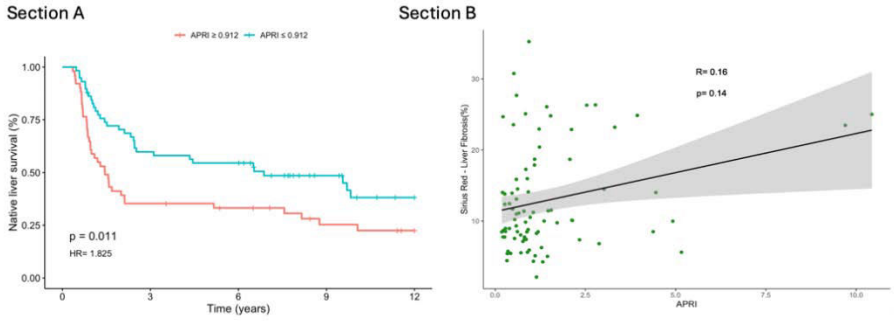

Figure 1.

SEXUAL FUNCTION AND FERTILITY IN YOUNG FEMALE ADULTS SURGICALLY TREATED FOR AN ORECTAL MALFORMATIONS

J.N. Gertler, J. Oddsberg, A. Gunnarsdóttir, A. Svenningsson, T. Wester, L. Örtqvist
(Stockholm, Sweden)

PURPOSE: The aim was to investigate sexual function and fertility in female adults operated on for anorectal malformations (ARM).
METHODS: This was a cross-sectional questionnaire-based study including female adult patients treated for ARM at our institution between 1994 and 2003. Sexual function in females was assessed using the Profile of Sexual Function (PFSF). Additional questions regarding fertility were answered by the participants. Patient characteristics were retrospectively retrieved from the medical records and descriptive statistics were used for analysis. Sexual function outcomes were compared to a control group from a previously published group of females. Composite outcome analysis was performed using previously published data to determine the potential impact of bowel function and health-related quality of life on sexual function. The ethics review authorities approved the study.
RESULTS:A total of 14 of 30 (46.7%) females responded to the questionnaires and had a mean age of 21.1 years (range 18-26). No association was found between PFSF and age or bowel function (Bowel Function Score), however, a strong correlation was found between PFSF and health related quality of life (HRQoL) with a Spearman correlation of ρ 0.82 (p=0.0011). The general satisfaction question was strongly associated to their total PFSF score (ρ = 0.71, p=0.0092)(**Fig.1**). Except for the "desire" item (p=0.015), the females in our cohort did not have significantly worse sexual function than the control population (**Fig.2**). 10/14 (71.4%) of females had had their sexual debut with a median debut age of 16 years and two of these women (20%) have been pregnant. All females had menarche with a median age of 12.5 years.
CONCLUSION: Sexual function in adult females was comparable to healthy controls except for the "desire" item where the cohort reported poorer outcomes. The cohort's sexual function had a direct association to their reported HRQoL where individuals with worse HRQoL also had poorer sexual function.

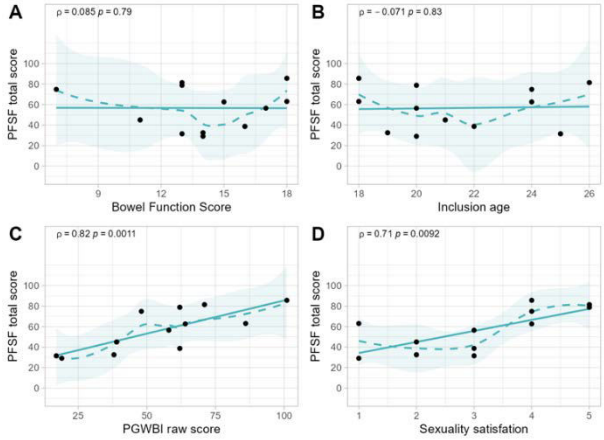

Fig. 1. Graphs showing degree of correlation of PFSF with A) BFS, B) inclusion age, C) HR-QoL and D) satisfaction of sexuality, respectively. Spearman correlation where rho (ρ) > 0.7 = strong correlation. Significance set as p < 0.05. (Abbreviations: PFSF – Profile of Female Sexual Function, BFS – Bowel Function Score, PGWBI – Psychological General Well-Being Index, HR-QoL – Health Related Quality of Life).

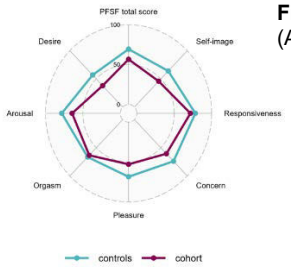

Fig. 2. Radial plot comparing cohort and controls PFSF
(Abbreviation: PFSF – Profile of Female Sexual Function).

WEDGE RESECTION FOR CONGENITAL PULMONARY MALFORMATIONS: IS SAVING LUNG PARENCHYMA THE BEST CHOICE FOR THE PATIENT IN THE SHORT AND LONG TERM? LOOKING FOR ANSWERS ON A LARGE SERIES

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(Milan, Italy)

PURPOSE: To compare the perioperative outcomes of wedge resections and lobectomies in infants with congenital pulmonary malformations (CPM).
METHODS: A retrospective study was conducted including all consecutive infants who had undergone surgery for a CPM in our pediatric tertiary referral center from 2007 to 2023, comparing perioperative outcomes between lobectomy (Group A) and wedge resection (Group B).
RESULTS: Of the 113 patients treated in the period of study, the records of 67 were complete for our analysis. At a median age of surgery of 8.0 (4.0-12.0) months, 44 (66%) patients were included in Group A and 23 (34%) in Group B. There was no difference between the two groups of patients regarding operative time and duration of anesthesia [Group A: 189.0 (155.0-280.0) minutes versus 151.0 (120.0-235.0) minutes, p-value: 0.16; Group A: 332.0 (255.0-426.0) minutes versus Group B: 277.0 (248.0-353.0) minutes, p-value: 0.24; respectively]. While the length of the NICU/PICU stay and the positioning of the chest tube were lower in Group B [Group A: 6.5 (2.0-22.0) minutes versus 2.0 (1.0-3.0) minutes, p-value: 0.017; Group A: 42 (100%) versus 19 (86%), p-value: 0.014; respectively], there was no difference in the overall length of hospital stay [Group A: 9.5 (6.0-13.0) days versus 10.0 (6.0-16.0) days, p-value: 0.83]. Neither the incidence of intra- and post-operative complications [n=2 (7%) versus n=1 (5%); p-value: 0.76; 11 (34%) versus n=8 (53%); p-value: 0.22; respectively] nor the need for re-do surgery [n=2 (5%) versus 3 (17%); p-value: 0.16] differ between the two groups of patients. In a median of 63.0 (47.0-112.0) months of follow-up, a similar incidence of postoperative chest wall anomalies was reported in the two groups of patients A [Group A: n=12 (31%) minutes versus n=5 (33%), p-value: 0.86].
CONCLUSION: Our data show that, in the short term, wedge resection does not seem inferior to lobectomy. However, the presence of residual alterations on second-level imaging requires a meticulous long-term follow-up, to exclude the presence of residual diseased tissue.

	Total (n=67)	Group A Lobectomy (n=44; 66%)	Group B Wedge resection (n=23; 34%)	p-value
Type of surgery, n (%)				0.003
Thoracotomy	30 (45%)	24 (55%)	6 (26%)	
Thoracoscopy	37 (55%)	20 (45%)	17 (67%)	
Associated anomalies at birth, n (%)	33 (52%)	21 (59%)	12 (57%)	0.59
CHD	14 (23%)	10 (27%)	4 (19%)	0.60
Urinary anomalies	7 (11%)	4 (10%)	3 (14%)	0.59
CNS anomalies	5 (8%)	4 (10%)	1 (5%)	0.49
Gastrointestinal anomalies	6 (10%)	4 (10%)	2 (10%)	0.98
Genetic anomalies	1 (2%)	1 (2%)	0 (0%)	0.48
Respiratory symptoms at birth, n (%)	9 (20%)	8 (26%)	1 (7%)	0.13
ASA score at surgery	2.0 (2.0-3.0)	3.0 (2.0-3.0)	2.0 (2.0-3.0)	0.26
Postoperative diagnosis, n (%)				**<0.001**
CPAM	23 (34%)	18 (41%)	5 (22%)	
Extralobar sequestration	6 (9%)	0 (0%)	6 (26%)	
Intralobar sequestration	11 (16%)	4 (9%)	7 (30%)	
Bronchial atresia	3 (4%)	1 (2%)	2 (9%)	
Bronchogenic cyst	1 (1%)	0 (0%)	1 (4%)	
Congenital lobar emphysema	4 (6%)	4 (9%)	0 (0%)	
CPAM + Sequestration	6 (9%)	6 (14%)	0 (0%)	
Other	1 (1%)	0 (0%)	1 (4%)	
Age at surgery (months)	8.0 (4.0-12.0)	6.0 (3.0-12.0)	9.0 (7.0-15.0)	0.072
Operative time (minutes)	182.5 (149.0-275.0)	189.0 (155.0-280.0)	151.0 (120.0-235.0)	0.16
Duration of anesthesia (minutes)	362.0 (255.0-445.0)	332.0 (255.0-426.0)	277.0 (248.0-353.0)	0.24
Extubation site (OR/NICU/PICU)				0.43
OR	3 (4%)	3 (7%)	0 (0%)	
NICU	5 (7%)	3 (7%)	2 (9%)	
Intraoperative complications, n (%)	3 (4%)	2 (7%)	1 (5%)	0.76
Type				0.51
Bleeding	2 (3%)	1 (2%)	1 (4%)	
Other	2 (3%)	2 (5%)	0 (0%)	
Chest tube n (%)	61 (95%)	42 (100%)	19 (86%)	**0.014**
Postoperative complications, n (%)	19 (40%)	11 (34%)	8 (53%)	0.22
Transfusion, n (%)	9 (31%)	8 (38%)	1 (12%)	0.18
Postoperative NICU/PICU, n (%)	29 (97%)	21 (95%)	8 (100%)	0.54
Length of NICU/PICU stay (days)	3.0 (2.0-9.0)	6.5 (2.0-22.0)	2.0 (1.0-3.0)	**0.017**
Length of hospital stay (days)	10.0 (6.0-15.0)	9.5 (6.0-13.0)	10.0 (6.0-16.0)	0.83
Redo surgery, n (%)	5 (8%)	2 (5%)	3 (17%)	0.16
Lung abnormalities at the follow-up chest X-ray, n (%)	30 (55%)	21 (55%)	8 (57%)	0.91
Lung abnormalities at the follow-up CT scan, n (%)	12 (75%)	7 (70%)	5 (83%)	0.55
Lung abnormalities at the follow-up MRI, n (%)	14 (64%)	9 (56%)	5 (83%)	0.24

Table. Baseline characteristics and perioperative outcomes of infants undergoing surgery for CLM.

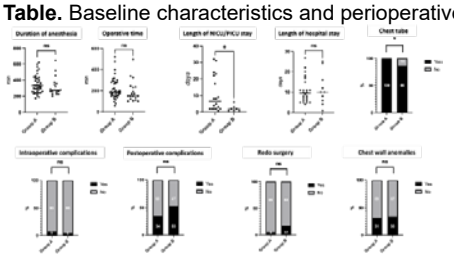

Figure. Perioperative outcomes of infants undergoing surgery for CLM, comparing lobectomy (Group A) and wedge resection (Group B).

NEURODEVELOPMENTAL AND PSYCHOSOCIAL OUTCOME AFTER NEONATAL SURGERY FOR CONGENITAL GASTROINTESTINAL MALFORMATIONS: A PROSPECTIVE SINGLE-CENTER COHORT STUDY

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PURPOSE: There is growing evidence suggesting that surgery in the neonatal period may be associated with an increased risk of long-term neurodevelopmental impairment, which in turn can have a significant impact on the quality of life (QOL) of these children and also their families. The aim of this study was to measure the neurodevelopmental and psychosocial outcome in school-aged children after neonatal surgery for congenital gastrointestinal malformations (GIMs) and to explore potential risk factors for adverse effects.
METHODS: Prospective cohort study of non-syndromic children without chromosomal abnormalities undergoing neonatal surgery for congenital GIMs in a single-center. Regular neurodevelopmental and psychosocial assessment included tools for measuring general intellectual function, attention, concentration, behavioral and emotional problems as well as parent- and self-reported QOL. Additionally, socioeconomic status, parental level of education and family history of migration were recorded. Multivariate regression analysis was conducted to identify GIM-related risk factors for neurodevelopmental impairment.
RESULTS: Thirty-two neonates with congenital GIMs (i.e. 13 abdominal wall defects, 8 esophageal atresia, 5 anorectal malformations, 4 small bowel atresia and 2 congenital diaphragmatic hernia) were enrolled and followed prospectively. The median age at most recent assessment was 8.5 years (range, 6-13). Overall, children had average intelligence levels, whereas they showed slight deficits in the domains of attention and concentration. The incidence of behavioral and emotional problems was lower compared to the general population. Children and parents reported good to excellent QOL, yet parents disclosed above-average levels of problems. A lower socioeconomic status and family history of migration was associated with higher levels of alertness and reaction speed. Children with low birth weight (i.e. <2500 grams), need for anal or esophageal dilatation and persistent fecal incontinence were more prone to emotional issues.
CONCLUSION: Neonates with GIMs undergoing surgery are at risk of attention and concentration deficits later in life. Reduced long-term psychosocial health may be related to repeated surgical interventions and continence problems.

WHAT CAN THE SIZE OF A URETEROCELE TELL US ABOUT ANATOMY AND OUTCOME? FINDINGS IN A COHORT OF 131 PATIENTS OVER 25 YEARS

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Purpose: Ureteroceles are rare congenital malformations and challenging in management. Besides endoscopically verified anatomy (ectopic vs orthotopic) and features of the dependent upper tract, there are no well-tested predictive variables. We aimed at evaluating the initially sonographically measured ureterocele size as a predictor for underlying anatomy, success of further management and complications.
Methods: All patients (n=131, 41m/90f) referred to a single tertiary department because of a ureterocele between 07/1995 and 07/2019 were included. Patients underwent initial evaluation including mercaptoacetyltriglycide scan and voiding cystourethrography. After the initial treatment, complications and symptomatic or high-grade VUR were indications for further treatment. Sonographical exams, occurrence of urinary tract infections and voiding problems, surgical and endoscopic data was recorded. Variables were examined using univariate and stepwise multivariate statistical analysis.
Results: The mean age at presentation was 11.22m (median 2m), the mean follow-up accounted to 78m (median 65m). The majority of ureteroceles were found with duplication anomalies (n=98, 74.8%), thereof 44.9% were ectopic. Of the single system ureteroceles (n=24, 18.3%), 12.5% were ectopic, 5 patients had bilateral ureteroceles. The mean transverse diameter of the ureteroceles was 16mm (SD 9.7, median 14, IQR 14). In a stepwise multivariate analysis ureterocele diameter significantly correlated with anatomy (ectopic / orthotopic, p=0.001, AUC 0.7), successful wait-and-see-strategy (p=0.003, AUC 0.82), successful incision (p=0.02, AUC 0.71) and further problems during later follow-up (p=0.03, AUC 0.69). Female gender was more significant than ureterocele size or anatomy considering the probability of success after primary ureterocele incision (p=0.002) and further problems (p=0.003) and the only variable correlating with UTIs (p=0.01). Ureterocele anatomy showed less significance than diameter in the analyses performed. In a predictive model based on the multivariate analysis a diameter of <10mm and >26mm showed a ≥90% probability of either orthotopic or ectopic.
Conclusion: Based on the largest single center cohort examined to date, with a long follow-up, ureterocele size is a strong, non-invasively detectable variable predicting the anatomic localization and success of primary wait-and-see strategy. Whereas female gender is more important concerning urinary tract infections and further problems, ureterocele diameter is more important than anatomy (ectopic / orthotopic).

POTENTIAL PREDICTORS OF NECROTIZING ENTEROCOLITIS IN EXTREMELY LOW—BIRTH—WEIGHT INFANTS: ANALYSES OF COAGULATION AND FIBRINOLYSIS MARKERS AT BIRTH AT A SINGLE INSTITUTION DURING THE PAST DECADE

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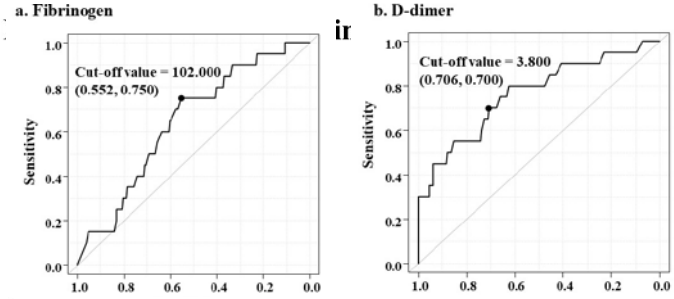
Purpose: A previous report found that inflammation and circulation disorders are related to the onset of necrotizing enterocolitis (NEC). We investigated predictors of NEC onset in extremely low-birth-weight infants (ELBWIs) by analyzing blood tests, including coagulation and fibrinolysis markers at birth.
Methods: We reviewed the medical records of ELBWIs born at our institution between 2013 and 2022. Patients' background characteristics and blood test results, including coagulation and fibrinolysis markers at birth, were compared between the NEC and control groups.
Results: A total of 246 ELBWIs were enrolled in this study (control group, n=226; NEC group, n=20). Background characteristics were compared between the control and NEC groups as follows: gestational age at birth, 25.4±1.5 vs. 25.3±1.9 weeks, *p*=0.736; body weight at birth, 733.8±159.5 vs. 663.9±152.1 g, *p*=0.062; and sex (male: female), 107:119 vs. 8:12, *p*=0.642. Coagulation and fibrinolysis markers at birth were compared between the control and NEC groups as follows: prothrombin time, 62.2%±19.4% vs. 54.2%±24.4%, *p*=0.169; prothrombin ratio, 1.65±0.55 vs. 1.39±0.27, *p*=0.052; activated partial thromboplastin time, 78.5±31.1 vs. 95.6±40.6 min, *p*=0.083; fibrinogen, 160.7±124.2 vs. 107.3±67.1 mg/dL, *p*=0.004; antithrombin III, 29.1%±7.8% vs. 28.4%±9.9%, *p*=0.083; D-dimer, 3.2±2.5 vs. 11.7±13.9 μg/mL, *p*=0.013; soluble fibrin, 30.0±26.9 vs. 35.7±25.4 μg/mL, *p*=0.372; thrombin antithrombin III complex, 9.0±7.9 vs. 20.5±28.1 ng/mL, *p*=0.082; plasmin-α2 plasmin inhibitor complex, 1.04±0.67 vs. 1.88±3.0 μg/mL, *p*=0.254; plasminogen activator inhibitor-1, 23.0±14.3 vs. 42.5±59.5 ng/mL, *p*=0.160; factor XIII, 25.7%±8.2% vs. 24.7%±9.7%; p=0.667. Significant differences were recognized in fibrinogen and D-dimer levels at birth between the control and NEC groups. Figure 1 shows the area under the receiver operating characteristic curves of fibrinogen (Figure. 1a) and D-dimer (Figure. 1b), which were analyzed as potential predictors of the onset of NEC.
Conclusion: In ELBWIs, fibrinogen and D-dimer levels at birth were potential predictors of the onset of NEC. The accentuation of the coagulation system and hyperfibrinolysis in the prenatal period due to inflammation or injury of vascular endothelial cell contribute to the onset of NEC in ELBWIs.



Receiver Operator Characteristic

	AUROC	95% CI	*p* - Value	Cut-off	Sensitivity	Specificity
Fibrinogen	0.641	0.525 - 0.757	0.004	102	0.750	0.552
D-dimer	0.763	0.639 - 0.886	0.013	3.8	0.700	0.706

A 12-YEAR COHORT STUDY ON THE ECONOMIC AND CLINICAL IMPACT OF JEJUNAL TUBE FEEDING IN PEDIATRIC PATIENTS

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Purpose: Jejunal tube feeding (JTF) is a crucial intervention for children with neurodisabilities and gut dysmotility. However, it necessitates frequent tube changes and regular hospital visits, significantly disrupting family life as parents often need to take time off work. This study aims to assess the socio-economic impact of JTF on families and the healthcare system.
Methods: This retrospective cohort study analysed all JTF patients from 2011 to 2023. Data was collected from electronic patient records, including demographics, procedure types, anaesthesia details, and complications. Hospital admission costs, tube change expenses, and patient travel costs were calculated using national tariff brackets and travel metrics.
Results: The study included 58 patients undergoing JTF procedures, with 19 primary and 39 secondary interventions. The median number of tube changes per year was two. Emergency procedures accounted for 42.3% (141/333) of cases, with 55.0% (183/333) requiring overnight hospital stays. The median annual hospital cost per patient was £12,246, leading to an annual expenditure of £860,086 for tube changes. The median annual travel cost per patient was £50.15. Frequent tube complications included displacement (45%), blockage (15%), and damage (13%). The majority of these complications occurred in tubes not replaced within the recommended six-month period. Additionally, 8.6% of patients experienced severe complications such as jejunal perforation and gastric ulceration.
Conclusion: JTF significantly impacts both the economic and physical well-being of families and the healthcare system, with emergency procedures exacerbating these effects. Adherence to scheduled tube changes and local hospital tube maintenance could mitigate these impacts. This study highlights the importance of patient education on the consequences of delayed tube changes and encourages reconsideration of surgical jejunostomy to reduce hospital visits and associated complications.

CAN HAIR FOLLICLE TRANSPLANTATION BE A PROMISING THERAPEUTIC APPROACH FOR NON-HEALING ULCERS AND BURN INJURY TREATMENT

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Purpose: Hair follicles play a pivotal role in skin regeneration and wound healing, by activating epidermal stem cells. This study investigates the regenerative potential of dermal papilla (DP) cells within hair follicles in promoting re-epithelialization and accelerating wound healing.
Methods: 24 male adult Wistar rats were subjected to abdominal skin wounds and divided into two groups: Group A:B treated with biocompatible dressing (BD): normal dressing (ND). Wound biopsies on Days 6, 10, 14, and 20 were analyzed histologically. Healing was graded by Shafer criteria, and hair follicles count in a 2mm² area (HFC) was quantified. The study assessed hair follicle or DP distribution in wound healing across three groups (Group C- Control).
Results: Normal control skin had a mean of 32.25 hair follicles count/2mm^2(HFC) 66.6% wounds showed an increased average HFC compared to normal skin. On Day 6, 3 biopsies with Grade 2:3:4 healing showed HFC of 81:71:46 for Group A, while Group B showed Grades 1:2:2 healing with HFC of 47:15:5 respectively that was comparable to Control Group C (p=0.056). Similarly, On Day 10, Group A had Grades 4:3:2 healing with HFC of 12:98:63 and Group B had Grades 2:2:2 with HFC of 4:8:22, with no significant difference (p=0.144). By Day 14, all three biopsies of wound showed grade 4 healing with HFC of 100:152:130 and Group b also showed grade 3 healing in all biopsies with HFC of 60:70:35 which was significantly higher HFC of 127.33:55 in BD: ND (p=0.017), with the control at 32.25. On Day 20, all biopsies were Grade 4, although the HFC of 38:68:86 depicting the low HFC causing healing delay. Group B showed Grades 1:1:2 healing with corresponding HFC of 66:30:0 (p=0.034). Wounds with higher grades of healing (Grades 3 and 4) exhibited a significantly higher HFC, particularly in BD group. These findings highlight the correlation between the presence of hair follicles and enhanced wound healing.
Conclusion: DP cells are critical for wound healing and re-epithelialization. Hair follicle transplantation presents a promising therapeutic approach for treating non-healing ulcers and burn injuries, advocating further clinical application and research to optimize this regenerative medicine technique.

