

**Long-gap esophageal atresia -  
The development of an experimental model of esophageal  
regeneration in vivo as an attempt to improve clinical outcome**

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av

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Avhandlingen baseras på följande delarbeten:

- I. **Jonsson, L. Friberg, L.G. Gatzinsky, V. Kötz, K. Sillén, U. Abrahamsson, K.**  
Treatment and follow-up of patients with long-gap esophageal atresia -15  
years' experience from the western region of Sweden.  
*Accepted for publication, October 2014, European journal of pediatric surgery.*
- II. **Jonsson, L. Gatzinsky, V. Jennische, E. Johansson, C. Nannmark, U.  
Friberg, L. G.**  
Piglet Model for Studying Esophageal Regrowth after Resection and  
Interposition of a Silicone Stented Small Intestinal Submucosa Tube.  
*Eur Surg Res, 2011. 46:169–179.*
- III. **Jonsson, L. Friberg, L. G. Gatzinsky, V. Jennische, E. Sandin, A.  
Abrahamsson, K.**  
Early regenerative response in the intrathoracic porcine esophagus-the  
impact of the inflammation.  
*Artificial Organs, 2014. 38: 439-46.*
- IV. **Jonsson L, MD, Dellenmark Blom M, RN, Friberg L-G, MD,  
Gatzinsky V, MD, Holmqvist O, MD, Jennische E, MD, Prof,  
Sandin A, MD, Abrahamsson K MD, Assoc Prof.**  
Macrophage phenotype is associated with the regenerative response in  
experimental replacement of the porcine esophagus.  
*Submitted for publication, October 2014, Artificial Organs*



# Abstract

**Background:** A subset (~8-15%) of the patients born with esophageal atresia (EA) suffer from a lack of esophageal tissue, which makes a primary anastomosis difficult to achieve. This is most common in patients born without a distal fistula between the trachea and the distal esophageal segment.

**Purpose:** To investigate the clinical course and outcome in patients born with long-gap esophageal atresia (LGEA) in the western region of Sweden and to develop an experimental model of guided tissue regeneration in the intrathoracic esophagus.

**Methods:** A retrospective study of 16 consecutive patients born with LGEA between 1995 and 2010 was performed. The patients had been followed according to a structured program at one and seven years of age. The experimental studies had been performed in growing piglets, where 3 cm of the intrathoracic esophagus had been replaced with a silicone stented Biodesign® mesh. The piglets were provided with a gastrostomy through a small midline laparotomy. Factors influencing the clinical and histological outcome had been recorded. In Paper II, six piglets underwent surgery, in Paper III, ten and, in Paper IV, six.

**Results:** No mortality was seen in the patients with LGEA. The mean age at definitive surgery was 147 days. The patients were small for gestational age. Eleven of sixteen (70%) had a delayed primary anastomosis as a definitive procedure, three had a gastroplasty and two underwent a colonic interposition. After surgery, anastomotic leakage was seen in seven of 16 (45%) patients and stricture developed in 11 of 16 (70%).

At follow-up, some catch-up in weight was seen at seven years of age, but no catch-up in stature was seen.

Spirometry performed at one and seven years of age showed obstruction or restriction in 9 of 14 (55%) measurements. The spirometry findings did not indicate any further need for surgery. Multiple breath washout was within the normal range in 11 of 15 (75%) measurements at one and seven years of age. Three of four (75%) of the patients with a pathological lung clearance index (LCI) at multiple breath washout required further surgery to prevent pulmonary damage due to aspiration. All patients either underwent surgery or were receiving continuous medical treatment for gastroesophageal reflux, and 7 of 16 (45%) had gastrostomy at the end of the study period. All patients were able to drink orally, but two of 16 (13%) were unable to eat solid foods.

In the first experimental study (Paper II), six animals lived for one to 17 weeks after surgery. Four animals were alive for at least four weeks and in two of them (50%) the stent was lost prior to four weeks. Piglets that lived longer than four weeks had recurrent stricture and required dilation. Histology showed connective tissue and intense angiogenesis in four piglets. In two of them, living four and 17 weeks respectively after surgery, the bridging area contained islets of immature-looking cells in the submucosa. The remaining three piglets only had inflammatory cells and fibrosis in the bridging area.

In Paper III, the piglets lived for three to 10 days after surgery. Three of 10 (30%) animals were sacrificed prior to plan due to mediastinitis. The surgical method was developed in such a way that the bridging graft could be sewn without leakage. If there was no significant leakage, the bridging graft was macroscopically surrounded by a tissue tube that connected the native esophageal edges. Histology showed connective tissue and inflammatory cells with intense angiogenesis in the bridging area. In addition, a thin layer of smooth muscle cells was seen around the bridging graft. In the piglets with significant leakage, there was an aggressive inflammatory pattern, with macrophages in the native muscle layers and islets of lymphocytes in the bridging area.

In Paper IV, all the piglets survived until sacrifice 20 days after surgery. In two of six (33%) piglets, there was stent loss prior to sacrifice. In the animals with a retained stent, the tissue tube between the native muscle edges was easily dissected macroscopically. If the stent was lost, the bridging area was narrow and attached to the surrounding tissues with firm adhesions. Histology in the piglets with a retained stent showed that the bridging area was organized in three layers with islets of smooth muscle cells organized in two layers in the wall. CD163-positive, M2 macrophages were seen close to the lumen. In those animals in which the stent was lost, the organization into three layers could not be seen. There were no M2 macrophages in the specimen, but calprotectin-positive, M1 macrophages could be seen throughout the wall of the bridging area.

**Conclusion:** Patients in our study born with LGEA required long hospitalization and suffered from symptoms related to gastroesophageal reflux during childhood. These individuals were small for gestational age. Some catch-up in weight was seen at seven years of age, but no catch-up in stature was seen. Multiple breath washout might be a valuable tool for the early detection of aspiration into the lungs in this patient group.

The experimental model for replacing a part of the intrathoracic esophagus in growing piglets showed that a remodeling inflammatory pattern, accumulation of muscle cells and a structured overall organization in the wall of the bridging graft can be achieved under favorable conditions. Leakage in the anastomoses and stent loss prior to 20 days changed the inflammatory profile and gave rise to scar tissue formation and stricture. Future studies are needed in order to see whether these differences account for a regenerative healing process, with functional tissue reforming in the esophagus.

**Keywords:** long-gap esophageal atresia, pulmonary physiology, esophageal replacement, guided tissue regeneration, macrophage phenotype, extracellular matrix

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