DISCERNING COMPLEMENTARY PATHWAY OF STEM CELL PROLIFERATION FOR HEPATIC REGENERATION BY PARTIAL HEPATIC RESECTION IN EXTRAHEPATIC BILIARY ATRESIA

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Purpose: Ongoing fibrosis despite successful Kasai for biliary atresia (BA) accounts for significant morbidity and mortality. We aimed to resect a part of liver along with Kasai to stimulate an alternative path of stem cell proliferation for hepatic regeneration

Methods: Institutional Ethics Approval was taken for an Ambispective study. Cases operated for BA from April 2014 to Feb 2022 were divided into two groups; A: in whom non-anatomical liver resection (segment 2,3) was done along with Kasai procedure and B: only Kasai was done. Preoperative; Post Operative (Day-7, I;3;6;12 months) bilirubin, Aspartate to Platelet Ratio Index (APRi) and survival were compared in both groups. Follow-up was done till June 2024.

Results: Of 70 cases of suspected BA, 4 neonatal cholestasis and 6 cases in whom only portal dissection with abdominal drain was done were excluded. 3 were refused surgical treatment due to advanced disease with portal hypertension. One parent refused surgical treatment despite being within 2 months. were excluded. Of eligible 56 BA cases, Group A:B was 29:27. The median (range) age at surgery was comparable as A: 101(54-242) and B: 92(47-310) with 14:7 females in Group A:B. Mean ($\pm$ SD) preoperative bilirubin in Group A:B was 12.2$\pm$5.3: 11.7$\pm$4.1 mg/dl. Mean Day-7 bilirubin in Group A:B was 8.4$\pm$3.6:1:9.3$\pm$3.2 mg/dl. Serum bilirubin at 1-month: 3-months was 6.3$\pm$3.6 v/s 9.6$\pm$4.7 mg/dl ($p<0.05$): 5.6$\pm$2.7 v/s 13.8$\pm$7.3 ($p<0.05$) in Group A v/s B. Preoperative APRi in Group A:B was 3.2$\pm$3.8:1.55$\pm$1.18. Day 7 APRi in Group A:B was 0.79$\pm$0. 48: 1.04$\pm$0.70. At 1 month post-operative, APRi was remarkably low in the study Group (A) 0.52$\pm$0.39v/s (B) 2.75$\pm$2.91. At a median follow-up of 93(42-122) months, 7/29:3/27 patients were alive in Group A:B with 7/7 :2/3 being jaundice-free and 0/7: 1/3 having cholangitis episodes respectively. Of the surviving patients,1:2 patients in Group A:B have undergone Liver transplant: Stem cell therapy.

Conclusion: Delayed presentation of BA is associated with high mortality. Partial hepatic resection may trigger an alternative path of stem cell proliferation for hepatic regeneration to improve survival and thus contribute as a bridge to liver transplantation.

THE ROLE OF ADENOVIRUS-ASSOCIATED CELLULAR SURFACE RECEPTORS AND DNA SENSING MOLECULES IN ACUTE APPENDICITIS

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PURPOSE: Virological evidence suggests a causative association between the double-stranded (ds)DNA adenovirus and acute appendicitis in children. We hypothesized correlations of gene expressions of adenovirus-associated cellular receptors and cytosolic viral double-stranded DNA sensing molecules with inflammatory pathways in children with acute appendicitis.
METHODS: After preoperative isolation of peripheral blood mononuclear cells from children aged 7-17 years with later histologically verified appendicitis (n=29), genome-wide gene mRNA expression data were analyzed. Correlations of receptors CD46 (adenovirus subgroup B) and coxsacki and adenovirus receptor (CAR, subgroups A, C-F) with T-cell marker CD3, T cell receptor (TCR) subunits alpha and beta, cellular lymphocyte counts and adenovirus immunological escape associated kinesin kif5B were investigated. Adenovirus-associated dsDNA sensing DDX41/STING with downstream signaling molecules TRIF and NF-kB, T cell markers and lymphocyte counts were analyzed. Differential analysis of non-necrotizing phlegmonous (PA, n=13) and necrotizing gangrenous appendicitis (GA, n=16) was performed. High correlation was defined at a Pearson correlation coefficient r of $\geq$ ($\leq$ -) 0.5, very high correlation at $\geq$ ($\leq$ -) 0.7. Level of statistical significance was $p<0.05$.
RESULTS: Correlations of CAR with CD3 ($r=0.67$, $p<0.0001$), with lymphocytes ($r=0.63$, $p=0.0002$) and mean correlations with TCR-alpha and -beta subunits ($r=0.64 \pm 0.15$ and $r=0.50 \pm 0.23$) were high. Correlations of CD46 with CD3 ($r=0.37$, $p=0.78$), lymphocytes (0.06, $p=0.049$) and TCR-alpha/ and -beta subunits ($r=0.37 \pm 0.12$ and $r=0.28 \pm 0.22$) were low to moderate with very high correlation with escape associated kif5B ($r=0.80$; $p<0.0001$). Correlation of DDX41 with STING was very high ($r=0.73$, $p<0.0001$) with subsequent very high correlation with TRIF ($r=0.85$, $p<0.0001$) and further downstream with NF-kB ($r=0.93$, $p<0.0001$). High correlations of NF-kB with lymphocytes ($r_{PA}=0.62$, $p=0.025$, $r_{GA}=0.18$, $p=0.51$), CD3 ($r_{PA}=0.85$, $p=0.0003$, $r_{GA}=0.30$, $p=0.25$) and TCR-alpha ($r_{PA}=0.81 \pm 0.1$, $r_{GA}=0.23 \pm 0.15$, $p<0.0001$) and -beta subunits ($r_{PA}=0.68 \pm 0.27$, $r_{GA}=0.25 \pm 0.16$, $p<0.0001$) were restricted to phlegmonous appendicitis.
CONCLUSIONS: The correlation analysis with cellular molecules is well compatible with evidence on adenovirus as general causative agent for acute appendicitis. T cell answer seems to be more effective in case of phlegmonous appendicitis compared with gangrenous inflammation. Adenovirus subgroup B probably represents an immunological escape variant.

EXPERIENCE WITH THE PYELO-URETERAL-MAGNETIC-ANASTOMOSIS (PUMA) DEVICE IN DIFFERENT PORCINE MODELS

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Purpose: We designed and tested a magnetic device to simplify laparoscopic pyelo-ureteral anastomosis by eliminating the need for suturing and knot tying.
Method: The device is built from two 4 mm diameter commercially available neodymium magnets, a JJ stent, a custom-made needle and a Malecot catheter tip. The 1st magnet is fixed to the stent whilst the 2nd magnet is free to move along the stent. The JJ stent with the first magnet is inserted into the ureter and the proximal end of the stent is stitched out from the ureter with the integrated needle. The free end of the ureter is closed with a clip. The stent is stitched into the renal pelvis the 2nd magnet is than inserted onto the stent. The device is removed via cystoscopy. The renal pelvis simply closed with barbed suture.
Results:
Simulation in plastic model of pyeloplasty: the mean anastomosis time dropped from 39.9±14.1 to 8.2±2.8 minutes ($p<0.0001$) and the quality increased from a median of 3 (range, 2-5) to 5 (range, 3-5) with the PUMA device ($p=0.0156$). The mean time-quality score (TQ) was significantly higher (i.e., less favourable) with the standard technique (67.8±34.4) compared with the PUMA method (9.6±5.1) ($p=0.0003$).
Animal model: Six mini- and 5 domestic pigs were used. Hydronephrosis was created by loose ligation of the proximal ureter. Patent anastomoses by laparoscopic insertion of the PUMA device were achieved in 4 mini-pigs and 2 domestic pigs. The magnets were removed through the ureterovesical junction 4 weeks after insertion. Widely patent anastomoses were achieved in 4 mini-pigs, and 2 domestic pigs. One domestic pig was lost in urosepsis after the ligation of the ureter, another one during the 2nd general anaesthesia after successful implantation of the device due to malignant hyperthermia. In 2 domestic pigs (£50 kg) the ureter was found only 2-3 mm diameter and the implantation of the device was not possible. The longest achievable follow up was 8 weeks after the removal of the device while the animal reached 120kg weight.
Conclusions: The PUMA device may simplify laparoscopic pyeloplasty, long-term patent anastomosis is achievable.

REMOTE ISCHEMIC CONDITIONING (RIC) ATTENUATES INFLAMMATION, PROMOTES PROLIFERATION AND ANGIOGENESIS ASSOCIATED WITH ESOPHAGEAL ANASTOMOTIC STRICTURES IN RATS

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Purpose: Anastomotic stricture after esophageal atresia (EA) surgery is the most common complication, attributed to various causes. Previous studies have reported that remote ischemic conditioning (RIC) reduces inflammation and promotes regeneration in the intestine, and is associated with vascular regeneration pathways. This study aims to investigate whether RIC applied after esophageal resection and anastomosis in rats improves esophageal stricture and to identify related pathways.
Methods: Fifty male Sprague-Dawley rats weighing 320-400g at 11 weeks old were divided into (a) control group without surgery (n=5), (b) resection and anastomosis only (R&A only, n=15), (c) R&A with one session of RIC (RIC1, n=15), and (d) R&A with two sessions of RIC (RIC2, n=15). RIC consisted of three cycles of 5-minute ischemia and reperfusion applied to the left hind limb. Surgery involved cervical esophageal exposure, resection, and anastomosis using 8-0 Prolene sutures. On the 7th postoperative day, esophageal samples were collected after injecting contrast medium for X-ray evaluation of stricture severity. Histological evaluation using H&E staining, immunohistochemistry, RT-PCR, and western blotting assessed markers associated with inflammation, proliferation, and angiogenesis.
Results: Anastomotic stricture significantly improved in the RIC-treated groups compared to R&A only group. PCR analysis showed significant reduction in IL-6 and TNF-alpha expression in RIC2 compared to R&A only group. Western blotting confirmed suppression of pSTAT3 related to IL-6, and pNF-kB and pIKKa/b related to TNF-alpha in RIC1 and RIC2 groups. Increased expression of proliferation markers such as Ki-67, cyclin A, B, CDK 2, Rb, and p130 associated with cell cycle activation was observed. Angiogenesis markers HIF and VEGF were also increased in RIC groups compared to R&A only group.
Conclusion: This study demonstrates that RIC applied after esophageal resection and anastomosis reduces inflammation at the anastomotic site, increases proliferation through cell cycle activation, and shows mild increase in angiogenesis.

T-CELL THERAPY IN PEDIATRIC SOLID TUMORS: UPDATE OF A PRECLINICAL PROSPECTIVE STUDY

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PURPOSE: Pediatric patients often face resistant/recurrent cancers. Additionally, those who recover are at increased risk of long-term toxicity from chemotherapy and radiation. Specific targeted therapies are needed to overcome these treatment-related side effects and improve survival. Tumor immuno-profiling has significantly improved current therapies in adult cancer; however, data obtained in adults are not always transferable in paediatrics. Our project aims to obtain primary tumor lines from pediatric patients, in order to investigate the efficacy of a newly developed T-cell product with multiple tumor specificities on tumor cells derived from samples of pediatric neoplasms.
METHODS: We have established a library of pediatric solid tumor cells from patients undergoing surgery, to develop stabilized cell lines. T lymphocytes, derived from healthy donors, were activated in vitro using the known tumor antigens WT1, Prame, Survivin1, Mage-A3, NY-ESO1 in the presence of cytokines. We compared lymphocyte activation by the classical plate culture method with one performed with the G-Rex bioreactor. We then evaluated cellular characteristics and function by cytofluorimetric analysis and immunological assays.
RESULTS: To date, we collected 33 neoplasms, 31 were available for the study: 6 neuroblastomas or neuroblastoma-like tumors, 4 sarcomas, 3 nephroblastomas, 4 lymphomas, and other rarer subcategories. We stored primary cells from these tumors, and were able to stabilize 5 tumor cells lines that we are employing in in-vitro studies.
At present, we were able to expand T cell lines from 4 healthy subjects; these T cells were specific for tumor antigens, and recognized and killed primary tumor cells and/or primary stabilized tumor cell lines from, nephroblastoma, neuroblastoma and osteosarcoma.
The expansion of cytotoxic T-cells (CTLs) was greater after the elicitation with G-Rex bioreactor rather than though classical method. They maintained a broad specificity for most of tumor antigens, and a favourable phenotype in terms of early memory T-cells.
CONCLUSIONS: We have been successful in showing feasibility of expanding multi-antigen specific CTLs with killing activity towards pediatric tumor cells and stabilized primary tumor lines. The setting implementation through G-Rex led to a greater CTLs expansion. Although preliminary, our results are promising. More studies on larger series are necessary.

DIPEPTIDYL PEPTIDASE IV INHIBITORS REDUCE HEPATIC FIBROSIS AND LIPID ACCUMULATION IN RAT INTESTINAL FAILURE-ASSOCIATED LIVER DISEASE MODELS

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(Tokyo, Japan)

PURPOSE: Intestinal failure-associated liver disease (IFALD) poses the highest mortality risk among patients with short bowel syndrome (SBS). In a previous rat SBS model study, we demonstrated the efficacy of dipeptidyl peptidase IV inhibitors (DPP4-I) in improving nutritional absorption and alleviating liver damage. This study aimed to investigate the effectiveness of DPP4-I against liver damage, specifically fibrosis and lipid accumulation, in a rat IFALD model involving SBS surgery and central venous catheter insertion.
METHODS: Fourteen Sprague-Dawley (SD) rats were divided into two groups, the Control group (n=7; normal saline (NS) + IFALD model; SBS + total parenteral nutrition) and the DPP4-I group (n=7; DPP4-I + IFALD model). All rats were euthanized 21 days post-surgery to obtain tissue samples. Hepatic fibrosis was evaluated based on Sirius Red and α-SMA staining. Hepatic lipid accumulation was assessed using the steatosis activity fibrosis (SAF) score, which is also used to assess nonalcoholic steatohepatitis (NASH). Inflammatory cytokines and incretins were examined using the ELISA method.
RESULTS: The 21-day post-surgery survival rate differed significantly between the groups (Control: 70%, DPP4-I: 87.5%). Liver tissue appeared yellowish in the Control group but reddish in the DPP4-I group. Two rats in the Control group exhibited progressive liver fibrosis in the periportal area with fibrous streaks. The mean area percentage of α-SMA immune-reactive cells, alanine transaminase (ALT) levels, and the SAF score were significantly lower in the DPP4-I group than in the Control group (α-SMA positive: 0.18±0.05% versus 0.28±0.03%, $P<0.01$; ALT: 33.3±9.6 IU/L versus 52.4±17.1 IU/L, $P<0.05$; SAF score: 2.67±1.66 versus 5.00±1.27, $P<0.05$). GLP-1 levels were significantly higher, and TGF-β levels were significantly lower in the DPP4-I group than in the Control group.
CONCLUSION: DPP4-I administration reduced liver fibrosis and steatosis in IFALD, possibly by inhibiting DPP4-I-induced adipogenesis and suppressing TGF-β. These findings may help elucidate the mechanism of IFALD.

	Survival rate(%)	α-SMA positive rate(%)	ALT(IU/L)	SAF score	GLP-1 (pM/mL)	TGF-β (pg/mL)
Control group	70.0	0.28±0.03	52.4±17.1	5.00±1.27	48.4±18.0	66.2±46.0
DPP4-I group	87.5	0.18±0.05#	33.3±9.6#	2.67±1.66#	102.0±83.7#	10.6±5.5#

AN ENGINEERED 3D SELF-ASSEMBLY TISSUE MODEL FOR VAGINAL WOUND HEALING

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PURPOSE: Many young female patients and children suffer from congenital malformations in the vaginal organ. These malformations can affect the well-being of the patient through obstruction, pain, stagnation or lack of proper reproductive and sexual functions. Tissue engineering can help to produce cellular grafts for expansion of needed tissue, while stimulating local regeneration. However, modelling the effects of different pro-regenerative stimuli requires a clinically relevant model of the organ. The objective of the study was to design and evaluate an in vitro 3D model for wound healing in human vaginal epithelium.
METHOD: We constructed a 3D tissue model of a human vagina using the self-assembly methodology. Primary human vaginal mesenchymal cells were isolated from patient biopsies (n = 3) and used to create a natively produced organ-specific submucosal equivalent. The cells were cultured with ascorbic acid for self-assembly of stromal collagen sheets. The cultured stromal sheets were then seeded with autologous epithelial cells and left to stratify on air-to-liquid interface. To assess the kinetics of wound healing, a 4-mm full thickness punch wound was introduced. To create a base for cell migration, a collagen type-I embedded polyglactin mesh was fused to the bottom of the models. The tissues were terminated at 2, 6, 24, 48, 96 h, and 1 week post-injury (n = 3). We evaluated the histology to assess the rate of wound closure, matrix deposition, cellular proliferation and migration.
RESULTS: The engineered vaginal tissue equivalents showed full epithelial coverage, with a high degree of similarity with the native stratified squamous epithelium, and a self-assembled stromal layer. The histology showed a collagen-rich extracellular matrix and a collagen IV positive basal layer. We were able to resolve the re-epithelization process at different time points with a full wound closure after 1 week.
CONCLUSION: We established a proof-of-concept model for wound healing in engineered human vaginal tissue and demonstrated the process of early wound re-epithelization in vitro.

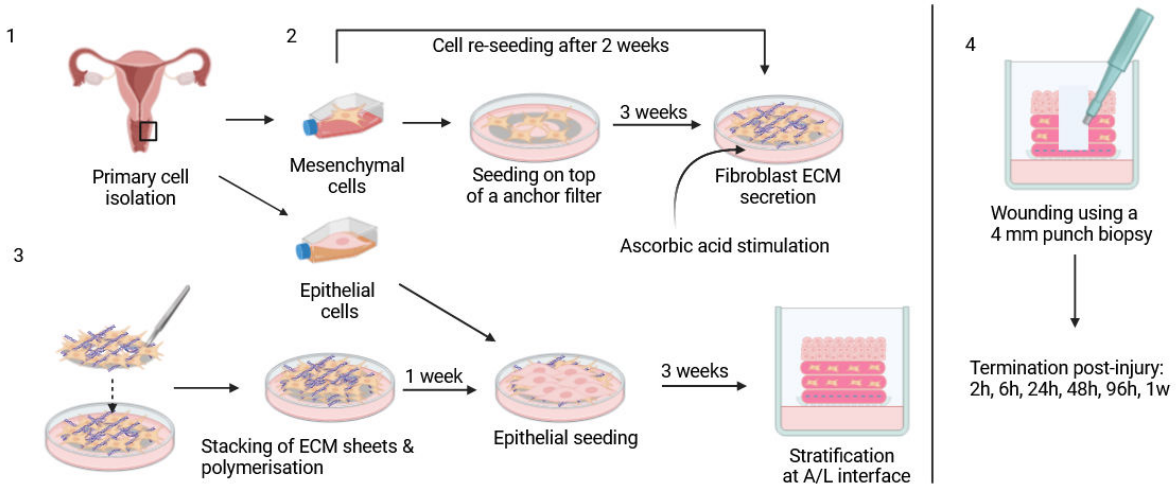

Figure: Graphical method summary.

HEPATOPROTECTIVE EFFECT OF CURCUMIN AND ITS COMBINATION WITH PIPERINE IN EXPERIMENTALLY INDUCED LIVER FIBROSIS IN RATS: A COMPARATIVE ANALYSIS

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Purpose: Curcumin (diferuloylmethane) is the major active constituent of turmeric. Oxidative stress and inflammation are the major pathogenic factors in developing liver fibrosis. Curcumin is a known anti-oxidant and anti-inflammatory agent. An experimental study was planned to establish the model of liver fibrosis through chemical intervention by giving carbon tetrachloride (CCl4). The hepatoprotective effect of Curcumin (Cur) and Curcumin with piperine (CurPip) was then assessed

Methods: After Institutional Animal Ethics Committee approval, a study was carried out on 50 Wistar rats aged 8-12 weeks, weighing 150- 200 grams. Rats were treated with Carbon tetrachloride 2 ml/kg, intraperitoneal, dissolved in saline (1:1 v/v), twice a week over eight weeks. Five animals were used to check whether the histopathological changes were established in the liver fibrosis model. 45 animals were divided into 5 groups with 5 rats in Group A (normal control group) and 10 animals in 4 groups (B-E) (n=10). Groups A; B (Diseased Control); C (Test group-1); D (Test group-2); E (Standard group) received Normal saline; Curcumin (200 mg/kg, p.o); Curcumin (200 mg/kg, p.o) and Piperine (20 mg/kg, p.o); Silymarin (a standard hepatoprotective agent) 50 mg/kg for 4 weeks after the development of hepatic fibrosis. At the end of the experiment, the rats were sacrificed. The liver was subjected to blinded histopathological examination and graded with a 6-feature,19-point Histopathological Grading Score [Sharma et al, PSI 2011:27, 451–461].
The liver function tests were measured at Day 14 and 28.

Results: The histopathological scores were 11.8 $\pm$1.3;7.3$\pm$1.8; 4.7$\pm$2.7;9$\pm$1.7 in B;C;D;E. The difference between Groups B vs C: D: E were statistically highly significant (p value < 0.0001;<0.0001; <0.001). The difference between Groups C vs D, C vs E and D vs E were statistically significant (p value < 0.05; <0.001;<0.05). The difference in Serum Bilirubin was statistically significant in Group B vs D (<0.05).

Conclusion: The CCl4 model of Liver fibrosis is a good model to study the effect of medications**.** Hepatoprotective effect of Curcumin alone and with a Piperine combination on an experiment model of liver fibrosis was demonstrated**.** The hepatoprotective effect of the CurPip combination was superior.

GLUCAGON-LIKE PEPTIDE-2 REINFORCES THE TIGHT JUNCTION OF THE SMALL INTESTINAL EPITHELIUM AGAINST THE STIMULATION OF LIPOPOLYSACCHARIDE

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Background: Bacterial translocation commonly occurs in patients with short bowel syndrome using long-term parenteral nutrition (PN). Several studies have shown that the glucagon-like peptide-2 (GLP-2) is effective for decreasing PN support and catheter-related blood stream infection (CRBSI). We investigated whether CRBSI in SBS patients is associated with the barrier function of the small intestine, such as the tight junction.
Purpose: The aim of this study is to clarify the efficacy of GLP-2 in protecting the intestinal barrier against the stimulation of lipopolysaccharide (LPS).
Materials and Methods: We used an experimental model using Caco-2 cells. Previous studies showed that differentiated cells from this line reproduce the basic structural and functional characteristics of enterocytes of the small intestine. We made the 4 groups as follows; the LPS only group (LPS 4 μg/mL), LPS/G50 group (LPS 4 μg/mL, GLP-2 50 nM), LPS/G100 group (LPS 4 μg/mL, GLP-2 100 nM), and LPS/G200 group (LPS 4 μg/mL, GLP-2 200 nM). We measured the level of cytokine mRNA (tumor necrosis factor-a [TNF-α], Interleukin-6 [IL-6], Interleukin-1β [IL-1β]) and the intercellular adhesive factors of tight junction (claudin-1, occludin, ZO-1). RNA was extracted, cDNA was synthesized, and real-time PCR was performed. Fluorescent immunostaining was performed using claudin-1.
Results: The interleukin-1β level of interleukin-1 in the LPS/G200 group significantly decreased compared to that of the LPS only groups (LPS/G200 0.30±0.12 vs. LPS only 1.52±0.89, $p<0.05$) (Fig.1). Although there were no significant differences, TNF-α and IL-6 demonstrated a concentration-dependent decreasing tendency. Tight junctions, the occludin and claudin-1 level in the LPS/G200 group significantly decreased compared to those of the LPS only group (occludin; LPS/G200 0.65±0.07 vs. LPS only 1.09±0.33, claudin-1; LPS/G200 0.75±0.15 vs. LPS only 1.20±0.27, respectively, $p<0.05$) (Fig.2). According to fluorescent immunostaining, the intercellular region coloring in the LPS / 200G group was significantly stronger than that in the LPS only group (Fig.3).
Conclusion: This in vitro study therefore demonstrated that GLP-2 is able to protect tight junctions and inhibit cytokine release against LPS stimulation.

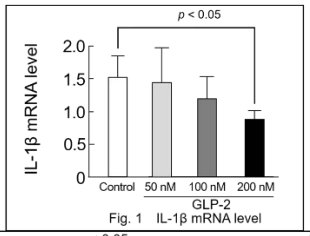

Fig. 1 IL-1β mRNA level

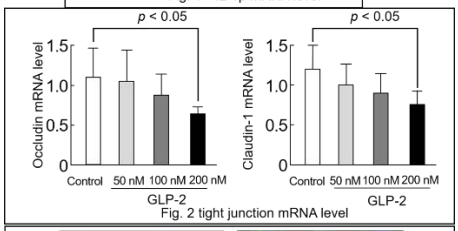

Fig. 2 tight junction mRNA level

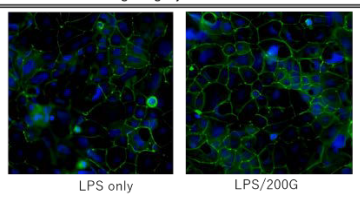

Fig. 3 Fluorescent immunostaining (claudin-1)

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DISSECTING THE DYNAMICS OF CELL DEATH PATHWAYS IN HIRSCHSPRUNG'S DISEASE: A COMPARATIVE ANALYSIS OF VIABLE AND NON-VIABLE CELLS UNDER PROINFLAMMATORY CONDITIONS

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Purpose: Hirschsprung's Disease (HSCR) is characterized by abnormal neural crest cell migration, leading to missing nerve cells in the intestine. This study investigates the cell death mechanisms under inflammatory conditions in HSCR, focusing on understanding how these mechanisms compare to control (CO) groups.
Methods: Using flow cytometry, we analyzed intestinal colonic organoid cultures derived from HSCR and CO groups. Our analysis focused on the quantification of RIPK1-independent and RIPK1-dependent apoptosis, as well as necroptosis in both viable and non-viable cells under acute and chronic inflammatory stress.
Results: Initial findings indicate that under acute proinflammatory stress, HSCR cells display a higher sensitivity to inflammation, as evidenced by an increased number of dead cells (Zombie+). In contrast, under chronic conditions, both HSCR and CO groups show adaptive changes, highlighting a shift towards survival mechanisms in response to prolonged inflammation. Notably, this adaptation in HSCR cells is characterized by a reduced RIPK1-dependent apoptosis in acute versus chronic conditions—a pattern not observed in the CO group. Furthermore, a dominance of RIPK1-independent apoptotic cells in both HSCR and CO suggests a cellular strategy aimed at mitigating inflammation. In non-viable cells, HSCR exhibits significant alterations in RIPK1-dependent apoptosis under chronic stress, indicating a heightened inflammatory response compared to CO.
Conclusion: Our study provides new insights into the regulation of cell death in HSCR under inflammatory stress, utilizing patient-derived organoids. The findings underscore the complex inflammatory response in HSCR, including an impaired cellular response to chronic inflammation and an inconsistent response to different levels of inflammatory stress. This study advances our understanding of the cellular mechanisms underpinning HSCR and may inform therapeutic strategies aimed at modulating inflammation in affected individuals.

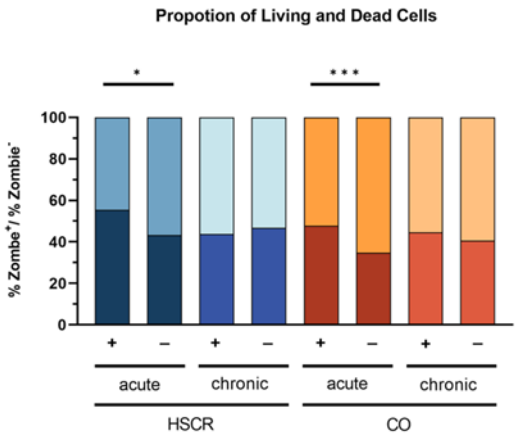

Figure 2. Proportions of dead and living cells. Analysis includes 5 spheorids for each measured subgroup. Cell proportions are shown for acute and chronic stimulation scheme before (-) and after treatment (+). Whole cell population was separated according to their zombie status. Zombie^{+} cells were marked as dead. Zombie- cells were marked as alive and undergoing cell death. Analysis reveals a significant increase in dead cells after acute proinflammatory stimulation for both HSCR (p=0.0029) and CO (p=0.0009).

EFFECT OF DRINKING WATER QUALITY ON FETAL DEVELOPMENT: A LITERATURE REVIEW

C. Riboni, S. Holland-Cunz, S. Gros
(Basel, Switzerland)

PURPOSE: Congenital malformations can, for example, result either from genetic predispositions or other disruption of development that might be associated with as-yet-unidentified environmental factors. These conditions are commonly observed in newborns at our clinic, but our current practice focuses solely on genetic analysis and does not incorporate epidemiological factors. Exposure to some pesticides such as nitrofen can cause congenital anomalies through various mechanisms including disruption of retinoic acid pathway or endocrine function. While many agricultural pesticides with potential teratogenic effects have been discontinued, instances of harmful substance transgressions in drinking water limits continue to be reported. These include glyphosate and atrazine, as well as their metabolites. Several studies have underlined a teratogen effect of both substances in animals. Moreover, they influence endocrine and reproductive function in humans. Atrazine may also be associated with gastroschisis. The scope of our work is to analyse if there are any known associations between pesticide present in tap water in Switzerland and the occurrence of malformations.
METHODS: We conducted a literature review on pubmed searching “epidemiological exposure AND congenital malformations”, “glyphosate and pregnancy”, “glyphosate AND congenital malformations”, “nitrofen and congenital malformation”, “tap water and congenital malformation”, “atrazine and pregnancy”. We checked the levels of pesticides and their metabolites in swiss drinking water based on water scrutiny data and information from local newspapers.
RESULTS: More and more studies underline a possible correlation between pesticides and some birth defects. At the same time, many potentially toxic substances continue to be detected in drinking water, sometimes even above allowed limits, as for atrazine. Data about concentration of their metabolites are not always reported.
CONCLUSION: The suspicion of a correlation between birth defects and pesticide exposure is not to be underestimated. However, data are mostly inconclusive due to the difficulty of documenting exposure, conflicting results from different studies and limited data availability. Therefore, more epidemiological studies with a larger sample size and precise measure of exposure are needed to define if there is an association between pesticides and congenital anomalies.

ALPHA-1-ANTITRYPSIN IMPROVES ANASTOMOTIC HEALING IN INTESTINAL EPITHELIAL CELLS MODEL

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(Hannover, Germany)

Purpose: Bowel resection and intestinal anastomosis are common in paediatric surgery. In children, anastomotic leakage occurs in up to 7%. Enhanced anastomotic healing may reduce the rate of anastomotic leakage. Human alpha1-antitrypsin (AAT) is a protease inhibitor that accelerates wound healing and has been shown to stimulate expression of proliferation-related genes. We investigated effects of AAT on intestinal epithelial cell confluence and the expression of the proliferation-related genes *CDKN1A, CDKN2A*, and *TIM1* in an *in vitro* model. We hypothesized that AAT improves and accelerates anastomotic healing in our *in vitro* model by enhancement of proliferation-related genes.
Methods: To resemble anastomotic healing on the cellular level, we established a scratch assay with intestinal epithelial cells. Non-tumorigenic HIEC-6 epithelial crypt cells derived from fetal intestine were cultured in 12-well plates at standard conditions. A scratch assay was generated by applying a defined scratch on the monolayer. One ml standard medium without (controls) or with AAT in different concentrations (0.5mg/ml, 1mg/ml, or 2mg/ml) was added. The scratches resembling the anastomotic suture line were investigated in an inverted microscope using an incubation chamber set. Images we captured at specific time intervals and analyzed. Cells were monitored for 24 hours using a Life-Cell Imaging System, with images taken every two hours. After RNA extraction of the corresponding cells and cDNA amplification, mRNA-expression of proliferation related genes *CDKN1A, CDKN2A*, and *TIM1* was measured by RT-PCR.
Results: In the presence of AAT, the scratch closed significantly faster compared to controls. Cells treated with 1mg/ml AAT showed 53% repopulation after 8 hours and 97% after 18 hours, while control cells showed 24% and 60% repopulation, respectively (Figure 1). AAT treatment increased the expression of *CDKN1A, CDKN2A*, and *TIM1 vs. control group.* Gene expression increased with elevated AAT-concentration up to 1mg/ml.
Conclusion: In our *in vitro* model, AAT accelerated the confluence of intestinal cells and increased the expression of proliferation related genes at an optimal concentration of 1mg/ml. AAT may bear therapeutic potential to improve anastomotic healing and reduce anastomotic leak rates in neonates.

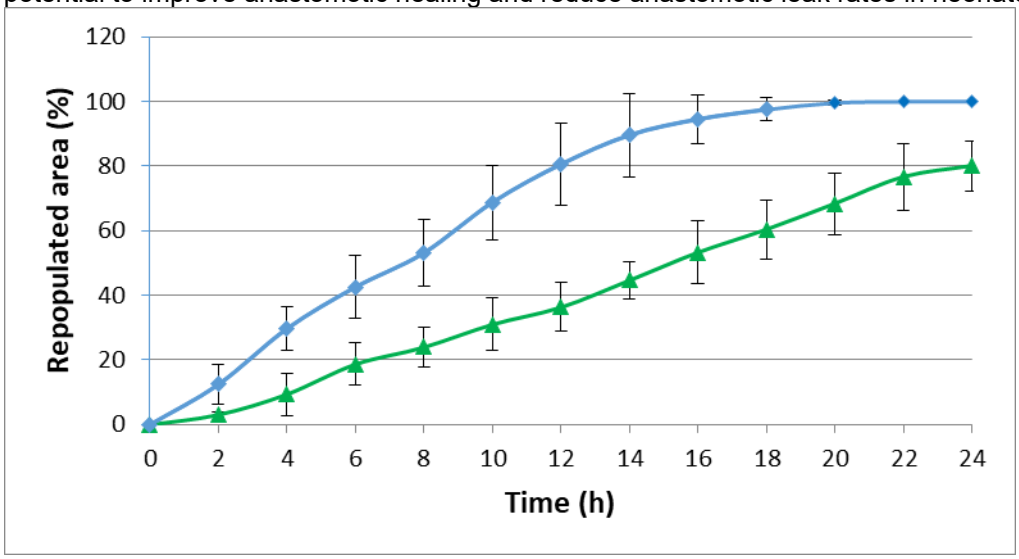


Figure 1: Effect of 1mg/ml AAT on gap closure: Percentage of cell-repopulated area in scratch space over time (- cells treated with AAT 1mg/ml, - controls standard medium).

PILOT IMPLEMENTATION STUDY INVESTIGATING THE INFLUENCE OF GENE POLYMORPHISM ON PEDIATRIC UROLITHIASIS IN A LOCAL COMMUNITY

M. Aboud, M. Kadhim
(Al Qadisiya, IRAQ)

PURPOSE: The study sheds light on the genetic basis of urolithiasis by understanding the molecular basis and identifying the genotypic and allelic frequency of gene polymorphism providing crucial preliminary insights into personalized prevention and management strategies for affected children in our community.
METHODS: This study was conducted on 45 nephrolithiasis patients, the others consisted of 45 healthy individuals without any history of systemic disease also enrolled as a control group. Genomic DNA samples were collected, and ARMS PCR for genotyping of PCRCSF-1 gene polymorphism was designed in this study using the NCBI SNP database and ARMS PCR primer1 design online. Statistical analysis is carried out with the (SPSS v 0.23). The significance level is considered when a P value is less than 0.05 (≤ 0.05). Our health authorities approved the study.
RESULTS: Regarding the *CSF-1* gene, the heterozygous genotype CA was more frequent in the patients group compared to the control group, and the difference was highly significant (P = 0.006). Therefore, genotype CA was a risk factor for renal stones. The homozygous genotype CC was more frequent in the patients' group compared to the control group, and the difference was highly significant (P < 0.001). Therefore, genotype CC was a risk factor for renal stones.
CONCLUSION: There is a synergistic effect of multiple risk alleles with pediatric nephrolithiasis susceptibility especially when they are frequently found within the same family. This pilot study marked a significant step in unraveling the genetic underpinnings of pediatric urolithiasis within a local community.

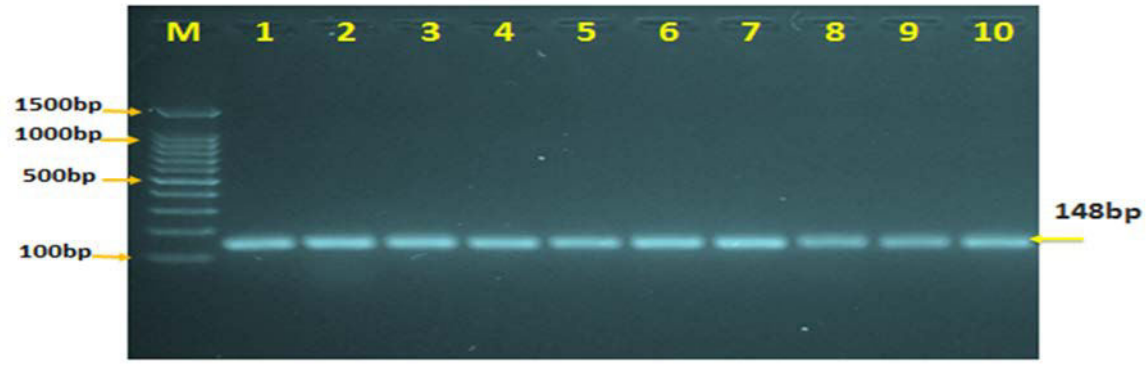


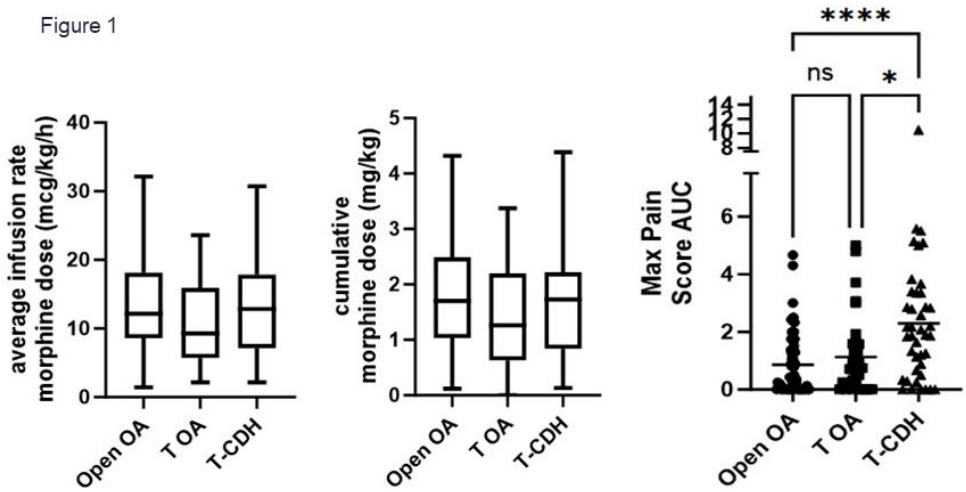
Figure: Agarose gel electrophoresis image that show the PCR product analysis of IL-18 gene from blood patient samples and healthy control sample. Where M: marker (1500-100bp), lane (1-10) some positive PCR amplification of IL-18 gene at 148bp PCR product size.

THORACOTOMY VS. THORACOSCOPY IN NEONATES: A 15 YEAR COMPARISON OF POST-OPERATIVE PAIN AND ANALGESIA

S. Tiboni, R. Verma, K. Bhandarkar, S. Eaton, S. Loukogeorgakis, D. Mullassery, S. Basson, S. Blackburn, K. Cross, J. Curry, S. Giuliani, S. Hannam, G. Williams, P. De Coppi
(London, United Kingdom)

PURPOSE: Thoracoscopy is postulated to enhance recovery after surgery, partly through reduced pain and opioid requirement. Despite increasing minimally invasive neonatal surgery there is a paucity of data to validate improved pain outcomes. Our aim was to look for any difference between thoracoscopy and thoracotomy approaches with regard to pain assessment and opioid requirement.
METHODS: This was a single centre retrospective study from 2009 to 2024 including all babies undergoing a thoracotomy for Oesophageal Atresia (OA-TOF) or thoracoscopy for OA-TOF or Congenital Diaphragmatic Hernia (CDH). We excluded patients undergoing simultaneous additional procedures. Outcome measures were Neonatal Pain Assessment (PAT) Scores, Opioid infusion rates and cumulative dosage over the first postoperative week, and time to extubation.
RESULTS: We collected 150 patients: 67 OA-TOF thoracotomy (O) and 74 thoracoscopy (T) (33 OA-TOF + 41 CDH). There were no significant differences between the cohorts: median (range) for birth weight (O: 2.27 [1.16-3.57] vs T: 2.24 [1.19-4.00] Kg), gestational age (O: 37[31-41] vs T: 38 [29-42] weeks or time to extubation (O: 3.8 [1-27] vs T: 3 [0-27] days. We found no significant difference in average morphine infusion rate or cumulative morphine dose. Thoracoscopic CDH patients had significantly higher maximum pain scores than thoracoscopic or open OA-TOF repair (Figure 1)
CONCLUSION: Our data suggests there are no benefits for neonatal thoracoscopy in terms of postoperative pain management. We have a complex CDH subgroup requiring extracorporeal membrane oxygenation (ECMO) so further analysis of confounding factors e.g. drain insertions and pre-operative sedation lengths is needed.

Figure 1



COMPARING THREE TYPES OF RETROPERITONEAL PYELOPLASTY (OPEN, LAPAROSCOPIC, AND ROBOT-ASSISTED LAPAROSCOPIC) IN CHILDREN WITH URETEROPELVIC JUNCTION OBSTRUCTION AT A SINGLE INSTITUTE: A FIRST REPORT

M. Tanaka, H. Koga, J. Ishi, S. Yoshida, K. Suda, S. Shibuya, T. Ochi, R. Arii, G.J. Lane, A. Yamataka
(Tokyo, Japan)

PURPOSE: Clinical effectiveness and safety of pyeloplasty (PP) for ureteropelvic junction obstruction (UPJO) were compared in children treated by open retroperitoneal (OR), laparoscopic retroperitoneal (LR) and robot-assisted laparoscopic retroperitoneal (RR) techniques.
METHODS: The medical records of matched pediatric UPJO patients treated by PP (OR: n=23, LR: n=33, and RR: n=24) between 2000 and 2022 were reviewed retrospectively to obtain demographic data, details of hospitalization, complication rates using the Clavien-Dindo classification, pre/postoperative hydronephrosis on ultrasonography, and split renal function/GFR on technetium-99m diethylene triamine penta-acetic acid (DTPA) renography. “Improvement” was defined specifically as postoperative status more than 5% better than preoperative status with resolution of symptoms. Total anastomotic time (TAT) and TAT/suture were calculated from intraoperative video recordings for LR and RP.
RESULTS: Hospitalization was significantly longer in OR; (15.4 days versus 10.1 days for LR and 8.5 days for RR; p<0.01) (Table). While there were no differences in overall complication rates (35% for OR, 18% for LR, and 17% for RR; p=0.24), 6 were above Clavien-Dindo grade III in severity (2 in OR, 3 in LR and 1 in RR). Redo surgery for persistent UPJO was required after OR (n=2), LR (n=1) and RR (n=1). TAT and TAT/suture were both significantly shorter with less variance for RR (p<0.05). Improvement was significantly better in RR (p<0.01).
CONCLUSION: This is the first study to compare 3 types of retroperitoneal PP for UPJO in matched children. RR was most effective with superior improvement because of stable suturing and less severe complications.

Table: Patient demographics and operative outcomes

	OR (n=23)	LR (n=33)	RR (n=24)	p Value
Patient Demographics				
Male/Female	18/5	22/11	17/7	0.656
Left/Right	15/8	25/8	22/2	0.147
Prenatal diagnosis	65% (15)	52% (17)	58% (14)	0.567
Preoperative Drainage (Nephrostomy / Double J Stent)	48% (10 / 1)	**6% (1 / 1)**	21% (2 / 3)	**<0.01**
Total anastomotic time (TAT) and TAT/suture				
TAT(min)	-	**136**	**61.3**	**<0.05**
TAT/suture(min)	-	**14.3**	**6.2**	**<0.05**
Post-operative Outcomes				
Analgesia(days)	2.2	2.2	2.1	0.868
Bowel movement (days)	1.4	1.6	1.6	0.192
Restart eating (days)	2.0	1.6	1.9	0.069
Serum CRP (mg/dL)	**3.9**	1.2	1.0	**<0.01**
Hospital stay (days)	**15.4**	10.1	8.5	**<0.01**

POSTPRANDIAL INTESTINAL PERFUSION IN TERM AND PRETERM INFANTS: IMPLICATIONS FOR PREVENTION OF NECROTIZING ENTEROCOLITIS

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(Toronto, Canada)

PURPOSE: Preclinical evidence has shown that prematurity is associated with an inadequate intestinal blood flow response to feeding, a risk factor for necrotizing enterocolitis (NEC). This study evaluated differences in postprandial intestinal perfusion between term and preterm infants, with the aim to inform NEC preventive strategies.
METHODS: Term and preterm (≤33weeks gestational age (GA) at birth) infants were recruited in this observational study at Xiamen Children's Hospital. Demographic and clinical data were collected at enrollment. Percent changes in superior mesenteric artery (SMA) time-averaged mean velocity (TAMV) and bowel wall perfusion in four abdominal quadrants were evaluated with color Doppler sonography before and 60-minutes after feeding in term (n=10) and preterm infants stratified by birth weight above (n=5) or below 1000g (n=5). Statistical analyses included chi-square tests for categorical variables, independent t-tests for continuous variables, paired t-tests for within-group and one-way ANOVA for between-group comparisons. Trial registration: NCT06319326.
RESULTS: Term infants had mean GA at birth of 38.60±1.17weeks versus 29.10±3.03weeks for preterm infants (p<0.001), mean birth weight 3205±490.15g versus 1316.5±617.42g for preterm infants (p<0.001), postnatal age at enrollment 8.1±1.8days versus 26.4±18.2days for preterm infants (p=0.005), and body weight at enrollment 3267±498.09g versus 1618±434.28g for preterm infants (p<0.001). Term infants primarily received formula (80%). Preterm infants received breastmilk (40%), formula (40%), or both (20%). Calorie intake was significantly higher in term versus preterm infants (103.8 vs. 94.5 kcal/kg/day; p<0.001). Mean SMA-TAMV percent changes were 58.1±86.7% (term), 37.7±74.6% (preterm>1000g), and 22.5±45.3% (preterm<1000g), without significant changes within or between groups (**Fig. a**). Term and preterm infants>1000g showed significantly higher intestinal perfusion post-feeding with mean percent change 36.55±32.97% (p=0.01) and 37.37%±19.69% (p=0.01) respectively. Preterm infants<1000g had mean percent change of 21.22±35.35% (not significant). One-way ANOVA showed no significant difference between groups (**Fig. b**).
Conclusion: We report significant increases in intestinal perfusion post-feeding in term and preterm infants >1000g, indicating a greater intestinal blood flow response to feeding. Preterm infants <1000g did not show a significant change, suggesting a poorer response to feeding. These findings suggest that infants at higher risk of NEC may have a poor intestinal blood flow response to feeding compared to those at lower risk.

Fig. a

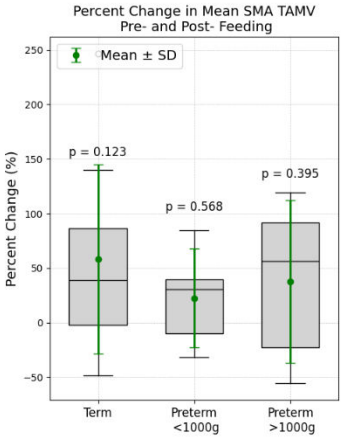
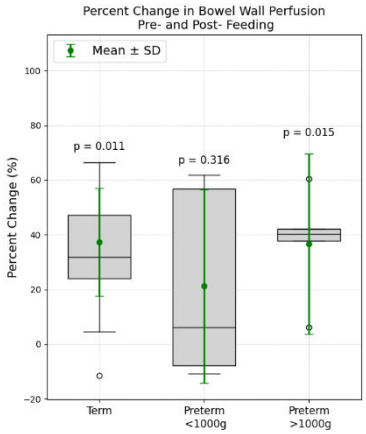


Fig. b



THE CONNECT TRIAL: FIRST PROGRESS REPORT

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Purpose: The optimal treatment of asymptomatic congenital pulmonary airway malformation (CPAM) remains a topic of ongoing debate among healthcare providers. With the aspiration to solve this issue, our team designed the first prospective study to compare elective surgical resection with the conservative management of asymptomatic CPAM lesions in children. This paper provides an overview of the steps taken and the lessons learned in the process.
Methods: After establishing the CONNECT (Collaborative Neonatal Network for the first European CPAM Trial) consortium, consensus was reached on a core outcome set which was incorporated in the CONNECT Trial. The trial aims to include children with a CT-confirmed CPAM diagnosis who remain asymptomatic, randomizing them to receive either conservative or surgical management. During a 5-year standardized follow-up, data will be collected on pulmonary morbidity, surgical complications, repeated imaging, histopathology, molecular genetics and validated questionnaires (on parental anxiety, quality of life and healthcare consumption). However, the primary endpoint is an exercise tolerance test at 5 years of age. Our group is currently doing further research into the Muscle Power Sprint Test (MPST) as the preferred method of measuring.
Results: The CONNECT Trial commenced at Erasmus MC in January 2023, with Radboud UMC in Nijmegen joining in September of that same year. The UZA/ZNA hospitals in Antwerp will begin patient enrolment soon. Currently, nine centres are awaiting ethical approval and ten more centres have expressed their interest in joining the trial. The rarity of CPAM and the nature of a randomized controlled trial create a challenging research environment. As more centres will join in the near future, we anticipate a significant increase in patient inclusions.
Conclusion: After several years of initialization, the CONNECT Trial is now gaining momentum. We expect this study to provide critical insights into the management of asymptomatic CPAM which could have relevant implications for clinical practice.

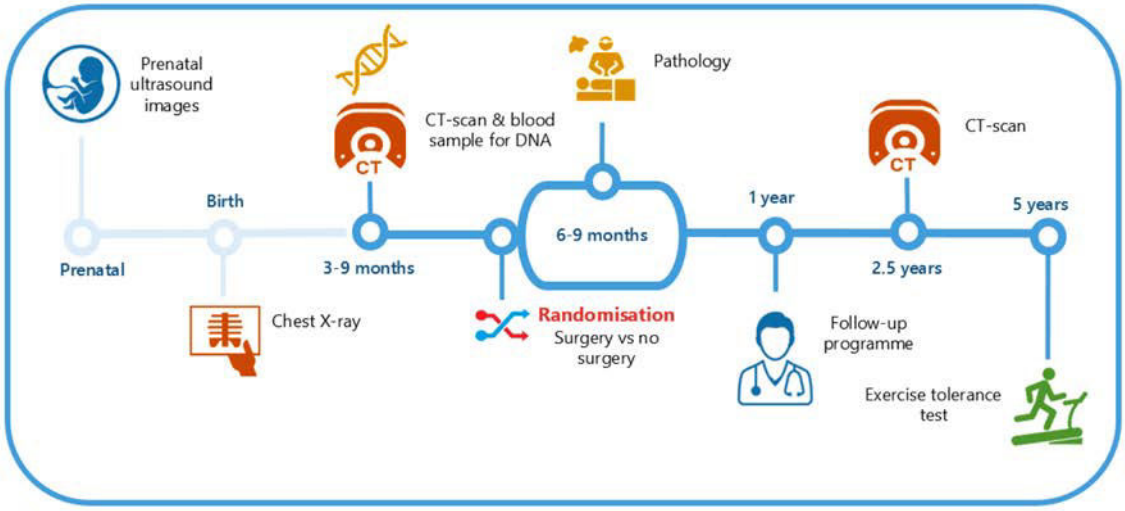

Figure 1 Timeline of the CONNECT trial.

FETAL CYSTOSCOPY (FC) AND VESICOAMNIOTIC SHUNTING (VAS) IN LOWER URINARY TRACT OBSTRUCTION (LUTO): A META-ANALYSIS OF LONG-TERM OUTCOME AND CURRENT TECHNICAL LIMITATIONS

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PURPOSE: To compare the survival, nephrological, and urological outcomes of children prenatally treated for lower urinary tract obstruction (LUTO) by using operative fetal cystoscopy (FC) and vesicoamniotic shunting (VAS).
METHODS: This study was a literature search using MEDLINE reference lists. All studies reporting the effectiveness, complications, and technical issues of FC and VAS in the prenatal treatment of LUTO were selected for a single-proportion meta-analysis. There was an independent selection of studies, data extraction, and quality assessment by two reviewers. We computed and declared effect sizes for estimating a single proportion using the Freeman-Turkey transformed proportion.
RESULTS: out of a total of 117 citations identified, 33 studies (n=489 fetuses) were included for a detailed evaluation of VAS, and 9 studies (n=183 fetuses) for FC. Overall, VAS was performed at a lower gestational age compared with FC [VAS: 19.24 (18.95-19.52) versus FC: 20.17 (19.60-20.64)]. FC was burdened by a higher incidence of intrauterine fetal demise [test Q: p=0.38; test θ: p=0.01; 0.43 (0.34-0.52)], termination of pregnancy [test Q: p=0.00; test θ: p=0.00; 0.76 (0.61-0.92)], and postnatal death [test Q: p=0.00; test θ: p=0.00; 0.78 (0.65-0.90)]. However, the incidence of chronic kidney disease was lower for infants undergoing FC compared to VAS [test Q: p=0.00; test θ: p=0.00; 1.10 (0.92-1.29)]. No definitive conclusion could be drawn regarding procedure-related and obstetric complications due to the heterogenicity of the study included.
CONCLUSION: Despite the inherent lack of randomized controlled trials and the necessity of analyzing small series with heterogeneous fetal phenotypes, our meta-analysis yields optimistic findings regarding the preservation of long-term kidney function with FC. This potential benefit of the FC over VAS may be associated with the fact that FC is also considered a diagnostic tool and can guide for termination of pregnancy for those with more complex diseases. The advancement of flexible and miniaturized technologies is expected to enhance outcomes related to periprocedural morbidities and mortalities, which have been impacted by suboptimal visualization of the posterior urethra and the limitations of current instrumentation.

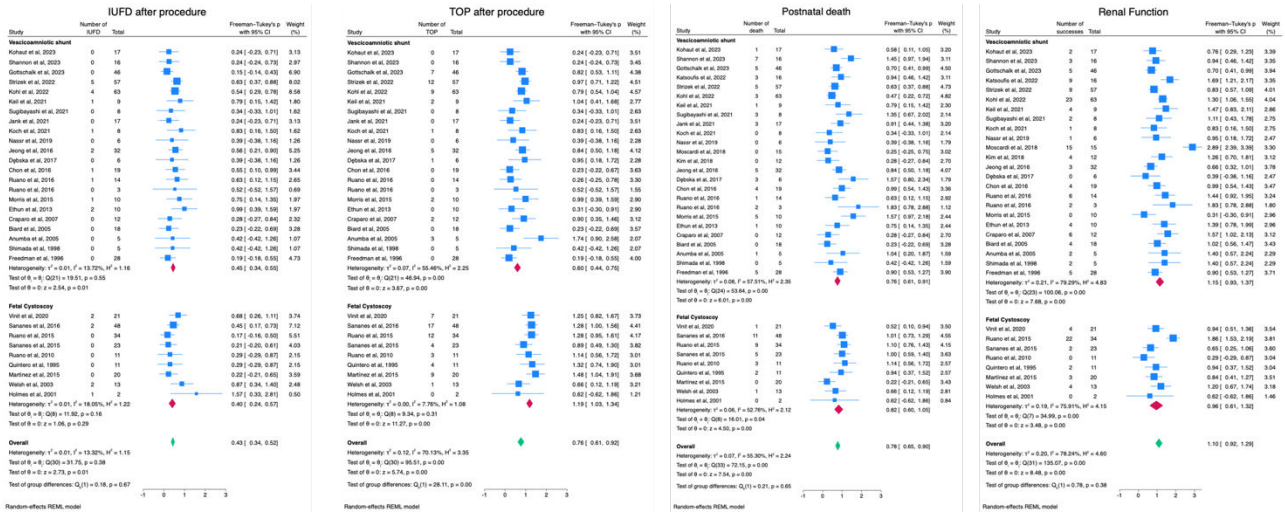

Figure. Forest plot of the available literature describing intrauterine fetal demise (IUFT), termination of pregnancy (TOP), postnatal death, and renal function of fetuses with lower urinary tract obstruction (LUTO) undergoing fetal cystoscopy (FC) or vesicoamniotic shunting (VAS).

NEONATAL INTESTINAL VOLVULUS: MIDGUT VOLVULUS WITH MALROTATION VERSUS SEGMENTAL VOLVULUS WITHOUT MALROTATION

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PURPOSE: Neonatal intestinal volvulus (NIV) can be classified as midgut volvulus (MV) associated with midgut malrotation or segmental volvulus (SV) due to other causes. MV and SV were compared to establish criteria for distinguishing between them.

METHODS: The medical records of NIV patients (n=85) treated at two institutions between 1994 and 2023 were reviewed retrospectively for details of diagnosis, pregnancy/delivery, clinical status, symptoms, and incidental morbidity. Continuous data were presented as medians and interquartile ranges (IQR). Fisher's exact test and the Mann-Whitney U test were used to compare MV (n=49) and SV (n=36).

RESULTS: Data were summarized in the table below. Abnormal prenatal ultrasonography (US) findings, such as bowel dilatation, cystic mass, polyhydramnios, and echogenic amniotic fluid, were more common in SV [6.1% versus 50.0%; $p<0.001$]. Gestational age and birth weight were significantly lower in SV. Abdominal distention was more frequently observed in SV both clinically [22.4% versus 94.4%; $p<0.001$] and on X-ray [8.2% versus 61.1%; $p<0.001$], while absence of abdominal gas on X-ray was more prevalent in MV [59.2% versus 36.1%; $p<0.048$]. SV required significantly more temporary colostomies and bowel resections. Postoperative outcomes were similar for MV and SV, with no significant differences in the incidences of short bowel syndrome, postoperative complications, or mortality.

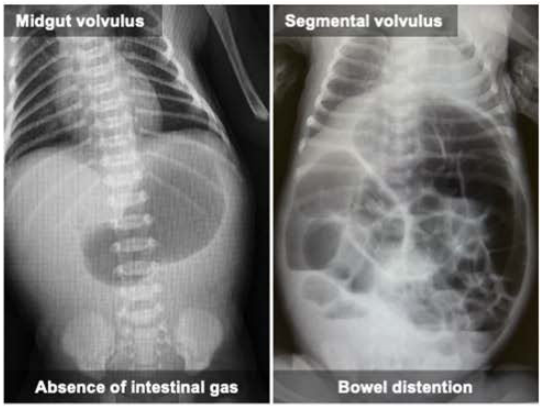
CONCLUSION: Prenatal US, clinical abdominal signs, and abdominal X-ray findings may aid in determining the type of NIV present.

Table: Comparison of midgut volvulus and segmental volvulus

	Midgut volvulus (n=49)	Segmental volvulus (n=36)	*p* value
Clinical characteristics			
Sex (male %)	37 (75.5%)	19 (52.8%)	0.038
Gestational age (weeks)	39.3 (38.3-40.0)	36.25 (34.2-37.7)	<0.001
Birth Weight (g)	2980 (2755-3300)	2620 (2018-2925)	<0.001
Abnormal prenatal US findings (%)	3 (6.1%)	18 (50%)	<0.001
Unstable vital signs* (%)	2 (4.1%)	12 (33.3%)	<0.001
Bilious vomiting** (%)	46 (93.9%)	22 (61.1%)	<0.001
Abdominal distention (%)	11 (22.4%)	34 (94.4%)	<0.001
Blood in stools (%)	16 (32.7%)	5 (13.9%)	NS
X-ray findings			
Bowel distension (%)	4 (8.2%)	22 (61.1%)	<0.001
Absence of intestinal gas (%)	29 (59.2%)	13 (36.1%)	0.048
Operative findings and outcomes			
Intestinal atresia	2 (4.1%)	15 (41.7%)	<0.001
Degree of volvulus rotation	360 (270-540)	540 (360-765)	0.003
Bowel resection (%)	7 (14.3%)	36 (100%)	<0.001
Stoma formation (%)	2 (4.1%)	9 (25%)	0.007
Death (%)	0 (0.0%)	1 (2.8%)	NS
Short bowel syndrome (%)	4 (8.2%)	2 (5.6%)	NS
Other complications (%)	5 (10.2%)	6 (16.7%)	NS

*: requirement for inotropic support for stabilization.
**: vomiting bile or bile withdrawn from the nasogastric tube.
NS: not significant

Figure: X-rays at birth



CAN WE PREVENT RECURRENCE AND CONTRALATERAL METACHRONOUS HERNIA IN THE PATIENTS AFTER LAPAROSCOPIC PERCUTANEOUS EXTRAPERITONEAL CLOSURE FOR INGUINAL HERNIA?

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PURPOSE: Recurrence and contralateral metachronous inguinal hernia (CMIH) are important postoperative complications in patients with inguinal hernia (IH) who undergo laparoscopic percutaneous extraperitoneal closure (LPEC). This study aimed to evaluate the incidence and causes of recurrence and CMIH after LPEC.
METHODS: This study included 2,308 patients who underwent LPEC from 2008 to 2019. Among the 2,308 patients, 176 had bilateral IH. Therefore 2,484 hernias were included in the analysis of recurrence. CMIH was analyzed in 2,094 cases with unilateral IH without a history of contralateral IH. More than 50 months have passed since the initial operation in all the cases. In cases of recurrence and CMIH, videos of the first and second surgeries were evaluated to categorize the causes.
RESULTS: Overall, 21 hernias (0.85%) recurred, and seven patients (0.34%) experienced CMIH. Based on the results of the video inspection, loose ligation was suspected as the cause of recurrence in eight cases (38.1% of recurrence), while injury and skipping of the peritoneum were suspected in five cases (23.8% of recurrence). These were considered preventable recurrences. The cause of recurrence could not be predicted in the remaining eight patients (23.8% of recurrences). Of the seven patients with CMIH, three (42.9%) had a mild depression around the internal inguinal ring, whereas a clear patent processus vaginalis was not confirmed. In the remaining four CMIH cases (57.1% of CMIH), no noteworthy findings were noted around the internal ring during the first surgery. All patients with CMIH developed an indirect inguinal hernia.
CONCLUSION: The incidence of recurrence and CMIH was consistent with that reported previously. The present study revealed that more than half of the recurrences, in which skipping of the peritoneum and loose ligation were suspected, could have been prevented by secure ligation during the first surgery. Extending the indications of prophylactic surgery for shallow depressions may reduce CMIH; however, the efficacy and safety of prophylactic surgery need to be further evaluated.

ROLE OF PERCUTANEOUS TRANSHEPATIC BILIARY DRAINAGE FOR MANAGING BILE LAKE FORMATION AFTER KASAI PORTOENTEROSTOMY

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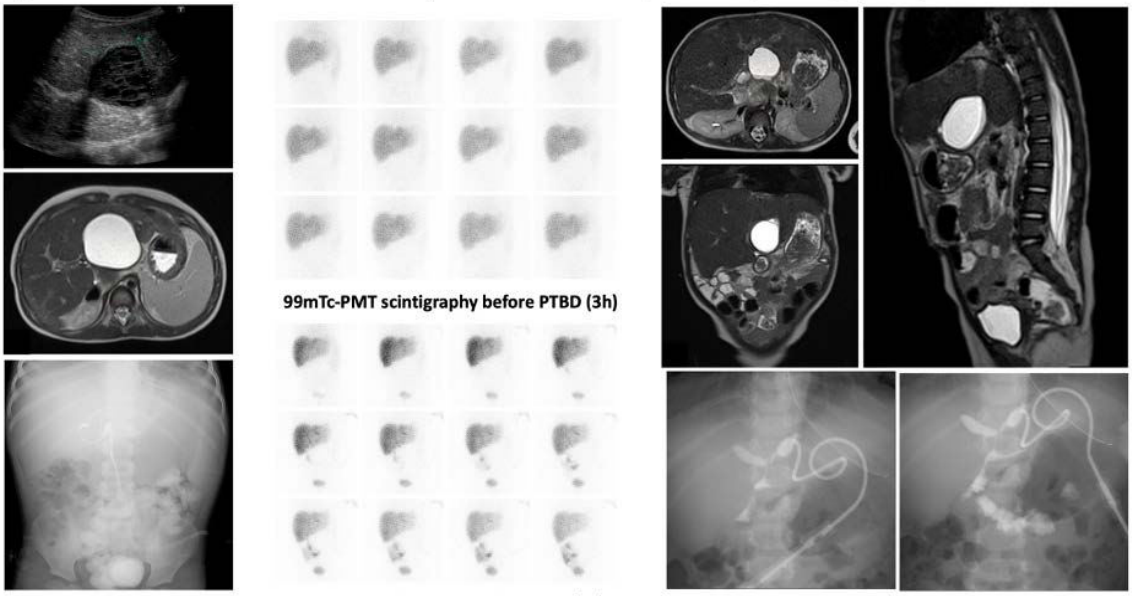
PURPOSE: Bile lake (BL) formation following Kasai portoenterostomy (KPE) can complicate the prognosis of biliary atresia (BA) patients by causing refractory cholangitis, potentially necessitating liver transplantation (LTx) due to deterioration in liver reserve. This study discusses the management of BL, with specific focus on percutaneous transhepatic biliary drainage (PTBD).
METHODS: The medical records of 64 KPE patients (open=31, laparoscopic=33) treated at a single center between 2004 and 2023 were reviewed retrospectively to identify BL cases. PTBD was indicated for BL cases presenting with jaundice or cholangitis refractory to antibiotic therapy and was performed under fluoroscopic and ultrasonographic guidance in collaboration with radiologists.
RESULTS: The incidence of BL was 14.1% (9/64 and did not differ significantly according to the type of KPE: 21.2% (7/33) for laparoscopic vs. 6.5% (2/31) for open (p=0.15). Median onset was postoperative day 273 (IQR: 170-920). One case resolved spontaneously following antibiotic therapy. Another case required redo KPE and eventually LTx, which removed the BL with the native liver. PTBD was performed in 7 of 9 patients (77.8%) at a mean of 20.7±18.7 months post-KPE, with successful resolution in 6 of 7 patients (85.7%). One case required repeated PTBD. Post-PTBD biliary peritonitis occurred in 3 of 7 patients 42.9% (3/7), necessitating emergency abdominal lavage.
CONCLUSION: Screening for BL is advisable in BA patients presenting with recurrent cholangitis and jaundice. PTBD is effective and has potential to prevent re-do surgery or LTx in patients affected by BL. Although the risk for complications such as biliary peritonitis warrants caution, PTBD seems a viable option for managing BL formation after KPE.

Figure: Two bile lake cases with successful percutaneous transhepatic biliary drainage (PTBD)



IMPACT OF SOCIOECONOMIC AND GEOGRAPHICAL FACTORS ON CLINICAL CARE OF BILIARY ATRESIA PATIENTS AFTER KASAI PORTOENTEROSTOMY OVER THE SHORT-TERM AND LONG-TERM DURATION: A CROSS-SECTIONAL STUDY

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(Chandigarh, India)

Purpose: Kasai portoenterostomy (KPE) is the initial and only available treatment for biliary atresia (BA) in low-middle-income countries. In this study, it was assessed whether socioeconomic status (SES) and geographical region (GR) have an impact on the continuation of the treatment and to make recommendations to mitigate the adverse factors.
Methods: Parents of the BA children who underwent KPE between 2018 and 2022 were interviewed. SES was assessed using the Kuppuswamy socioeconomic scale (KSES), while GR was divided into urban, rural, and remote areas according to the settlement profile. The clinical care parameters assessed were age-at-surgery, number of follow-up visits, frequency of cholangitis, loss to follow-up, liver transplantation, and mortality.
Results: A total of 43 patients were included in the study; according to GR, 10, 28, and 3 children were from urban, rural, and remote areas, respectively. According to KSES, 2,13,14,12 and 2 children were from the upper, upper middle, lower middle, upper low, and lower SES, respectively. During follow-ups at 3, 6,12, and 24 months, it was observed that the highest mortality was from the lower-middle SES group, while the maximum percentage of loss to follow-up was from remote areas. After 24 months, it was observed that 19 children were expired, 15 were lost to follow-up, and only nine were under follow-up; five and four from urban and rural, respectively, while 2,3,3 and 1 from upper, upper middle, lower middle, and upper lower SES, respectively. The patients who had better KSES scores presented at an early age, experienced less frequency of cholangitis and were available at most follow-up visits.
Conclusion: The mortality, morbidity, and loss to follow-ups were less in the urban and higher KSES groups compared to rural and remote groups with lower KSES. The better KSES and urban patients presented at an early age, came for regular follow-up, and had less frequent cholangitis. To improve survival; education, economic status, and access to hospitals should be considered in the planning and management of patient.

GLOBAL PREVALENCE OF ESOPHAGEAL ATRESIA AND ITS ASSOCIATED RISK FACTORS: A SYSTEMATIC REVIEW AND META-ANALYSIS

S. Yan, H. Liang, N. Ganji, Y. Huang, Z. Chen, T. Wu, Z. Lyu, J. Chen, A. Pierro, H. Zhu
(Fujian, China)

PURPOSE: Esophageal atresia (EA), a significant congenital anomaly affecting neonatal upper gastrointestinal tract, lacks comprehensive global epidemiological research. This systematic review aimed to determine the global prevalence of EA and identify potential risk factors.
METHODS: Following PRISMA guidelines, PUBMED, MEDLINE, The Cochrane Library, and Web of Science databases were searched for cross-sectional surveys and case-control studies on EA prevalence and associated risk factors published before January 2024. Studies were screened using inclusion/exclusion criteria, assessed for quality using AXIS and Newcastle-Ottawa scales, and analyzed via Meta-analysis in R 4.0.4 and RevMan 5.3. This review is registered with PROSPERO (CRD42024501506).
RESULTS: Fifteen cross-sectional surveys and twenty-one case-control studies were included. Spanning 22 countries, global EA prevalence ranged from 1 in 10,000 to 54 per 10,000 births, with Jordan reporting the highest prevalence **(Figure)**. The pooled EA prevalence was 2.67 per 10,000 births (95% CI 0.0001658-0.0004307), based on 6,038 EA cases among 24,071,820 births. Significant factors included maternal parity (nulliparous: pooled OR=1.65, 95% CI 1.49-1.82; >1 parity: pooled OR=0.63, 95% CI 0.57-0.70), maternal age (20-30 years: pooled OR=0.86, 95% CI 0.76-0.97; >30 years: pooled OR=1.23, 95% CI 1.09-1.30), and gestational age <37 weeks (pooled OR=9.72, 95% CI 8.42-11.23). Furthermore, this systematic review identified associations between EA risk and prenatal bronchodilator use (OR=2.39, 95% CI 1.23-4.66), clomiphene use (OR=2.3, 95% CI 1.3-4.0), prenatal urinary/genital tract infections (OR=1.31, 95% CI 1.01-1.69), and assisted reproductive technologies (singletons OR=6.8, 95% CI 2.8-15.0; multiples OR=2.2, 95% CI 0.9-5.2).
CONCLUSION: Current evidence indicates global esophageal atresia prevalence ranges from 1 to 3 per 10,000 births, with significant variability among countries. Global research on EA remains limited, with only 11% of countries contributing prevalence data. Maternal parity, maternal age, prenatal medication use, pregnancy-related infections, and premature birth emerged as significant risk factors. These findings provide a basis for developing strategies for EA prevention and management. Further studies from diverse geographic regions are imperative to enhance understanding, facilitate prenatal and postnatal counseling, and inform clinical practice.

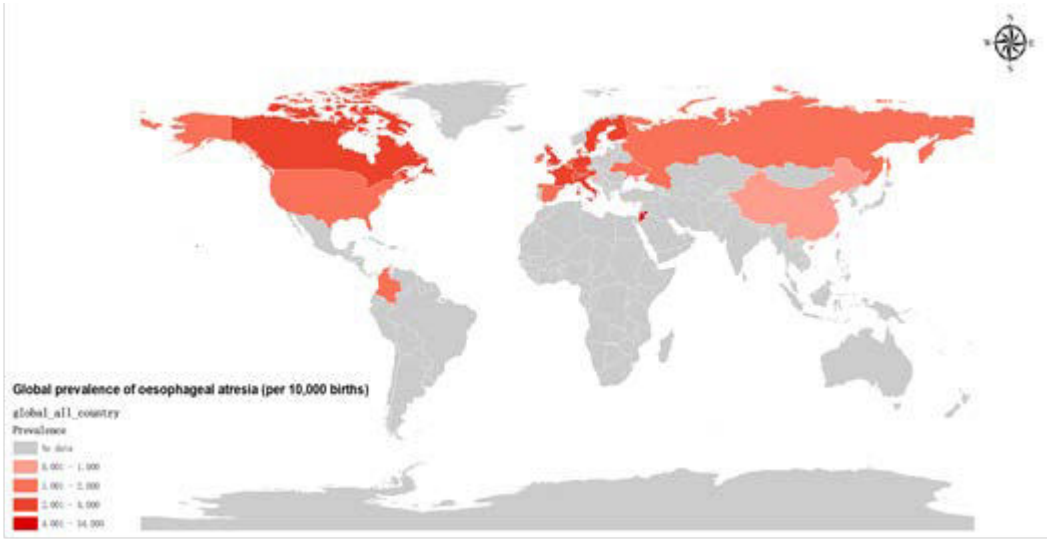

Figure Global prevalence of esophageal atresia.

LONG-TERM OUTCOMES OF FEMALE AND MALE CONGENITAL DIAPHRAGMATIC HERNIA SURVIVORS

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PURPOSE: There is limited data on biological sex-specific differences in congenital diaphragmatic hernia (CDH). Current literature reports conflicting data on the mortality and morbidity of female and male CDH patients. We aimed to explore the long-term outcomes of female and male CDH survivors.
METHODS: We linked a clinical database of congenital surgical anomalies to a population-level data repository to investigate the long-term outcomes of CDH survivors born between 1991-2022. We determined risk ratios (RR), plotted Kaplan-Meier curves for the time to first diagnosis and constructed Cox proportional hazard models to determine hazard ratios (HR). We controlled for socioeconomic status and CDH defect size. For recurrent events, we performed Poisson regression (RaR) and Cox proportional hazard modelling.
RESULTS: We included 96 CDH survivors with Bochdalek-type hernia (39 females and 57 males). There were no differences between females and males in the risks for intestinal obstruction (RR=1.48, p=0.39; HR=1.84, p=0.3; RaR=1.24, p=0.3), esophageal dysfunction (RR=0.75, p=0.43; HR=0.67, p=0.4), nutritional dysfunction (RR=0.83, p=0.20; HR=0.60, p=0.061) and skeletal anomalies (RR=0.82, p=0.73; HR=0.67, p=0.5). Female and male survivors had the same risk for asthma (RR= 1.01, p=0.98; HR=0.92, p=0.8) and acute respiratory infections (RR=0.87, p=0.12; HR=1.01, p>0.9). Male patients showed a trend towards an increased risk for pneumonia compared to female CDH survivors (RR=1.87, p=0.02; HR=2.02, p=0.063). Male survivors who had asthma or pneumonia had recurrent events more frequently as per the Poisson regression (RaR=2.25, p<0.001; RaR=1.39, p=0.007, respectively). The percent of female and male CDH survivors with neurodevelopmental delay (females 26%; males 34%) and mental health diagnoses (females 54%; males 53%) were high but did not differ between the sexes (neurodevelopmental delay RR=1.34, p=0.37; HR=1.15, p=0.7; mental health RR=0.98, p=0.9; HR=1.4, p=0.2). We are currently comparing the reported outcomes to a sex-disaggregated healthy control population which we aim to present.
CONCLUSION: Male CDH survivors may have more frequent recurrences of asthma and pneumonia. Otherwise, we observed no differences in the long-term respiratory, gastrointestinal, neurodevelopmental and mental health outcomes between female and male CDH survivors.

FIFTEEN YEARS OF SURGERY FOR CONGENITAL PULMONARY MALFORMATIONS (CPM): RETROSPECTIVE VIEW AND CURRENT VALUE OF THE SURGICAL APPROACH

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PURPOSE: To compare the thoracotomic and thoracoscopic approach in a large case series of infants undergoing surgery for congenital pulmonary malformations (CPM), focusing on perioperative outcomes.
METHODS: A retrospective study was conducted including all consecutive infants who had undergone surgery for a CLM in our pediatric tertiary referral center from 2007 to 2023, comparing perioperative outcomes between thoracotomy (Group A) and thoracoscopy (Group B).
RESULTS: Of the 113 patients treated in the period of study, the records of 76 were complete for our analysis. At the median age of surgery of 8.0 (5.0-13.0) months, 32 (42%) patients belonged to Group A and 44 (58%) to Group B. Although the operative and the anesthesiological time were significantly lower in infants in Group A [160.0 (135.0-185.0) minutes versus 191.5 (133.5-287.5) minutes, p-value: 0.048; 255.0 (230.0-306.0) minutes versus 339.5 (274.5-437.5) minutes, p-value: <0.001; respectively], the length of the NICU/PICU stay and hospital stay was significantly lower in infants in Group B [8.0 (2.0-23.0) days versus 2.0 (1.0-3.0), p-value: 0.003; 10.0 (7.0-18.0) versus 5.5 (4.0-10.5) days; p-value: 0.007; respectively]. Neither the incidence of intra- and post-operative complications [n=0 (0%) versus n=3 (8%), p-value: 0.20; n=8 (33%) versus n=11 (37%), p-value: 0.80; respectively] nor the need for re-do surgery [n=1 (4%) versus 4 (10%); p-value: 0.36] differ between the two groups of patients. However, in a median of 61 (35.0-100.0) months of follow-up, the occurrence of chest wall anomalies was significantly higher in group A [n=11 (42%) versus 7 (18%); p-value: 0.037].
CONCLUSION: Our series confirms that thoracoscopy is comparable to thoracotomy in terms of safety and efficacy, even if the smallest, most critical, and anatomically complex patients tend to be approached openly. We believe that thoracotomy should be reserved for fewer and fewer patients, also considering the higher long-term sequelae on the thoracic wall.

	Total (n = 76)	Group A Thoracotomy (n = 32; 42%)	Group B Thoracoscopy (n = 44; 58%)	p-value
Type of surgery, n (%)				
Lobectomy	42 (55%)	24 (75%)	18 (41%)	**0.032**
Segmentectomy	3 (4%)	0 (0%)	3 (7%)	
Wedge resection	18 (24%)	6 (19%)	12 (27%)	
Miscellanea	12 (16%)	2 (6%)	10 (23%)	
Postoperative diagnosis, n (%)				**0.002**
CPAM	22 (29%)	14 (44%)	8 (18%)	
Extralobar sequestration	9 (12%)	0 (0%)	9 (20%)	
Intralobar sequestration	14 (18%)	2 (6%)	12 (27%)	
Bronchial atresia	3 (4%)	1 (3%)	2 (5%)	
Bronchogenic cyst	3 (4%)	1 (3%)	2 (5%)	
Congenital lobar emphysema	5 (7%)	4 (12%)	1 (2%)	
CPAM + Sequestration	6 (8%)	5 (16%)	1 (2%)	
Other	1 (1%)	1 (3%)	0 (0%)	
Age at surgery (months)	8. 0 (5.0-13.0)	5.0 (0.0-10.0)	9.0 (6.0-16.0)	**<0.001**
Operative time (minutes)	174.0 (135.0-243.0)	160.0 (135.0-185.0)	191.5 (133.5-287.5)	**0.048**
Anesthesiological time (minutes)	298.0 (248.0-380.0)	255.0 (230.0-306.0)	339.5 (274.5-437.5)	**<0.001**
Extubation site, n (%)				0.53
NICU	3 (4%)	2 (6%)	1 (2%)	
PICU	7 (9%)	2 (6%)	5 (11%)	
Intraoperative complications, n (%)	3 (5%)	0 (0%)	3 (8%)	0.20
Bleeding	2 (3%)	0 (0%)	2 (5%)	0.46
Other	2 (3%)	1 (3%)	1 (2%)	
Chest tube, n (%)	70 (97%)	30 (100%)	40 (95%)	0.23
Postoperative complications, n (%)	19 (35%)	8 (33%)	11 (37%)	0.80
Transfusion, n (%)	11 (31%)	5 (28%)	6 (35%)	0.63
Postoperative NICU/PICU, n (%)	35 (97%)	17 (100%)	18 (95%)	0.34
Length of NICU/PICU stay, days	3.0 (2.0-8.0)	8.0 (2.0-23.0)	2.0 (1.0-3.0)	**0.003**
Length of hospital stay, days	8.0 (5.0-13.0)	10.0 (7.0-18.0)	5.5 (4.0-10.5)	**0.007**
Redo surgery, n (%)	5 (8%)	1 (4%)	4 (10%)	0.36
Postoperative chest wall deformities, n (%)	18 (28%)	11 (42%)	7 (18%)	**0.037**
Follow-up (months, median)	61.0 (35.0-100.0)	75.5 (47.0-112.0)	60.5 (25.5-84.5)	0.17

Table. Baseline characteristics and perioperative outcomes of infants undergoing surgery for CLM.

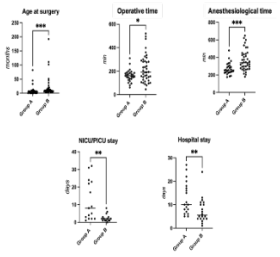

Figure. Baseline characteristics and perioperative outcomes of infants undergoing surgery for CLM.

THE ROLE OF TUMOR INFLAMMATORY MICROENVIRONMENT (TME) IN CONGENITAL LUNG MALFORMATIONS (CLM): UNCOVERING THE BASIS FOR POSSIBLE MALIGNANT TRANSFORMATION

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(Milan, Italy)

PURPOSE: This study investigates the role of the tumor inflammatory microenvironment (TME) in congenital lung malformations (CLM), focusing on activating inflammatory pathways critical for lung tumor development. The goal is to inform clinical management and decision-making regarding elective surgeries in asymptomatic infants by exploring the connection between CLM formation and the potential for malignant degeneration.
METHODS: CLM tissue samples from patients treated at Vittore Buzzi Children's Hospital were analyzed between January 2017 and June 2024. Histological analysis was conducted using H&E staining and markers for different types of inflammatory cells. Differences in inflammatory cell infiltration were compared between CLM and healthy lung tissues, considering CLM subtype, patient age at diagnosis, and history of lung infections.
RESULTS: The histopathological samples of 41 children operated on in our pediatric tertiary referral hospital were evaluated. The mean age at surgery was 9 months (IQR: 7-12). The histopathological diagnosis revealed 4 (10%) congenital bronchial atresia, 2 (5%) bronchogenic cysts, 10 (24%) congenital pulmonary airway malformations, 9 (22%) pulmonary sequestrations, 10 (24%) combined anomalies including CPAM and pulmonary sequestration, 6 (15%) complex parenchymal and vascular airway anomalies. Six (15%) infants have a previous history of recurrent respiratory symptoms. In comparison with healthy lung tissues, an infiltration of different inflammatory cells was identified in the resected specimens of children with CLM. This pattern was consistent across different CLM subtypes and was influenced by patient age and previous lung infections.
CONCLUSION: The study underscores the significance of examining the inflammatory response activation in CLM tissues, considering factors such as CLM type, patient age at diagnosis, and history of lung infection, to explore any potential link between CLM and malignant transformation. Investigating the TME may help determine if elective surgical resection in asymptomatic infants could be advantageous in reducing the risk of future complications, including malignancy. This research advances the understanding of CLM and provides a foundation for future studies focused on therapeutic interventions targeting inflammatory pathways.

PREOPERATIVE IMAGING ASSESSMENT OF THE RISK OF THE ADAMKIEWVICZ ARTERY INJURY IN LOWER MEDIASTINAL NEUROBLASTOMA

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(Tokyo, Japan)

PURPOSE: “Lower mediastinal neuroblastoma infiltrating costovertebral junction between T9 and T12 vertebral levels” was added as an image-defined risk factor because of the risk of the Adamkiewicz artery (AKA) injury. Since 2017, at our institution, we have been using preoperative imaging evaluation to assess the risk of the AKA injury and to develop a surgical strategy. We conducted a retrospective review of these patients to establish appropriate surgical treatment for neuroblastoma with the risk of the AKA injury.
METHODS: Nine patients with mediastinal neuroblastoma were treated between 2017 and 2023. Of 6 patients whose tumor located between T9 and T12, 4 patients (age at surgery; 19, 22, 52, 73 months) underwent surgery rather than a biopsy were selected. All patients were evaluated by multiplanar reconstruction CT (CT-MPR). To visualize the AKA more clearly, spinal digital subtraction angiography was performed in two patients. From preoperative image evaluation, it was determined which side the AKA was being fed from, and the ipsilateral tumor at risk was limited to partial or subtotal resection.
RESULTS: According to the international neuroblastoma risk group (INRG) staging system, there were three and one patients in stage L2 and stage MS, respectively. *MYCN* non-amplification was confirmed by initial tumor biopsy in all patients. All patients were classified into the intermediate-risk according to the INRG risk classification. Paraneoplastic symptoms included opsoclonus-myoclonus-ataxia syndrome in 2 patients and diarrhea caused by vasoactive intestinal peptide secretion in 1 patient. In one patient, it was confirmed preoperatively that the feeding artery of the AKA was on the contralateral side of the tumor, so complete resection was achieved. Three of four patients underwent subtotal resection of the ipsilateral tumor according to the preoperative evaluation of the AKA injury risk. One patient with paraplegia due to intraspinal tumor compression preoperatively was undergoing treatment for a tumor that has recurred. The other 3 patients had no paralytic symptoms and no tumor recurrence. All paraneoplastic symptoms disappeared postoperatively. The median postoperative follow-up period is 42.0 months.
CONCLUSION: Appropriate evaluation of preoperative imaging studies can avoid the risk of the AKA injury in lower mediastinal neuroblastoma.

ABDOMINAL COMPLIANCE OPTIMIZATION FOR LAPAROSCOPY SURGERY IN PEDIATRIC PATIENTS USING SWARM INTELLIGENCE

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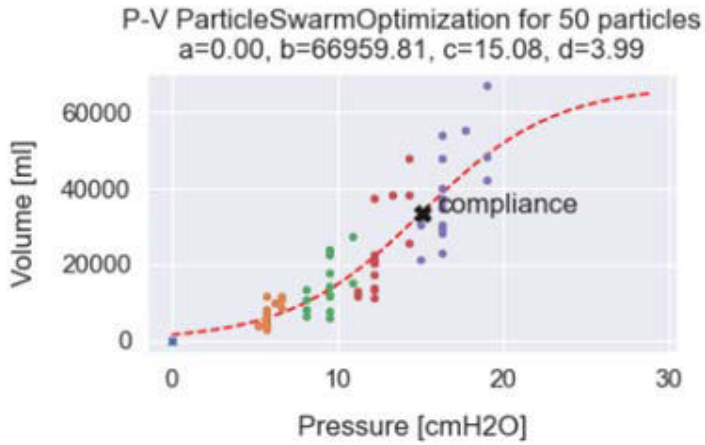
PURPOSE: In recent years, medical treatment has shifted from standardized methods to personalized medicine, tailored to individual clinical conditions and characteristics. While pediatric surgery has adopted this evolution, laparoscopy has lagged. Traditionally, the relationship between intra-abdominal pressure (IAP) and intra-abdominal volume (IAV) is treated as a single curve. This study proposes a new approach for pediatric laparoscopy, using individual data to estimate abdominal compliance, to improve safety and outcomes.
METHODS: Several optimization algorithms, including Trust Region Reflective, Dogleg, Levenberg-Marquardt and Particle Swarm Optimization (PSO) with 50, 100 and 200 particles, were applied to estimate abdominal compliance in cmH2O and the corresponding pressures in mmHg. Data were collected from 16 pediatric patients aged 7 to 18 years during laparoscopic procedures. The collected data included anteroposterior (A-P) length, bis-spino-iliac line length, xipho-bisiliac length, and pubic symphysis length. The pressure-volume curve was approximated with the Venegas equation, which represents a sigmoid. The performance of the algorithms was evaluated using pseudo-R^2, mean squared error (MSE), and root mean squared error (RMSE).
RESULTS: The Trust Region Reflective, Dogleg and Levenberg-Marquardt algorithms estimated compliance values of 19.03 cmH2O, with corresponding pressures of approximately 13.99 mmHg. However, they do not help in maintaining the correct physics of the problem because it was not possible to setup minimum and maximum pressure and volume values (including negative values). The PSO with 50, 100 and 200 particles produced slightly lower compliance values of 15.08 cmH2O, 15.03 cmH2O and 15.07 cmH2O, with pressures of approximately 11.08 mmHg, 11.05 mmHg, and 11.09 mmHg, respectively. However, such approach respects the physics of the problem. The PSO is, therefore, a better approach for estimating abdominal compliance. The comparison between 50, 100, and 200 particles does not change the performance. In this regard, is better to use the model with 50 particles because it is faster and smaller.



CONCLUSION: The PSO algorithm offers good performance and realistic behavior in estimating abdominal compliance. These results suggest that PSO is a valid optimization technique for estimating abdominal compliance in laparoscopic procedures, helping to reduce the risk of abdominal hypertension and improve patient clinical outcomes.

DIAGNOSIS AND TREATMENT OF CONGENITAL TRACHEOBRONCHIAL REMNANTS WITH OR WITHOUT THE PRESENCE OF ESOPHAGEAL ATRESIA

J.K. Youn, D. Ko, H.-Y. Kim
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Purpose: This study aims to investigate the diagnosis and treatment of pediatric patients with tracheobronchial remnants (TBR), comparing those with and without a history of esophageal atresia (EA).
Methods: A retrospective review of medical records was conducted for patients diagnosed with TBR and operated on at Seoul National University Hospital from January 2000 to December 2023. Data collected included demographic information, surgical details, and postoperative outcomes.
Results: During the study period, a total of 39 patients underwent surgery for TBR, with 9 of these patients having had prior surgery for EA. The median age at diagnosis was 1.7 years, with the EA+TBR group being younger (0.8 vs. 1.7 years, P=0.044), though the timing for surgery was not statistically different between groups (1.2 vs. 1.9 years, P=0.230). Vomiting was the most common symptom, observed in 31 patients (79.5%). The EA+TBR group had more associated anomalies compared to TBR-only patients (77.8% vs. 16.7%, P=0.001). All patients underwent a contrast study for diagnosis, and all lesions were located in the lower third of the esophagus. Open surgery was performed on 23 patients, while minimally invasive surgery (MIS) was performed on 16. Postoperative leakage occurred in 2 cases, one from each group, both of which improved with fasting and conservative treatment. Anastomotic stricture was found in 5 patients, with the EA+TBR group experiencing significantly more strictures than the TBR-only group (33.3% vs. 6.7%, P=0.038), all of whom underwent balloon dilatation. Postoperative gastroesophageal reflux (GER) requiring medication was present in 38.5% (55.6% vs. 33.3%, P=0.235) of the patients. The average z-scores for height and weight measured during follow-up were below average, at -0.46 and -0.81, respectively.
Conclusion: Vomiting is the most common symptom in TBR patients, and surgery for TBR yields reliable outcomes. Patients with EA were diagnosed earlier, and MIS was feasible for both groups. Postoperative support for growth and treatment for anastomotic stricture and GER is necessary.

ROLE OF PS6, COX2, MMP 9, MTOR IN CHOLEDOCHAL CYST MALFORMATIONS: AN IMMUNOHISTOCHEMICAL EVIDENCE

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(New Delhi, India)

PURPOSE: Cycloxygenase-2 and phosphorylated 40s ribosomal protein S6 (PS6) are two proinflammatory and proliferative molecules. This study aims to show the expression of these molecules in CDC malformations.
METHODS: A study on specimens of patients operated for CDC malformations (CM). Immunohistochemical (IHC) stains with antibodies to COX-2 and PS6 were done both on CM and gall bladder (GB) specimens following proper protocol. For standardization, intensity of IHC were scored: 0 (negative), 1(weak), 2 (moderate) and 3 (strong) positives and distribution was scored from 0 (negative), 1+ (<10 to 25%), 2+ (26-50%), 3+ (51-75%) and 4+ (76-100%) of cells. All the specimens were analyzed by a single pathologist. Chi square test was applied for the discrete variables and analyzed by STATA version 17.
RESULTS: Specimens from twelve (n=12) patients were analyzed. Mean age of the patients was 6.9 years with a female preponderance (M:F-1:3). PS6 IHC showed an overexpression, with high intensity signals from 7 specimens of CM versus 1 of GB specimen (p=0.001). There was a 4+ distribution in 9 CM specimens versus 1 in GB (p=0.0003). COX-2 however, was not expressed in most of the CM and GB specimens. 8 of CM and 10 of GB specimens showed 0 intensity of COX-2 (p=0.47). Similarly, 8 of CM and 11 of GB specimens showed 0 cell distribution of COX-2 (p=0.23).
CONCLUSION: Immunohistochemical staining demonstrated the overexpression of PS6 in specimens of CDC malformations. COX-2 however, was not overexpressed in the specimens. This finding provides an insight for the inflammatory and proliferative etiology of PS6 in choledochal malformations.

RECOGNITION OF FREE AIR ON NEONATAL ABDOMINAL RADIOGRAPHS USING A CONVOLUTIONAL NEURAL NETWORK

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Purpose: This study aimed to develop and assess the performance of a Convolutional Neural Network (CNN), ResNet50, in accurately recognizing free air on neonatal abdominal radiographs using limited image data.
Methods: In this diagnostic accuracy study, we retrospectively analyzed a dataset containing 924 abdominal neonatal X-rays from 177 patients. Among these X-rays, 26 were anteroposterior views showing *"Free-Air"*, 27 were lateral views showing *"Free-Air"*, 662 were anteroposterior views with *"No Free-Air"*, and 209 were lateral views with *"No Free-Air"*. A deep neural network, ResNet50, was trained on this dataset to distinguish between *"Free-Air"* and *"No Free-Air"* cases. The images were de-identified, and any text was removed to ensure the model focused solely on the relevant X-ray features. Given the data imbalance, we employed a stratified five-fold cross-validation technique to evaluate the model's resilience to data variations. No augmentation was applied. The model was trained over 200 epochs with a batch size of 64, using the Adam optimizer with a learning rate of 1e-5 and a cross-entropy loss function with label smoothing. The model was implemented in Python using the PyTorch library. The model's performance was evaluated using the Area Under the Receiver Operating Characteristic Curve (AUC-ROC).
Results: Using the anteroposterior views, our model predicted *"Free-Air"* with an AUC score of 0.67 (Fig.1). Using lateral views, the model achieved an AUC score of 0.72 (Fig.2). These results underscore the CNN's ability to accurately recognize the presence or absence of pneumoperitoneum on neonatal abdominal X-rays.
Conclusion: Our model performed better on lateral views than anteroposterior views. The model performance could improve adding more *"Free-Air"* cases and applying data augmentation techniques. However, considering the results achieved in the presence of data imbalance and with no data-augmentation, our findings suggest that the assessed CNN model presents a potential solution for improving the detection and decision-making process for the management of pneumoperitoneum in neonates. These findings could contribute to the expanding field of Artificial Intelligence-driven medical imaging, with potential applications for enhancing patient outcomes.

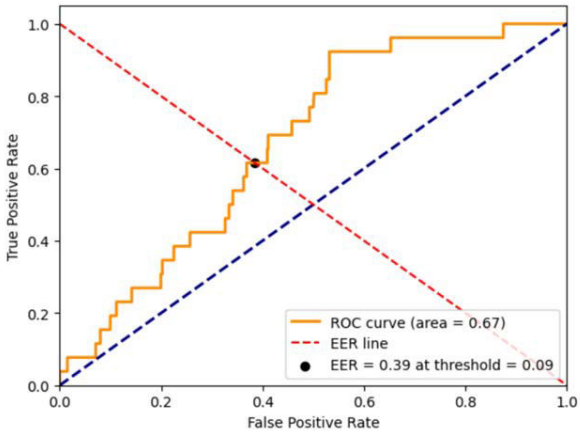

Figure 1. ROC Curve for Anterior View

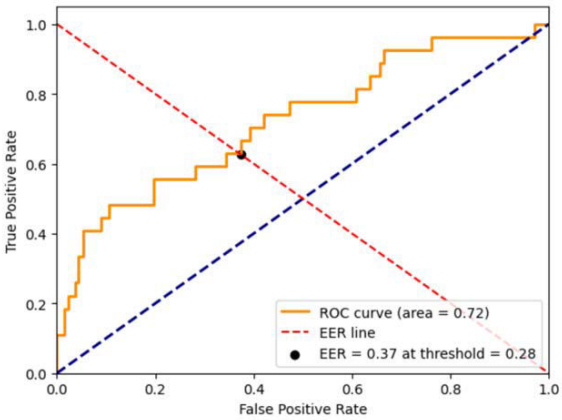

Figure 2. ROC Curve for Lateral View

VIRTUAL REALITY AND PATIENT-SPECIFIC-ORIENTATION IN PEDIATRIC SURGERY

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(Milan, Italy)

PURPOSE: Virtual reality (VR) promotes anatomical spatial orientation and three-dimensional (3D) preoperative navigation of an anatomical district, through a patient-specific orientation. As we know from the adult experience, VR increases surgeons' confidence by more than 60 times and modifies decision making in 10% of cases. We present the application of VR at our Pediatric Surgical Center.
METHODS: Our retrospective analysis of 3D VR models and pre-operative planning obtained in the period 2021-24 included patients affected by both malformative and tumoral pathologies. A preoperatively navigable model was created for each patient starting from 3D-reconstructed CT/MRI images. The model was viewed before, during and after the surgical procedure by the entire team, who were asked to list its advantages in relation to traditional imaging.
RESULTS: Forty-five VR models were generated, 32 for malformations (15 pulmonary, 7 bilio-pancreatic, 3 renal, 2 intestinal, 2 splenic cysts, 3 others) and 13 for neoplastic diseases (5 pulmonary, 5 neuroblastic, 2 hepatic, 1 renal). The models demonstrated a superior anatomical definition to that provided by CT/MRI for the pulmonary, hepatic and renal hilum. In 70% of cases it modified the surgical approach studied with traditional imaging, highlighting potential vascular incidents and defining the parenchyma-vessel relationships. VR clearly indicated the possibility of renal sparing, in contrast to CT; vice versa, VR visualization of the pelvic collector system and the ureter, compared to standard radiology, deserves further fine-tuning of the method.
CONCLUSION: The use of preoperative VR constitutes a moment of training and simulation for the reduction of intraoperative laparoscopic and open errors and offers a simulation model useful for the comfort of the surgical team.

ARTIFICIAL INTELLIGENCE FOR PEDIATRIC SURGICAL PLANNING: EXAMPLE OF A MATHEMATICAL MODEL

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(Milan, Italy)

PURPOSE: The management of operating rooms (OR) is one of the most studied topics in Operations Research applied to healthcare. Particularly, scheduling elective surgeries in a Children's Hospital can be a challenge, because disruptions can occur due to emergencies and no-shows. Aim of our research was to create a mathematical model to best schedule patients, considering possible disruptions and, in case something disrupts the plan, defining how to best manage the rescheduling process.
METHODS: Our study originates from a collaboration between a high-volume Pediatric Surgery Department and a University Department of Electronics, Information and Bioengineering. The disruptions we consider are: 1) emergencies (external inputs that may impact the planned schedule by delaying subsequent operations and occupying OR capacity); 2) no-shows (last-minute cancellation of a planned operation). Elective surgeries are scheduled considering the time spent on the waiting list and the patient's priority, based on the severity of their condition and their surgical deadline, generating a nominal schedule. This schedule is optimized in conjunction with a series of back-up schedules, to guarantee that OR activity immediately recovers in case of a disruption.
RESULTS: An Integer Linear Programming-based approach for the problem is proposed. We enumerate a representative subset of the possible emergency and no-show scenarios and for each of them a back-up plan is designed. The approach reschedules patients minimizing disruption with respect to the nominal schedule and applies an as-soon-as-possible policy in case of emergencies to ensure that all patients receive timely care. The approach shows to be effective in managing disruptions, ensuring that the waiting list is managed properly, with a balanced mix of urgent and less urgent patients.
CONCLUSIONS: This approach provides an effective solution for scheduling patients in a pediatric hospital, taking into account the unique features of such facilities.

SURGICAL OUTCOMES FOR NON-HIGH-RISK ABDOMINAL NEUROBLASTOMA IN RELATION TO THE RENAL VESSELS AND THE TUMOR

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(Tokyo, Japan)

PURPOSE: To evaluate the surgery of non-high-risk abdominal neuroblastoma, we investigated the surgical outcome based on the relationship between the tumor and the renal vessels.
METHODS: We performed a retrospective analysis of neuroblastoma cases operated at our hospital from March 2002 to December 2023 based on medical records. Cases who had surgical resection were divided into the following three groups according to imaging at the time of diagnosis and before operation; Group E: tumor encased in renal vessels, Group C: tumor contacted with renal vessels, Group S: tumor were separate from renal vessels.
RESULTS: Among 256 neuroblastoma cases during the applicable period, non-high risk 27 cases who underwent surgery rather than a biopsy for abdominal tumor (13 of whom received preoperative chemotherapy) were included, and the relationship between renal vessels and tumor was assessed using computed tomography. There were 16 boys and 11 girls, 385 (436-746) days old at initial diagnosis; International Neuroblastoma Risk Group was very low in 5 cases, low in 12 cases, and intermediate in 10 cases. There were no perioperative deaths and 2 deaths with an overall survival rate of 92.6%. The number of cases were group E 11, C 9, S7 At the time of diagnosis, and group E 5, C 14, S 8 before operation. There were no significant differences in operative time and blood loss between the three groups E, C, S and in either the time of diagnosis or before operation. The renal complications of combined concurrent renal resection and post-operative renal atrophy were five cases of group E at the time of diagnosis, two cases of group C and three cases of group E before operation. The odds ratio for renal complications was significant at 27.9 for group E at the time of diagnosis and 15.0 for group E before operation, while group C was not significant difference in both timings.
CONCLUSION: In the non-high-risk neuroblastoma, tumor encased in renal vessels cases have the highest risk of renal complications, followed by tumor contacted in renal vessels.

ERYTHROPOIETIN-CONTAINING HYDROGEL – A NEW "EPOCH" IN THE TOPICAL TREATMENT OF CHILDHOOD THERMAL INJURIES? A STUDY PROPOSAL

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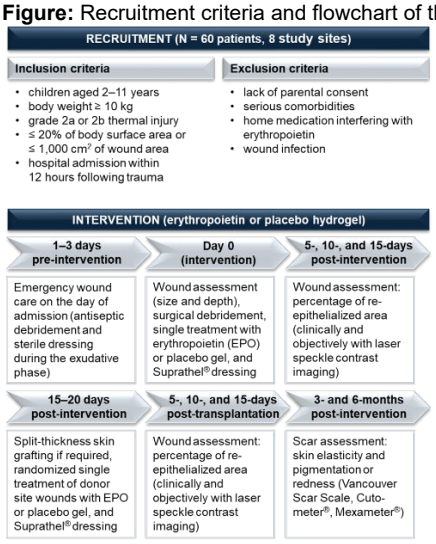
Purpose: Thermal injuries are a major cause of childhood morbidity and mortality worldwide. However, even with optimal wound care, adequate healing of deep thermal skin wounds is often not achieved. Currently, there remains a lack of topical treatment options for these injuries that specifically enhance skin regeneration during the first critical weeks following trauma. Erythropoietin, an endogenous hormone and clinically approved erythropoietic drug with tissue-protective, anti-inflammatory, anti-apoptotic, and pro-angiogenic off-label use properties, has emerged as a promising therapeutic agent to address this problem in adults. Yet, to date, this approach has not been systematically evaluated in children.
Methods: We present the design of the EPOCH (ErythroPOietin for CHildren) study, a multicenter, randomized, double-blind, placebo-controlled Phase IIa clinical trial to evaluate the efficacy and safety of erythropoietin-containing hydrogel (150 IU of recombinant human erythropoietin [NeoRecormon®] per 1 g of gel per 25 cm^2 of wound area) in the topical treatment of second-degree thermal injuries and split-thickness skin graft donor sites in children aged 2 to 11 years. In- and exclusion criteria and the study flowchart are shown in the figure.
Results: The primary endpoint of the study is defined as the percentage of re-epithelialized wound area on day 15 following topical treatment of grade 2b thermal injuries with erythropoietin-containing hydrogel compared to placebo-containing hydrogel. Secondary endpoints include the proportion of completely re-epithelialized sites, the degree of re-epithelialization of grade 2a injuries and split-thickness skin graft donor sites, local tissue oxygenation, and hemoglobin concentration on days 5, 10, and 15 post-intervention. Additionally, scar quality, skin elasticity, and pigmentation are assessed 3 and 6 months after treatment.
Conclusion: The randomized, controlled EPOCH study investigates a new, affordable, non-invasive therapeutic approach that may significantly improve the treatment of grade 2 thermal injuries in children. Through enhanced skin regeneration and more rapid wound healing, topical erythropoietin might reduce the need for surgery, shorten the duration of hospitalization, and minimize scar formation. We hypothesize that this novel therapy can ultimately help to prevent severe physical impairment and disfigurement of patients, along with their associated psychosocial sequelae.

Figure: Recruitment criteria and flowchart of the EPOCH clinical trial.



CONGENITAL DIAPHRAGMATIC HERNIA AND CLEFT LIP AND PALATE: LOOKING FOR A COMMON GENETIC ETIOLOGY

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(Stockholm, Sweden)

PURPOSE: Congenital diaphragmatic hernia (CDH) and cleft lip and/or cleft palate (CL/P) are midline closure defects occurring during the first trimester of pregnancy. The genetic factors influencing their co-occurrence and the outcomes for patients with both anomalies (CDH+CL/P) remain unclear. The aim of this study was to identify associated genetic abnormalities and to investigate the prevalence and outcomes for CDH+CL/P patients.
METHODS: Data from the Congenital Diaphragmatic Hernia Study Group (CDHSG) registry was collected. Prevalence of CL/P in CDH patients was determined. Chromosomal abnormalities and additional malformations in CDH+CL/P were explored. Patient characteristics and outcomes were compared between CDH+CL/P and isolated CDH (CDH-) using Fisher's Exact Test for categorical data, t-test for continuous data with a normal distribution, and Mann-Whitney U-test for continuous data with a skewed distribution. A p-value <0.05 was considered significant.
RESULTS: Genetic anomalies in CDH+CL/P included trisomy 13, 8p23.1 deletion, and 4p16.3 deletion. CL/P prevalence in CDH patients was 0.7%. CDH+CL/P had lower survival rates than CDH- (54.5% vs 77.7%, p<0.001) with a nearly fourfold increased risk of death within 7 days (CI 2.45-6.08, p<0.001). CDH+CL/P patients were less often supported with extracorporeal life support (ECLS) (15.2% vs 29.3%, p<0.001), had higher non-repair rates (32.3% vs 11.3%, p<0.001), and longer hospital stays (median 49 vs 35 days, p=0.003) compared to CDH-.
CONCLUSION: Genetic anomalies, such as trisomy 13, 8p23.1 deletion, and 4p16.3 deletion, are seen in association with CDH and orofacial clefts. CL/P in CDH patients is rare and is associated with poorer outcomes compared to CDH-, seemingly influenced by goals of care decision-making.

PUBLIC AWARENESS OF TISSUE ENGINEERING: LITERATURE REVIEW AND OESOPHAGEAL ATRESIA PATIENT-FOCUSSED ENGAGEMENT

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Purpose: Little is known about patient and public perspectives toward the complex and ethically sensitive concepts involved in tissue engineering (TE). We therefore conducted a systematic search to understand the extent to which published literature has explored this concept, prior to holding a patient engagement event to assess expert patient/carer attitudes towards the use of TE oesophageal grafts for long-gap oesophageal atresia (LGOA).
Methods: A systematic literature search using terms 'tissue engineering' AND 'public' AND ('awareness,' OR 'perspectives' OR 'attitude') was conducted (updated July 2024). A patient-focussed talk on the use of TE grafts in a pre-clinical porcine model of LGOA was delivered at an open access engagement event held by the UK Tracheo-Oesophageal Fistula Support (TOFS) group in November 2023. Pre-existing knowledge and attitudes towards TE were assessed among key stakeholders (patients >11 years, parents) by a live Mentimeter link. Data displayed as percentages responders for that question. Perceptions of TE were assessed pre- and post- seminar on a 5-point Likert scale, displayed as median (range), compared using the Mann-Whitney test ($p<0.05$ significant).
Results: Literature reporting on the degree of public awareness of TE was remarkably limited with only two outdated studies (2013) reporting awareness of the regenerative medicine concepts. Only one case study of TE cartilage use was described, where patient and lay citizen workshops highlighted differences between areas of importance between the two groups, with all parties reporting it to be a useful endeavour. Patients and parents in our survey (n=43) had a high overall awareness of TE (85% (n=43) and were receptive to TE approaches; 89% reported no concerns about the laboratory growth of their/child(s) cells. Perceptions of TE significantly improved after the presentation from 4 (2-5, n=32) to 5 (3-5, n=28) on a Likert scale, $p<0.0001$, with 96% declaring interest in involvement in a focus group for the development of a TE product for LGOA patients.
Conclusions: Patient perspectives towards TE and its approaches have not been well reported in the literature but were well received within our studied group. This highlights the importance of early patient involvement to guide development of first-in-human trials.

SUCCESS OF ANTEGRADE CONTINENCE ENEMA (ACE) IN PEDIATRIC PATIENTS WITH DEFECATION DISORDERS

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Purpose: We aimed to evaluate outcomes and success of antegrade continence enema (ACE) in pediatric patients with defecation disorders.
Methods: After ethical approval, 181 patients undergoing ACE procedure during 1997-2019 were included in this retrospective institutional study. Successful ACE treatment was defined by adequate bowel emptying and fecal continence.
Results: Median operative age (laparoscopic 80%) was 8 years (IQR 5-13). Main underlying etiologies included meningomyelocele (36%), anorectal malformations (31%), Hirschsprung's disease (14%), and idiopathic constipation (7%), and 21% had learning disability. Most prevalent surgical complications affecting 33% of patients included stenosis (19%), infection (18%), granuloma/mucosal prolapse (18%), and leakage (15%). 21% experienced problems with catheterization, emptying, pain, or motivation. Among 122 patients continuing ACE, 29% reported any fecal incontinence and 9% used protective aids. 14 patients were converted to enterostomy after failed ACE treatment (n=7) or additional surgery (n=7), 4 discontinued dysfunctional ACE, 1 opted for rectal washouts, and 1 were lost to follow-up. After median follow-up 8 years (IQR 5-11), successful ACE treatment was achieved by 122 (67%), of whom 38 had discontinued ACE usage as unnecessary after 5 (IQR 3-10) years. In univariable regression analysis, appendicostomy leakage (HR 2.4, p=0.047), but not underlying etiology or learning disability, predicted unsuccessful ACE treatment.
Conclusions: Our study underscores the efficacy of ACE in treating pediatric defecation abnormalities, with over two-thirds of patients achieving successful treatment response, and over one-fifth being able to successfully discontinue ACE usage after median 5 years. Appendicostomy leakage predisposed to unsuccessful ACE treatment.

SURGERY FOR CONGENITAL LUNG MALFORMATIONS (CLM) IN ASYMPTOMATIC INFANTS: A RETROSPECTIVE STUDY ON A LARGE COHORT OF PATIENTS

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(Milan, Italy)

PURPOSE: To report perioperative outcomes of infants with congenital lung malformations (CLM) undergoing surgery due to respiratory symptoms in comparison with asymptomatic peers.
METHODS: A retrospective study was conducted including all consecutive infants who had undergone surgery for a CLM in our pediatric tertiary referral center comparing perioperative outcomes between asymptomatic infants (Group A) and infants undergoing surgery due to respiratory symptoms (Group B).
RESULTS: Of the 113 patients treated in the period of study, the records of 68 were complete for our analysis. At a median age of surgery of 7.0 (4.0-11.0) months, 54 (79%) were included in Group A and 14 (21%) in Group B. While more infants in Group B underwent open procedures (Group A: n= 17 (31%) versus Group B: n = 7 (50%), p-value: 0.046) and the operative and duration of anesthesia were significantly lower in this group of patients [Group A: 170.0 (135.0-227.5) minutes versus Group B: 146.5 (90.0-155.0) minutes, p-value: 0.026; Group A: 302.0 (261.0-365.0) minutes versus Group B: 235.0 (179.0-245.0) minutes, p-value: <0.001; respectively], the length of the postoperative intubation [Group A: 1.0 (1.0-3.0) days versus 5.5 (3.5-7.0) days, p-value: 0.028], the length of NICU/PICU stay [Group A: 2.0 (1.0-5.0) days versus 23.0 (5.0-24.0) days, p-value: 0.013], and the length of hospital stay [Group A: 7.0 (5.0-11.0) days versus Group B: 16.0 (10.5-20.5)) days, p-value: 0.027] were significantly higher. Neither the incidence of intra- and post-operative complications [n=2 (6%) versus n=0 (0%); p-value: 0.54; 14 (35%) versus n=2 (33%), p-value: 0.94; respectively] nor the need for re-do surgery [n=3 (7%) versus 2 (18%); p-value: 0.26] differed between the two groups of patients. However, at a median of 55.0 (29.0-112.0) months of follow-up, a higher incidence of postoperative respiratory symptoms was reported in Group B [Group A: n=8 (23%) versus n=5 (62%), p-value: 0.028].
CONCLUSION: Our data confirm that differences exist in perioperative outcomes between symptomatic and asymptomatic patients, thus making prophylactic surgery preferable to conservative treatment in CLM.

	Total (n = 68)	Group A Asymptomatic (n = 54; 79%)	Group B Symptomatic (n = 14; 21%)	p-value
Age at surgery (months)	7.0 (4.0-11.0)	7.0 (5.0-10.0)	1.0 (0.0-37.0)	0.25
Type of surgery, n (%)				**0.046**
- Thoracotomy	24 (35%)	17 (31%)	7 (50%)	
- Thoracoscopic	28 (41%)	25 (46%)	3 (21%)	
- Laparoscopic	1 (1%)	1 (2%)	0 (0%)	
- Thoracoscopic & mini thoracotomy	2 (3%)	2 (4%)	0 (0%)	
- Laparotomy	2 (3%)	0 (0%)	2 (14%)	
Operative time (minutes)	160.0 (127.0-195.0)	170.0 (135.0-227.5)	146.5 (90.0-155.0)	**0.026**
Duration of anesthesia (minutes)	287.5 (243.0-344.0)	302.0 (261.0-365.0)	235.0 (179.0-245.0)	**<0.001**
Length of postoperative intubation (days)	2.5 (1.0-5.0)	1.0 (1.0-3.0)	5.5 (3.5-7.0)	**0.028**
Conversion, n (%)	4 (9%)	4 (10%)	0 (0%)	0.42
Intraoperative complications, n (%)	2 (5%)	2 (6%)	0 (0%)	0.54
Chest tube insertion, n (%)	54 (95%)	44 (94%)	10 (100%)	0.41
Postoperative complications, n (%)	16 (35%)	14 (35%)	2 (33%)	0.94
Postoperative diagnosis, n (%)				0.18
- CPAM	17 (25%)	14 (26%)	3 (21%)	
- Extralobar sequestration	10 (15%)	9 (17%)	1 (7%)	
- Intralobar sequestration	10 (15%)	7 (13%)	3 (21%)	
- Bronchial atresia	2 (3%)	2 (4%)	0 (0%)	
- Bronchogenic cyst	4 (6%)	4 (7%)	0 (0%)	
- Congenital lobar emphysema	4 (6%)	1 (2%)	3 (21%)	
- CPAM + Sequestration	5 (7%)	4 (7%)	1 (7%)	
Postoperative admission in NICU/PICU, n (%)	34 (100%)	27 (100%)	7 (100%)	
Length of NICU/PICU stay, days	3.0 (1.0-8.0)	2.0 (1.0-5.0)	23.0 (5.0-24.0)	**0.013**
Length of hospital stay, days	8.0 (5.0-15.0)	7.0 (5.0-11.0)	16.0 (10.5-20.5)	**0.027**
Respiratory symptoms after discharge, n (%)	13 (30%)	8 (23%)	5 (62%)	**0.028**
Redo surgery, n (%)	5 (9%)	3 (7%)	2 (18%)	0.26
Postoperative chest wall anomalies, n (%)	12 (23%)	8 (19%)	4 (44%)	0.094
Follow-up (months)	55.0 (29.0-112.0)	54.0 (28.0-112.0)	70.0 (47.0-84.0)	0.34

Table. Baseline characteristics and perioperative outcomes of infants undergoing surgery for CLM.

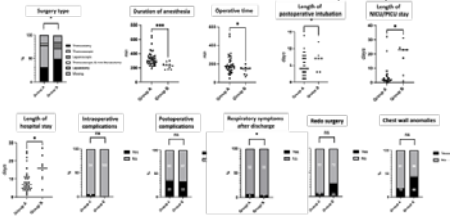

Figure. Perioperative outcomes of infants undergoing surgery for CLM, comparing asymptomatic (Group A) and symptomatic (Group B) children.

THE COMPLEXITIES OF FETAL ANALGESIA: INTEGRATING PHYSIOLOGICAL INSIGHTS AND CLINICAL ETHICS

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PURPOSE: To evaluate and synthesize current evidence on fetal pain and the clinical imperatives - surgical and anesthesiological - driving the practice of providing fetal analgesia during surgeries
METHODS: We employed a narrative review approach, analyzing existing evidence on fetal pain, including the development of neural structures, physiological responses to noxious stimuli, and concerns regarding the use of anesthetics and analgesics in such a vulnerable population. This review incorporated peer-reviewed articles, clinical studies, and expert opinions from physiologists, surgeons, and anesthesiologists.
RESULTS: The review identified significant physiological evidence suggesting that nociceptors develop as early as 7 weeks of gestation, with thalamocortical connections forming around 24 to 26 weeks. Early fetal responses to stimuli, such as increased heart rate and elevated cortisol levels, indicate distress even before full cortical development. The role of the subplate in sensory processing suggests some form of sensory experience before complete thalamocortical connectivity. Empirical data from fetal surgeries reveal physiological stress responses to noxious stimuli, advocating for the ethical imperative of administering analgesia. The pharmacokinetics and pharmacodynamics of analgesics in fetuses differ significantly from those in adults, necessitating careful selection and dosing. Anesthetists face complexities in ensuring both effective pain management and minimal risk to the fetus.
CONCLUSION: The debate over fetal analgesia encompasses a complex intersection of physiology, surgery, and anesthesiology. While physiological insights suggest a developmental timeline for pain perception, clinical practice emphasizes the need to minimize fetal distress during surgery, even in the early stages of development. An integrated approach, informed by ongoing research and interdisciplinary collaboration, is essential to ensure maternal and fetal well-being during fetal surgery.

ASSESSING THE ROLE OF ROUTINE POSTNATAL CHEST X-RAY IN ASYMPTOMATIC CHILDREN WITH CONGENITAL LUNG MALFORMATIONS

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Purpose: Despite its limited sensitivity, chest X-ray (CXR) is often the first postnatal imaging modality during the initial observational hospital stay of newborns with a prenatally diagnosed congenital lung malformation (CLM). The clinical implications of CXR in asymptomatic neonates with CLM are uncertain. We assessed the justification for the postnatal use of CXR in this population.
Methods: We included patients with CLM confirmed through chest computed tomography angiography (CTA) or histopathological analysis who were asymptomatic at birth, underwent routine postnatal CXR, and participated in our standard of care prospective structured longitudinal follow-up program. Children with major associated morbidities were excluded. Primary outcomes were the positive and negative predictive values (PPV and NPV) of CXR findings for symptom development at four weeks and six months of age. Secondarily, we sought to establish whether CXR findings were associated with undergoing additional diagnostics during the initial observational hospital stay or prolonged postnatal hospital admission.
Results: Among 121 included patients, CXR showed no abnormalities in 35 (29%), nonspecific abnormalities in 23 (19%), and probable CLM in 63 (52%). The PPV of CXR for symptom development at four weeks and six months was 0.05 and 0.25, respectively. Corresponding NPVs were 0.96 and 0.91. An association was identified between CXR findings and undergoing further diagnostics during the initial observational hospital stay (p= 0.047). The findings of these additional diagnostics did not influence subsequent clinical management. CXR findings were not associated with prolonged initial hospital stay (p= 0.40).
Conclusion: The routine postnatal use of CXR in asymptomatic patients with prenatally diagnosed CLM can be omitted. The low PPV of CXR for symptom development within the first four weeks and six months of life, coupled with the lack of impact of aberrant findings on subsequent clinical management, supports this approach. CXR remains useful in exploring underlying causes of unexplained neonatal clinical deterioration.

SPATULATED UMBILICAL CORD TECHNIQUE EMBRACING WHARTON'S JELLY MESENCHYMAL STEM CELLS FOR PRIMARY REPAIR OF GASTROSCHISIS: A MULTICENTER EXPERIENCE

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Purpose: The goal of the surgical management of gastroschisis is to return the bowel into the abdomen without jeopardizing the viscera. We aimed to prospectively analyze the outcomes of primary closure of gastroschisis using Spatulated Umbilical Cord technique (SUCT).
Methods: This pilot interventional study was conducted prospectively at 3 centres from February 2016 to February 2023 with follow up till February 2024. Inclusion criteria included those cases where primary fascial closure had risk of compartment syndrome (CS). Exclusion included those with intestinal atresia. Outcome measures were success rates (no CS), post-operative ventilation, days of parenteral nutrition (PN), time to full enteral feeds and complications. The SUCT involved opening the amnion layer from the base at 9 O'clock position, exposing the Wharton's jelly and 'spatulating' using longitudinal incision and then securing as a patch over the defect.
Results: The SUCT was conducted in 10 patients. It was successful in 80% (8/10). The median duration of mechanical ventilation in 8 patients was 3 days (range 2-6). There were two patients operated on the bedside who were not ventilated. Median time-to-start enteral feeds (n=9): 8 (2-11) days. Median time to achieve goal enteral feeding(n=9): was 20(5-48) days while partial PN (n=8) was given for 17(10-46) days. Regarding complications, 2 patients needed re-laparotomy after the development of CS. Application of silo with delayed closure was done in one of them and the other one died on the operating table during the re-laparotomy. Other complications included wound infection (2), intestinal obstruction (1), persistent umbilical hernia (1) and sepsis-associated mortality (1). At a median follow-up of 61 (36-92) months, 8 survivors were healthy.
Conclusion: Primary closure of gastroschisis using SUCT is safe, feasible, cost-effective with comparable outcomes to other techniques. Mesenchymal stromal cells from Wharton's jelly have wound-healing properties through paracrine signaling and enhanced migration of fibroblasts.

COMPARATIVE OUTCOMES OF LAPAROSCOPIC GASTROSTOMY IN PEDIATRIC PATIENTS: A WEIGHT-BASED ANALYSIS

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Purpose: We compared the outcomes of laparoscopic gastrostomy button insertion between patients weighing “less than 5kg” and “more than 5kg” to determine if weight influences complication rates and overall procedural success.
Methods: After REB approval, we conducted a retrospective chart review of all patients who underwent laparoscopic gastrostomy insertion between 2020-2023 at our institution. Primary outcomes were perioperative complication rates. Secondary outcomes included: length of the procedure, representation to the hospital or the need for further interventions within 30 days of the initial procedure. Fisher's exact test and t-test were used. *P* value <0.05 was considered significant.
Results: A total of 151 patients were included. Of which 40 patients were less than 5 kg. Mean weight at time of operation was 3.8 ± 0.6 and 11.5 ± 6.6 for the “less than 5kg” and “more than 5kg” group respectively. There were no significant differences in gender distribution, operation time, rates of major or minor complications, intraoperative issues, and hospital readmission rates within 30 days post-discharge between the two groups. The “less than 5kg” group showed a significantly higher prevalence of prematurity (62% vs. 12%, P=0.0001) (table 1). Tube dislodgement was the most common major complication.
Conclusions: Laparoscopic gastrostomy is a viable and safe procedure in pediatric patients regardless of weight. The lower weight and the higher prematurity rate in the “less than 5kg” group did not increase the complication rates, suggesting that laparoscopic gastrostomy can be safely performed in smaller, potentially more fragile pediatric patients.

Table 1: Demographics with primary and secondary outcomes of the two groups.

Parameter	Less than 5 kg *n*= 40	More than 5 kg *n*=111	P
Demographics			
Male, *n* (%)	22 (55)	52 (46)	0.4
Premature, *n* (%)	25 (62)	14 (12)	0.0001
Syndromic, *n* (%)	16 (40)	46 (41)	1
Outcomes			
Major complications, *n*	0	7	0.1
Minor complications, *n* (%)	4 (10)	15(13)	0.7
Intraoperative complications, *n*	0	1	1
Length of procedure, minutes (mean ± SD)	32 ±7.7	30 ±7	0.3
Representation to hospital within 30 days of discharge, *n* (%)	10 (25)	39 (35)	0.3
Contrast study within 30 days of operation, *n* (%)	4 (10)	11 (9)	1
Return to the operating room within 30 days of the procedure, *n* (%)	1	2	1

Minor Complications = Superficial infection, hyper granulation tissue, leaking, or feeding difficulty.
Major Complications = Tube dislodgement, gastrocolic fistula, prolapse, other organs injury, or death.
Intraoperative complication = posterior wall gastric perforation

ANAL CANAL DUPLICATION: CHRACTERISTICS, MANAGEMENT AND OUTCOMES OF A RARE MALFORMATION

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PURPOSE. Anal canal duplication (ACD) is a rare and inadequately characterized congenital anomaly with <100 reported cases in the literature. We aimed to review disease characteristics, surgical management, and outcomes of ACD.
METHODS. After ethical approval, all ACD patients treated in 2 tertiary referral centers by same surgeons from 2001-2023 were retrospectively reviewed.
RESULTS. Altogether, 10 patients, all females, were diagnosed at median age 29 months (IQR 7-74) (**Table**). In each case, ACD located in midline, posterior to anal opening resembling miniature normal anus. At presentation, 2 patients had infectious symptoms whereas others were asymptomatic. Diagnostics included fistulography, sacral x-ray, ultrasound, and more recently magnetic resonance imaging. ACD was associated with presacral sacrococcygeal teratoma (SCT) in 3 patients, of whom 2 had genetically confirmed Sotos syndrome and 1 Currarino syndrome. In all patients, ACD with or without associated SCT was excised using posterior sagittal approach. Histopathology showed epithelial and smooth muscle structures consistent with anal canal. Postoperatively, all but 1 patient with superficial wound infection recovered uneventfully. After median follow up of 40 months (IQR 3-110), 9 patients were symptom-free, while 1 patient with severe sacral dysplasia and Currarino syndrome had poor fecal continence and neurogenic bladder.
CONCLUSION. ACD is an important differential diagnosis when assessing anorectal disorders in girls. Careful preoperative evaluation is required to assess associated anomalies such as presacral SCT and sacral anomalies. Postoperative outcomes of isolated ACD were excellent, while associated sacral dysplasia/SCT may predispose to functional problems.

Table. Patient characteristics.

Patient	Age at diagnosis (months)	Age at surgery (months)	Associated anomalies	Preoperative symptoms	Surgery	Follow up (months)
1	6	7	no	none	PSE of ACD	107
2	47	48	no	none	PSE of ACD	127
3	-	5	no	none	PSE of ACD	40
4	27	28	no	none	PSE of ACD	-
5	73	81	no	none	PSE of ACD	1
6	8	11	no	none	PSE of ACD	2
7	0	2	SCT and Currarino	none	PSE of ACD and presacral teratoma	99
8	29	36	no	none	PSE of ACD	8
9	74	74	SCT, Sotos syndrome	infection, discharge	PSE of ACD and presacral teratoma	113
10	153	155	SCT, Sotos syndrome	infection, discharge	PSE of ACD and presacral teratoma	3

SCT = sacrococcygeal teratoma, PSE = posterior sagittal excision, ACD = anal canal duplication

INCIDENCE OF COVID-19 IN CHILDREN WITH CONGENITAL SURGICAL ANOMALIES DURING THE PANDEMIC

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PURPOSE: Children with congenital surgical anomalies may be at increased risk of infection due to their medical conditions and frequent medical interventions. This study aimed to compare COVID-19 infection and vaccination rates between these children and date-of-birth-matched controls.
METHODS: We performed a retrospective case-control study of children diagnosed with congenital surgical anomalies from 1991 to 2022. COVID-19 infection rates and vaccination status, available from an administrative dataset, were compared using Chi-squared tests. Additionally, we compared case- and control-mothers' COVID-19 infection rates and vaccination status to determine if the maternal outcomes may have contributed to the children's outcomes.
RESULTS: A total of 800 cases and 8000 controls were identified. We found significant differences in COVID-19 infection rates for specific anomalies compared to controls. COVID-19 infections occurred in 19% of children with gastroschisis compared to 8.7% of controls (p<0.001). Similarly, 16% of Hirschsprung disease cases had COVID-19 compared to 9.2% of their controls (p=0.03). In contrast, only 2.4% of children with congenital diaphragmatic hernia had COVID-19 compared to 9.5% of their controls (p=0.008). Vaccination rates were similar for all anomalies and their controls. Maternal COVID-19 infections were more common in case-mothers of children with anorectal malformation (19% vs 11%, p=0.017) and gastroschisis (17% vs 11%, p=0.013) than control-mothers. Vaccination rates were not significantly different except for case-mothers of children with congenital lung lesions (88% vs 74%, p=0.034).
CONCLUSION: Our results identified gastroschisis and Hirschsprung disease survivors as possibly being at increased risk of community-acquired infections. These results warrant further investigation, as lessons learned from the unique experience of COVID-19 should inform the care of all children in future pandemic events.

	Children's COVID-19 infections			Children's vaccinations		
	Cases	Controls	p	Cases	Controls	p
Anorectal malformation	13/95 (14%)	79/950 (8.3%)	0.08	47/95 (49%)	464/950 (49%)	>0.90
Congenital diaphragmatic hernia	3/125 (2.4%)	119/1250 (9.5%)	0.008	54/125 (43%)	643/1250 (51%)	0.08
Congenital lung lesion	6/44(14%)	30/440 (6.8%)	0.12	25/44(57%)	222/440 (50%)	0.40
Esophageal atresia	8/89 (9.0%)	77/890 (8.7%)	>0.90	40/89 (45%)	472/890 (53%)	0.15
Gastroschisis	37/198 (19%)	172/1980 (8.7%)	<0.001	90/198 (45%)	1003/1980 (51%)	0.20
Hirschsprung disease	15/92 (16%)	85/920 (9.2%)	0.03	46/92 (50%)	502/920 (55%)	0.40
Intestinal atresia	7/97 (7.2%)	68/970 (7.0%)	>0.90	42/97 (43%)	433/970 (45%)	0.80
Omphalocele	9/60 (15%)	49/600 (8.2%)	0.08	29/60 (48%)	292/600 (49%)	>0.90

※Chi-squared tests

CHILDREN WITH CONGENITAL SURGICAL ANOMALIES ARE MORE PRONE TO TRAUMA THAN CONTROLS

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Purpose: Childhood trauma is common and associated with significant mortality and morbidity. While children with congenital neurocognitive deficits are known to face a heightened risk of trauma compared to their healthy peers, the impact of congenital surgical anomalies (CSA) is understudied. This study aimed to assess whether children with CSA have higher rates of traumatic injuries compared to age-matched controls.
Methods: We performed a retrospective study of all children born with a CSA between 1991 and 2022. We compared the risk of cases with traumatic injuries to controls. The CSA included gastroschisis, omphalocele, esophageal atresia with or without tracheoesophageal fistula, Hirschsprung disease, congenital diaphragmatic hernia, intestinal atresia and anorectal malformation. We calculated the adjusted risk ratio (RR) and adjusted rate ratio (RaR) of overall trauma and specific mechanisms (burn, fall, assault, crush, struck, drowning and suffocation).
Results: A total of 799 cases and 7900 controls were included. Children with CSA were more likely to experience any traumatic injury (OR=2.19, $p<0.001$) and encountered traumatic events more frequently (RaR=2.00, <0.001) than controls. Although crushing, drowning and suffocation were infrequent in both cases and controls, CSA cases were more prone to falls (OR=1.70, $p=0.04$) without a significantly increased frequency (RaR=1.55, $p=0.08$). Moreover, cases were more likely to suffer from burns (OR=4.01, $p<0.001$) and experienced burns more frequently (RaR=3.53, $p<0.001$).
Conclusion: Children with CSA face an elevated risk of traumatic injuries, highlighting the importance of educating healthcare professionals and parents about preventative measures in this population.

POSTNATAL OUTCOMES OF PRENATALLY DIAGNOSED CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT

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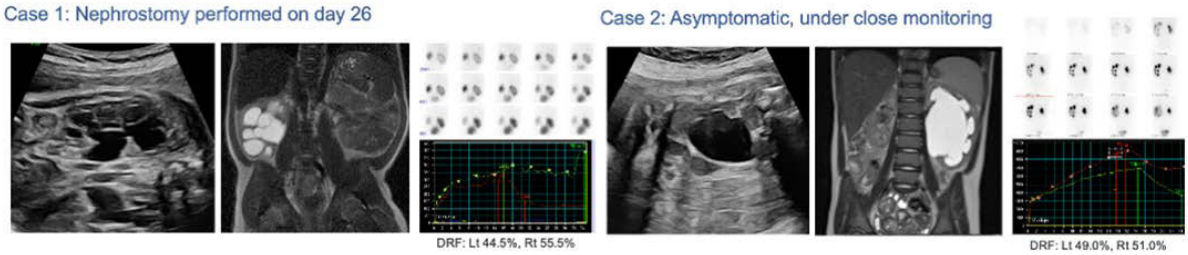
PURPOSE: Prenatal detection of congenital anomalies of the kidney and urinary tract (CAKUT) has increased with advancements in ultrasonography (US), but many do not actually require treatment after birth. Outcomes of prenatally diagnosed CAKUT were reviewed as there is a paucity of reports about the prognosis of CAKUT.
METHODS: CAKUT diagnosed prenatally between 2017 and 2023 (n=45) were reviewed, retrospectively.
RESULTS: Mean gestational age at the time of prenatal diagnosis was 27.1±5.5 weeks. CAKUT identified by US were renal pelvis dilation (29/45; 64.4%), cystic kidney (13/45; 28.9%), duplicated ureters (3/45; 6.7%), and ureter dilation (2/45; 4.4%). Some cases had multiple CAKUT. Anomalies were left-sided in 48.9%, right-sided in 28.9%, or bilateral in 22.2%. Postnatal outcomes were ureteropelvic junction obstruction (UPJO; n=14), multicystic dysplastic kidney (MCDK; n=11), duplex collecting system (n=4), other diagnoses (n=4), spontaneous resolution (n=5), and lost to follow-up (n=7). All patients with MCDK remained asymptomatic for a median of 5.1 (range: 2.8-6.6) years. For UPJO (n=14), 8/14 (57.1%) had pyeloplasty, 5/14 (35.7%) with hydronephrosis requiring urgent nephrostomies as neonates (figure), and one case had robotic-assisted pyeloplasty after becoming symptomatic after a urinary tract infection (UTI) when 2 years old. For duplex collecting system, 1/4 required nephrostomy, 1/4 underwent Deflux injection due to UTI, and 2/4 remained asymptomatic. Of the 29 cases with prenatal renal pelvis dilation, nephrostomy cases (n=8) were compared with non-nephrostomy cases (n=21). Differences in the gestational age at diagnosis were not significant (28.5±5.1 vs 28.1±4.6, p=0.72), but differences in the maximum anteroposterior diameter (APD) of the renal pelvis were significant (23.0±13.4 vs 13.7±7.4, p=0.013).
CONCLUSION: Prenatal US findings largely correlated with postnatal diagnoses. APD may have potential as an indication for surgical intervention in renal pelvis dilation patients.



LOW RATE OF COMPLICATIONS OF BALLOON DILATATIONS FOR ANASTOMOTIC STENOSIS IN ESOPHAGEAL ATRESIA PATIENTS: TIME TO CHANGE THE MANAGEMENT?

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PURPOSE: Anastomotic stenosis (AS) is a common complication after primary repair of esophageal atresia (EA). Some AS even require multiple balloon dilatations (BD) before achieving a persistent esophageal width, burdening families and hospital capacities likewise. Endoscopies with BD are usually in-patient procedures due to the potential risk of esophageal swelling and perforation. This study aimed to investigate the rate of complications of BD after EA repair and test the hypothesis, that BD may be performed as an outpatient procedure.
METHODS: In-house SOP (standard operating procedure) for endoscopy with BD for AS implies three postinterventional courses of intravenous antibiotics (Ampicillin/Sulbactam) with a mandatory overnight stay. A retrospective review of all patients with AS after primary EA repair undergoing BD from 01/01/2017-31/05/2024 was performed (study applied for ethical approval). All patient charts were screened for intra-, postinterventional and anesthetic complications and graded according to Clavien-Madadi score, with Grade I-IIIa being minor and Grade IIIb-V being sentinel events.
RESULTS: 45 EA patients (age-range 4weeks- 94months) were treated in our department during the study period. Of these, 17 patients (37.8%) developed AS requiring at least one BD. 12 of these 17 patients required ≥ three BD (70.6%). 108 BD were performed during the study period (dilatation range 6mm-16mm). During BD, in 65 cases (60.2%), slight mucosal bleeding but no esophageal perforation or relevant bleeding requiring blood transfusion was reported (Clavien-Madadi grade IB). No post-interventional bleeding or dysphagia was observed after BD. One patient (5.9.%) developed fever post-interventionally related to concomitant bronchoscopy with bronchoalveolar lavage during endoscopy (Clavien-Madadi Grade IB). Three patients (17.7%) experienced self-limiting anesthesia-related events (saturation drops, bronchospasm, Clavien-Madadi grade II) during endoscopy itself but not BD, with one patient experiencing severe apnea requiring resuscitation due to bacterial bronchitis (Clavien-Madadi grade IV).
CONCLUSION: No major complications attributed to BD were observed in our EA cohort with AS. An uneventful anesthesia provided, BD for AS in an outpatient setting is safe. In-house BD SOP including adaption of antibiotic prophylaxis to single-shot or oral application as well as post-interventional monitoring time will be reevaluated according to this data.

PRIMARY OBSTRUCTIVE NON-REFLUXIVE MEGAURETERS: ARE URETERAL DIAMETER AND VISIBLE PERISTALSIS ON ULTRASOUND VALID PREDICTORS FOR OUTCOME?

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Purpose: Primary obstructive, non-refluxing Megaureters (POM) are common among congenital malformations of the urinary system and defined as >7mm in prevesical diameter. Whilst they are associated with an increased risk of urinary tract infections (UTI), their prognosis as to spontaneous resolution is favorable.
However, predictive variables enabling non-surgical management on the one hand and timely intervention before loss of kidney function on the other hand are not available. This study aims to evaluate prevesical diameter at time of diagnosis during the first 8 months of life and presence of visible peristalsis on ultrasound for their potential to predict pyelonephritis and indication for surgery.
Methods: A total of 170 patients diagnosed with megaureter between 2012 and 2018 were included. After excluding refluxive, ectopic, secondary megaureters (n=81) and ureteroceles (n=26), 63 patients (female/male 24/76%) remained for analysis. Megaureter diameter was measured retrovesically, peristalsis was defined as visible, repetitive coaptation of the ureteral walls in pelvic sonography. Surgery was indicated after recurrent pyelonephritis, loss of renal function or lack of regression during follow-up. Parameters were analyzed using univariate tests and a stepwise multivariate logistic regression model.
Results: Median age at initial consultation was 1.57 months, median duration of follow-up was 34 months. During follow-up 49 episodes of pyelonephritis (36, 73% during 1st year-of-life, 11, 22% breakthrough infections, 2, 4% during second year-of-life or later) occurred in 45% (n=27) of patients and 17% (n=11) underwent ureteral reimplantation at a mean age of 21.5 (12-60, median 60) months. The mean ureteral diameter was 11mm (SD+/-4.5mm), peristalsis was present in 50.7% (n=32). Neither univariate analysis nor a stepwise, logistic regression model incorporating age, grade of hydronephrosis, ap-diameter and sex did show any association of diameter or peristalsis with pyelonephritis, breakthrough infection or surgery (ureteral diameter p=0.71, peristalsis p=0.54).
Conclusion: Prevesical ureteral diameter or the presence of peristalsis visible on ultrasound might not be useful predictors of the frequency of episodes of pyelonephritis or the probability of definitive surgery in primary obstructive megaureters for patients presenting under 1 year of age.

DO MICRORNAS HOLD THE KEY TO UNDERSTANDING HYPOSPADIAS?

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PURPOSE: MicroRNAs (miRNAs) are noncoding RNAs with vital regulatory role for eukaryotic gene expression. In the recent years, miRNA's regulatory role in hypospadias have posed interesting attention. Hypospadias is a malformation of the male external genitalia, characterizing by a displacement of the urethral meatus. This systematic review aimed to summarize what is known about the relationship between miRNAs and hypospadias.
METHODS: The search strategy consisted of both MeSH and free text terms, including hypospadias AND microRNA through PubMed and EMBASE. Three authors evaluated the studies identified through the search strategy based on titles and abstracts. The authors, then assessed the complete articles and selected studies conforming to the eligibility criteria. The original data on miRNAs associated with hypospadias were extracted from selected articles.
RESULTS: In five studies, the sample species were human while in two studies, they were mouse and rat and in one study both human and rat samples were applied. The human studies indicated the relationship between miR-145, miR-6756-5p, miR-182, miR-210, miR-143-3p, miR-566 and hypospadias. The animal studies suggested miR-145, miR-200c and miR-494 as critical regulators in hypospadias. All these human and animal studies showed these miRNAs can play a vital role in occurrence of hypospadias through different signaling pathways such as, TGF-β, MAPK, PI3K/AKT, AR, BMP, Insulin/IGF, and hypoxia (Figure).
CONCLUSION: MiRNAs could serve as potential biomarkers related to hypospadias and could play a role for potentiating wound healing after surgery. Further study can suggest a novel direction for understanding the etiology of hypospadias and improving treatment modalities.

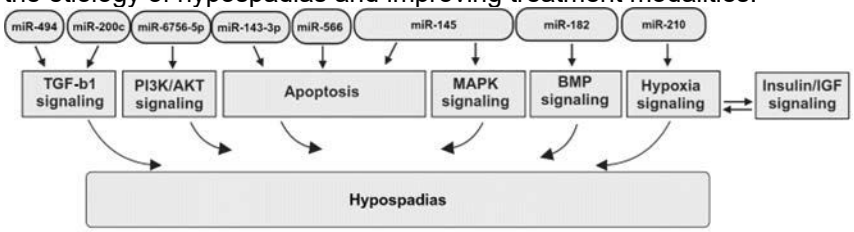

Figure. A schematic diagram presenting the regulatory role of different miRNAs in hypospadias.

DYNAMIC CHEST RADIOGRAPHY FOR PEDIATRIC PATIENTS: CLINICALLY APPLICABLE FOR THORACIC AND RESPIRATORY DISEASE?

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PURPOSE: The dynamic chest radiolography (Konica Minolta) is a real-time sequential high-resolution digital X-ray imaging system of the thorax. It captures videos of respiratory movements. In adult patients with respiratory disease, application for assessment of diaphragm and chest wall motion, measurement of pulmonary ventilation and perfusion are reported. However, application for pediatric patients is still unknown. The purpose of this study was to elucidate the clinical application of chest radiograpty dynamic analysis system for pediatric thoracic and respiratory disease such as congenital diaphragmatic hernia, congenital cystic lung disease, and pectus excavatum.
METHODS: We prospectively collected patients after surgical treatment for pediatric respiratory diseases and normal controls, and performed dynamic chest radiography in the upright position using a dynamic chest radiography analysis system. The radiography protocol consisted of still radiography and 10-second video recording under natural breathing. The changes in lung density associated with breathing and cardiac movement in the recorded videos were analyzed to evaluate the effective ventilation volume, pulmonary blood flow distribution, and the possibility of adhesion.
RESULTS: We have performed dynamic radiology evaluation in 20 patients including 7 cases of pectus excavatum, 4 cases of congenital diaphragmatic hernia, 2 cases of congenital cystic lung disease and in 5 normal controls., The median age was 11.5 years (range 2-19 years), and 6 were preschool children aged 6 years or younger. For preschool children, it took time and ingenuity to create an environment in which radiography was possible after entering the examination room, but evaluation was possible in all cases. The dynamic radiography conditions were 85-100 kV and 0.5-07 mAs, and the total entrance dose was within the amount of two plain chest radiographs. Analysis protocol for adult patients did not work in preschool children so that analysis protocol for children seemed necessary to evaluate the respiratory function in pediatric cases.
CONCLUSIONS: The dynamic chest radiography was applicable for pediatric patients. Data correction in larger number of patients is necessary to create the analysis protocol for small children, which will be useful for long-term follow-up of pediatric pediatric thoracic and respiratory disease.

IMPACT OF CONGENITAL HEART DISEASE ON ESOPHAGEAL ANASTOMOSIS IN CHILDREN WITH ESOPHAGEAL ATRESIA

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PURPOSE: Congenital heart disease (CHD) is one of the poor prognostic factors in esophageal atresia (EA), but the impact of CHD on esophageal anastomosis is unclear. We investigated the risk factors for esophageal anastomotic complications (EAC) in children with EA and CHD.
METHODS: Nineteen children with C-type EA and CHD treated at our hospital from 2000 to 2022 were retrospectively analyzed. The EAC were defined as suture failure, anastomotic stenosis or tracheoesophageal fistula reopening, and the patients were divided into two groups based on the presence or absence of the EAC. The patient backgrounds, operative findings, perioperative blood test findings, postoperative management of esophageal anastomosis, and outcomes were examined and compared between the two groups.
RESULTS: Among 19 patients, 6 (32%) developed the EAC. For the CHD, 15 (79%) had ductus-dependent cyanosis or an increased pulmonary blood flow. In all patients, EA surgery preceded CHD surgery after birth, and 6 (32%) opted for two-stage EA surgery. Esophageal anastomosis was performed at a median age of 3 days (days 0-234) and a median weight of 2254 g (1045-6625 g). There were more boys in the complicated group than in the uncomplicated group (83% vs 15%, p=0.01) and the preoperative PaO_2 was significantly lower (53.1 mmHg vs 69.5 mmHg, p=0.01). Intraoperatively, lower pH (7.29 vs 7.4, p=0.03) and higher $PaCO_2$ (51.2 mmHg vs 38 mmHg, p=0.006) were found in the complicated group than in the uncomplicated group, respectively. The postoperative median and minimum Hb were significantly lower in the complicated group (11.2 g/dL vs 13.7 g/dL, p=0.048 and 10.2 g/dL vs 11.8 g/dL, p=0.03).
CONCLUSION: Boys, low preoperative PaO_2, low intraoperative pH, high intraoperative $PaCO_2$ and low postoperative Hb can be risk factors for the EAC in C-type EA complicated CHD. With this knowledge in mind, it is important to optimize the perioperative status of the patient. Also, further observational research with larger numbers of cases is needed focusing on possible perioperative risk factors.

A 10-YEAR EVALUATION OF PEDIATRIC CHOLECYSTECTOMY

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Purpose: The incidence of gallstone disease within the pediatric population is rising, likely attributed to increasing obesity rates and improvements in diagnostic imaging techniques. This study evaluates the necessity of cholecystectomy in pediatric patients with gallstone, examining the correlation between clinical symptoms, diagnostic imaging, histological findings, and postoperative outcomes.
Methods: A retrospective cohort study was conducted at a tertiary pediatric institution, reviewing patient records from 2013 to 2023. This analysed patient demographics, preoperative symptoms, ultrasound findings, liver function tests, associated comorbidites, surgical techniques and postoperative outcomes. Data analysis was performed using R 4.4.0.
Results: The study reviewed 52 pediatric patients, of whom 67% were female, with an age range of 3-16 years (median age=13). Recurrent abdominal pain was reported by 94% of patients, while 6% were asymptomatic. Notably, 18 patients exhibited jaundice, of which 5 had pancreatitis and cholecystitis. Comorbidities were present in 46%, including sickle cell disease (29%), hereditary spherocytosis (12%), and metachromatic leukodystrophy (6%).
Ultrasonography identified gallstones in 92% (48/52) of the patients, excluding those with metachromatic leukodystrophy. Histological examination confirmed gallstones in 67% (35/52) of the patients, including two cases with negative ultrasound results. Preoperative ultrasound suggested cholecystitis in 8% (4/52) of the cases, whereas histology confirmed it in 69% (36/52), indicating ultrasonography's limited sensitivity (11.76%) and negative predictive value (40%) but its high specificity and positive predictive value (100%).
Laparoscopic cholecystectomy was performed in 94% (49/52) of the patients, with 25% (13/52) also undergoing splenectomy. Three procedures were converted to open surgery due to splenectomy complications. Postoperatively, 98% of the patients reported symptom relief, with only one patient experiencing recurrent symptoms, later diagnosed as irritable bowel syndrome. No other significant complications were observed during the follow-up period.
Conclusion: This study highlights a significant disparity between preoperative ultrasound findings and histological results. Nonetheless, cholecystectomy remains a safe and effective intervention for selected symptomatic pediatric patients, with the majority experiencing resolution of symptoms post-surgery.

UTILITY OF URETERAL DIAMETER RATIO FOR CLINICAL DECISION-MAKING IN CHILDREN WITH VESICOURETERAL REFLUX: A SYSTEMATIC REVIEW AND META-ANALYSIS

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PURPOSE: The prevalence of vesicoureteral reflux (VUR) in children is 0.4-1.8%. VUR can be conservatively managed or may require endoscopic or surgical management. There is no clear consensus for its management based on the severity of reflux. Distal Ureteral diameter ratio (UDR) has been recently investigated in several studies as a more objective parameter for prognosis and management of VUR. This systematic review and meta-analysis aims to investigate the usefulness of UDR as a tool to prognosticate and manage VUR.

METHODS: The literature search was conducted by two independent investigators according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Four scientific databases (PubMed, EMBASE, Web of Science, and Scopus) were systematically searched for relevant studies using the keyword “distal ureteral diameter”. Inclusion criteria were all studies in which UDR was used in prognostication and/or management of VUR. An independent assessment of the methodological quality was performed by two authors using the Newcastle Ottawa Quality scale. RevMan 5.4 software was used to perform the meta-analysis.

RESULTS: Thirteen studies were included in systematic review and eight were included in the meta-analysis. Majority of the studies were retrospective. Five, three, and three studies, respectively, analysed the association of UDR with the need for intervention, risk of breakthrough UTI (bUTI), and the chance of spontaneous resolution. Pooling of data demonstrated statistically significant association of UDR with need for intervention (WMD=0.10, 95%CI = (0.06,0.14), P <0.00001), the risk of bUTI (WMD=0.09, 95%CI = (0.07,0.11), P <0.00001), and spontaneous resolution (WMD=0.12, 95%CI = (0.06,0.19), P =0.0003). The estimated heterogeneity for all outcomes was high and statistically significant. The methodological quality of the included studies was good in the majority (12/13) of the studies.

CONCLUSION: The results of the present systematic review indicate that UDR can be used as an objective parameter to predict spontaneous resolution, risk of bUTI, and need for intervention in VUR. However, further studies are required to predict the cut-off values for these respective indications.

FACTORS INFLUENCING OUTCOMES IN NEONATAL ABDOMINAL SURGERY: A RETROSPECTIVE COHORT STUDY

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Neonatal abdominal surgery was often indicated from congenital conditions, presenting significant risks of morbidity and mortality. However, comprehensive surgical data and identification of complication-related risk factors still receive little attention.
Purpose: Our objectives were to characterize neonatal patients undergoing abdominal surgery and to identify prognostic factors influencing complications and mortality in those patients.
Methods: A retrospective study was conducted on newborn patients aged between 0-30 days undergoing index surgery at a university hospital from 2013 to 2021. Categorical data were compared between the without-complication group and the with-complication group using the Chi-square tests and Fisher-Exact tests. Continuous data were analyzed using Wilcoxon rank sum tests. A significance level of $P < 0.05$ was employed. Complications were defined as any events requiring intervention, either surgical or medical. Logistic regression was used to identify prognostic factors for complications and mortality.
Results: There were 154 neonates undergoing 178 index procedures. Nineteen patients had more than one condition. The 5 most common conditions requiring abdominal surgery included abdominal wall defect (31%), colorectal diseases (16%), intestinal atresia (14%), necrotizing enterocolitis (8%), and meconium peritonitis (7%), respectively. The incidence of patients with at least one complication was 44.81%, and the mortality rate was 7.74%. By using logistic regression, factors affecting complications included birth weight <1,000g (p=0.01), congenital diaphragmatic hernia (CDH) (p=0.04), and operative time >110 mins (p=0.03). Significant influences on mortality were birth weight <1000g (p=0.02), cardiovascular anomalies (p=0.02), CDH (p=0.001), and platelet count <100,000/ml (p=0.03).
Conclusion: This study provides valuable insights into factors influencing outcomes in neonatal abdominal surgery. Birth weight <1000g, CDH, and operative time >110 mins were identified as significant contributors to complications. Birth weight <1000g, cardiovascular anomalies, congenital diaphragmatic hernia, and platelet count <100,000/ml strongly influence mortality. These findings underscore the complexity of neonatal abdominal surgery with various conditions. Understanding these prognostic factors can predict the outcome of neonatal abdominal surgery. Improvement of their outcome may be achieved in the future.

LAPAROSCOPIC-ASSISTED URETERORENOSCOPY FOR STONE REMOVAL IN A 10-MONTH-OLD INFANT WITH CYSTINURIA AND ACUTE POSTRENAL KIDNEY FAILURE: A VIDEO CASE STUDY

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(Hannover, Germany)

PURPOSE: Urolithiasis in early infancy is a rare condition and management is difficult due to limited possible therapeutic approaches. In adults, several techniques for stone removal are available such as percutaneous nephrolitholapaxy, ureterorenoscopy and extracorporal shock wave lithotripsy. In young infants, some of these methods are difficult to perform due to smaller anatomy and limited availability of suitable instruments.
In the case presented, a 10-month old girl was referred to our center due to anuria and acute postrenal kidney failure. Bilateral hydronephrosis and kidney stones were detected on sonography. In this video, we present an unconventional approach for kidney and ureteral stone removal in a 10-month-old infant by laparoscopically assisted flexible ureterorenoscopy.
METHODS: Initial attempt to place bilateral DJ-stents was only successful on the right side. Subsequent laparoscopy was performed using a transumbilical approach. The left renal pelvis and proximal ureter were identified. After ureterotomy, a flexible 12Charr. ureterorenoscope (9.5Charr, LithoVueTM, Boston Scientific) was introduced. Subsequently, three stones were salvaged from the collecting system as well as two stones from the distal ureter using a stone basket. Due to the positioning of the ureterotomy a laparoscopic pyeloplasty was performed and a DJ-catheter was inserted. Stone analysis revealed cystinuria. Postoperatively, the initial creatinine level of > 300 µmol/l decreased to normal values within three days. Three months later, nine stones (three ureteral stones, six stones in the collecting system) were removed from the right side in the same fashion as described above.
RESULTS: At three months and one-year follow-up, the patient was stone free on sonography with regular morphology of the kidneys and the urinary tract. Under kidney stone metaphylaxis, no further symptoms occurred during follow-up to date (2.5 years).
CONCLUSION: Laparoscopic-assisted flexible ureterorenoscopical stone removal of the urinary tract in infants is feasible and safe. It represents a viable option for emergency treatment of acute renal failure due to urolithiasis. Here, we present a novel minimal invasive approach for kidney and ureteral stone removal in small infants as alternative for treatment, if other approaches for stone removal fail.

ELASTIC STABILE INTRAMEDULLARY NAILING IN THE TREATMENT OF DIAPHYSEAL FRACTURES IN CHILDREN

B.M.C. Evers, S. Minaev, A. Isaeva (Hannover, Germany)

Aim of the Study was to investigate the outcome of elastic stabile intramedullary nailing (ESIN) in children with diaphyseal fractures (DF) of bones.

METHODS

This was a retrospective cohort study. We analyzed the medical records and plain radiographs of 101 children with DF of the tibia (59.4%), femur (34.7%), or humerus (5.9%) (aged between 5 and 12 years) who were stabilized with ESIN. There were 69(68.3%) boys, girls - 32(31.7%). At follow-up conducted 4-25 months after surgery, we obtained an X-ray or CT.

MAIN RESULTS

Two nails of the same diameter were used. The nails were prebent such that the apex of the convexity was at the level of the fracture. We inserted the intramedullary rods in a retrograde fashion up to the level of the fracture line. Then, closed reduction, controlled by image-intensifier imaging, was performed. To counteract postoperative rotational forces at the site of the ESIN osteosynthesis, we put an immobilization bandage on a broken limb for two weeks.Main results line 7: Add - Therefore mobilization does not induce torsional forces on the fracture site. Patients are performing full weight-bearing in 2-3 weeks after surgery. Inflammatory complications were not noted.

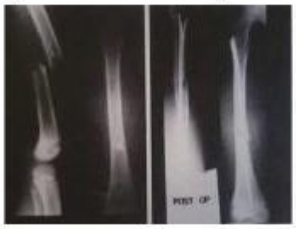
CONCLUSIONS

Thus, ESIN in children with DF of the tibia, femur, or humerus is a minimally invasive procedure that protects the soft tissue and produces large scars .Another advantage of this technique is avoiding growth- plate injuries and avascular necrosis of the metaphysis Remodeling is realized by piezoelectric currents inducing osteoclastic and osteoblastic activity we suggest.

RESULTS 2

REMODELING MECHANISM BY PIEZOELECTRIC ACTIVE CRISTALS AND CURRRENTS WITHIN THE BONY MATRIX

Velocity and completeness of remodeling highsignificantly depends on the grade and pressure generated by the deformity due to gravitational forces. In a varusdeformity it is quic and complete, in a rotationaldeformity it is slow and may be incomplete. This can be explained by the following Remodeling Mechanism: Piezoelectric active cristals - in general siliciumcristals - generate currents more intensive in the injured and more bended side than in the healthy side. These electric currents are conduced by afferent preganglionic parasympathetic fibers to the parasympathetic ganglion and measured. Then the parasympathetic ganglion by efferent postganglionic fibers is sending signals to regulate osteoclastic and osteoblastic activity in the side with more intensive electric currents wit consecutive remodeling til the electric activity in both sides - injured and healthy - is equal.



A SYNDROMIC TRIAD OF PERSISTENT UROGENITAL SINUS, HERLYN-WERNER-WUNDERLICH AND PRUNE BELLY SYNDROME IN A NEONATE

E. Grömping, H.C. Schmidt, J. Hagens, K. Reinshagen, C. Tomuschat
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Purpose: Despite their low frequency, congenital malformations are the cause of infant mortality and infertility. Diseases such as Prune-Belly syndrome (PBS), Herlyn-Werner-Wunderlich syndrome (HWWS) and persistent urogenital sinus (PUS) are even more rare. They are each associated with different comorbidities and have a high complication and mortality rate. A complex case with features of PBS, HWWS and PUS is reported here, with a focus on pediatric surgical care. The aim is to provide an exemplary basis for diagnostics and a therapeutic treatment approach to gain usable knowledge for the validation of surgical techniques and the optimization of case management.
Methods: Prenatal screening, a detailed postnatal clinical examination of the newborn and a trio exome analysis were performed, and the surgical procedures and postoperative follow-up were described in detail.
Results: This study presents a unique phenotype of the combination of PBS, HWWS and PSU and their treatment. Conservative and surgical interventions in the urogenital and gastrointestinal systems initially relieved the respiratory system and improved hemodynamics, facilitating the neonate's adaptation to postnatal conditions. Later, corrective interventions on critical anatomical malformations were added to improve physiological organ function. During the follow-up period, complications were mainly limited to acute infectious events and the effects of developmental delay. A trio exome analysis revealed no polymorphism in the child and the father, but a maternal polymorphism in plasminogen activator inhibitor-1 4G/5G.
Conclusion: Surgical interventions in pediatrics play a crucial role in the treatment of complex congenital anomalies. This case demonstrates the importance of a multidisciplinary approach and tailored surgical procedures and highlights the need for specialized surgical teams to achieve optimal outcomes. Future research should focus on the long-term outcomes of such surgical interventions to further refine and improve treatment protocols for complex multiple or solitary anomalies.

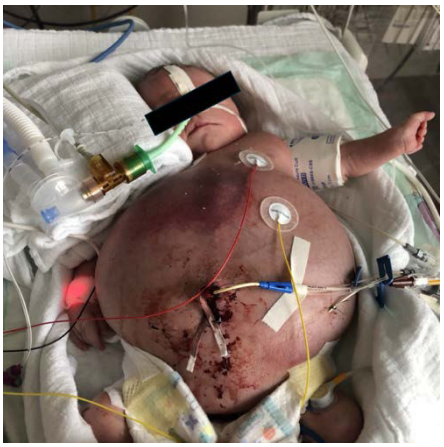

Fig. 1 Triad of syndromes consisting of Prune-Belly, Herlyn-Werner-Wunderlich and persistent urogenital sinus leading to urethral outflow obstruction and via the uterine tubes to intraperitoneal redirection and urinary ascites.

PROLONGED COMPLICATION INTUSSUCEPTION CAUSED BY MECKEL'S DIVERTICULUM IN 1 YEAR-OLD GIRL WITH LAPAROTOMY AND ANASTOMOSIS ILEO-COLICA: A CASE REPORT

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(Surabaya, Indonesia)

PURPOSE: Intussuception is serious condition with 10% nonidiopathic caused by pathologic anatomical lead points such as Meckel's Diverticulum. Meckel's Diverticulum is mostly asymptomatic in children, 30% of cases show symptoms. Treatment consists of non-surgical approach and surgical exploration. We reported intussuception caused by Meckel's Diverticulum with necrotic complication of small bowel.
CASE REPORT: 1 year-old girl presented at emergency-room with complaints of vomiting, initially diagnosed as GI-tract infection. On day-2 of treatment, there were signs of ileus obstruction and re-assessment was carried out, founded an acute intussusception, thus an urgent laparotomy-exploration was performed. During operation, Meckel's Diverticulum was found as leadpoint with necrotic ileal-segment. Ileal resection followed by end-to-end anastomosis and pathology anatomy sampling was done. She was discharged on day-6 without significant complications.

Figure 1 : X-ray Abdomen, showed obstructive ileus

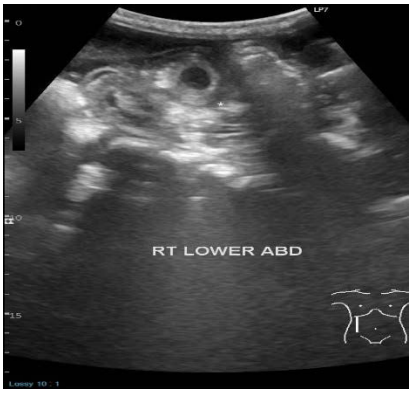

Figure 2: Ultrasound visualized hyperechoic intraluminal image, target sign in right abdominal, lateral umbilical, intususeption in colo-colica, and bowel dilatation.

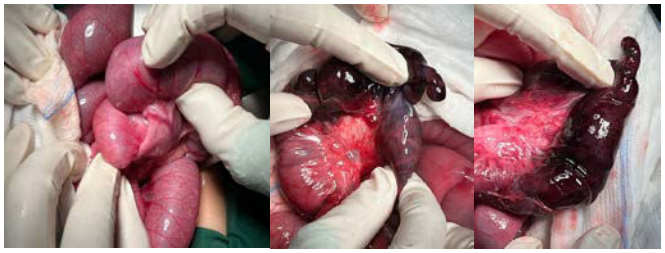

Figure 3: Exploration-laparotomy, small bowel dilated and ileo-ileocolica to colon ascendens intususeption, 40 cm to the caecum and found lead point diverticle meckle approximately to 20 cm. Distal decompression was carried out and ileum was resected 20 cm from necrotic lesions until viable ileum segments followed by end-to-end anastomosis.

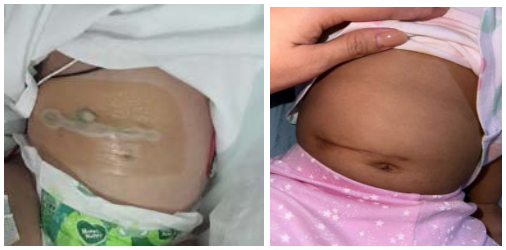

Figure 4: Abdominal photo 1 day and 1 month post-surgery.

DISCUSSION: Intussuception caused by Meckel's Diverticulum was identified intra operation with strawberry bloody stool thus appear 8 hours after intestinal obstruction occurs. Meckle's Diverticulum with necrotic complication is indication for emergency surgery exploration.
CONCLUSION: Delayed finding intussusception mostly found in necrotizing intestines which required resection with anastomosis could increase morbidity and complications. Early recognition is important to prevent complications of acute intussusception.