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ETIOLOGICAL ASPECTS OF ESOPHAGEAL ATRESIA

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Stockholm 2010

Published by Karolinska Institutet. Printed by Repro Print AB

ISBN 978-91-7409-838-9

Till Niclas, Wilhelm och Märta

ABSTRACT

Esophageal atresia (EA) is a severe congenital malformation, characterized by a discontinuity of the esophagus. To identify possible preventive measures, it is important to understand the etiology of EA, but little is known about risk factors. The principal aim of the present thesis is to contribute to a better understanding of the etiology of EA. The role of potential etiological maternal risk factors for EA in the infant has been approached. Studies I and II also concerned aspects of EA that warrant being addressed in a large and population-based investigation, covering the incidence, mortality, and cancer risk as well as the characteristics of an unselected cohort of patients. EA is rare, which makes it difficult to study. In Sweden, however, there is a unique possibility of conducting large population-based studies through the nationwide registers available. In all studies included in the present thesis, Swedish nationwide population-based registers were used, linked through personal identity numbers.

In study I, a population-based cohort study of 1,126 EA patients, the incidence of EA and the associated mortality and cancer risk were assessed. The mean incidence was found to be 3.16 per 10,000 live births, without any temporal changes (p for trend=0.94). EA patients had an almost 12 times higher risk of mortality compared to the background population (SMR 11.8, 95% CI 10.3-13.5). Survival improved substantially, however, during the study period (p for trend=0.0001). Occurrence of associated anomalies and very low birth weight were linked with a worse prognosis. Although uncertain, the risk of cancer did not seem to be increased in patients operated on for EA (SIR 0.9; 95% CI 0.2-2.6).

Studies II, III, and IV were all population-based, nested case-control studies, including over 700 cases of EA, conducted to assess the association between selected maternal exposures and the risk of EA in the infant.

In study II the risk factors maternal parity, age and ethnicity were approached. There seemed to be an increased risk of EA among infants of mothers having their first delivery. An over 30% decrease in risk of EA was found for mothers delivering their second (OR 0.68; 95% CI 0.56-0.83) or third child (OR 0.64; 95% CI 0.49-0.83), compared to first time mothers. The risk of having an infant with EA was found to increase with maternal age. Infants of women giving birth when 35-40 years and >40 years old showed a 2-fold (OR 2.09; 95% CI 1.09-3.99) and 3-fold (OR 3.04; 95% CI 1.37-6.74) increase in risk of EA, respectively, compared to those of mothers <20 years. There was a 66% increase in risk of isolated EA in infants of mothers of Caucasian (OR 1.66; 95% CI 1.06-2.61), compared to non-Caucasian ethnicity. In study II the characteristics of an unselected cohort of infants born with EA were described. Infants born with EA had a lower birth weight and were more often prematurely born, of male gender and twins, compared to infants born without this malformation.

In study III the potential maternal risk factors tobacco smoking, obesity and low socioeconomic status were assessed. No associations were found between these exposures and the risk of having an infant with EA.

In study IV we addressed the risk of having an infant with EA among women with diabetes. Maternal diabetes during pregnancy seemed to increase the risk of EA in the child. The adjusted risk of EA was 70 % higher among infants of women with diabetes than among those of women without the disease (OR 1.7; 95% CI 1.0- 2.9).

LIST OF PUBLICATIONS

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals (I-IV).

I. Jenny Oddsberg, Yunxia Lu, Jesper Lagergren
Aspects of esophageal atresia in a population-based setting: Incidence, mortality, and cancer risk
Manuscript submitted

II. Jenny Oddsberg, Chongqi Jia, Emma Nilsson, Weimin Ye, Jesper Lagergren
Influence of maternal parity, age and ethnicity on risk of esophageal atresia in the infant in a population-based study
Journal of Pediatric Surgery (2008) 43, 1660–1665

III. Jenny Oddsberg, Chongqi Jia, Emma Nilsson, Weimin Ye, Jesper Lagergren
Maternal tobacco smoking, obesity, and low socioeconomic status during early pregnancy in the etiology of esophageal atresia
Journal of Pediatric Surgery (2008) 43, 1791–1795

IV. Jenny Oddsberg, Yunxia Lu, Jesper Lagergren
Maternal diabetes and risk of esophageal atresia
Manuscript submitted

CONTENTS

LIST OF ABBREVIATIONS

BMI - body mass index
CI - confidence interval
EA - esophageal atresia
GER – gastro-esophageal reflux
HR - hazard ratio
ICD - International Classification of Diseases
IVF – in vitro fertilization
OR - odds ratio
SES - socioeconomic status
SIR - standardized incidence ratio
SMR - standardized mortality ratio
TEF – tracheoesophageal fistula

INTRODUCTION

Esophageal atresia (EA) is a severe congenital malformation, characterized by a discontinuity of the esophagus. It is the most common congenital malformation of the esophagus. Many infants with EA also have other associated anomalies.[1] EA demands major surgery in the newborn infant. The survival seems to have improved over the years, but gastrointestinal or respiratory morbidity is still frequently seen after EA repair and might contribute to a decreased quality of life.[2]

To identify possible preventive measures, it is important to identify why EA develops. The etiology, however, remains poorly understood. Data from twin and family studies suggest that hereditary factors do not play a major role.[1] Environmental risk factors thus seem to be important in the etiology of EA. There are, however, few studies addressing the role of environmental exposures of women during pregnancy and the risk of EA in the infant and in the studies conducted the results have been conflicting. The principal aim of this thesis is to contribute to the understanding of the seemingly heterogeneous and complex etiology of the malformation EA.

BACKGROUND

HISTORY

The first documented case of EA, confirmed at post-mortem examination, was recorded by Gibson in "The anatomy of the humane bodies epitomized" in 1697. It was described as follows "About November 1696, I was sent for to an infant that would not swallow. The child seemed very desirous for food, and took what was offered it in a spoon with greediness, but when it went to swallow it, it was liked to be choked, and what should have gone down returned by the mouth and nose…"[3]

It was not until 1939 that the first children born with EA survived. In 1941 Cameron Haight in the United States performed the first successful primary repair of the esophagus and in 1948 EA was operated on for the first time in Sweden. [3, 4]

ANATOMY/ CLASSIFICATION

EA is a group of congenital anomalies characterized by a discontinuity of the esophagus. There are several different anatomical variants of EA. They have been classified by Vogt (numbers+ lower case letters) and Gross (capital letters) on the basis of the type of atresia and the presence or absence of a fistula to the trachea, a so called tracheo-esophageal fistula (TEF) (Fig 1.).[5] In this thesis, all types of EA, with or without TEF, are referred to as EA.

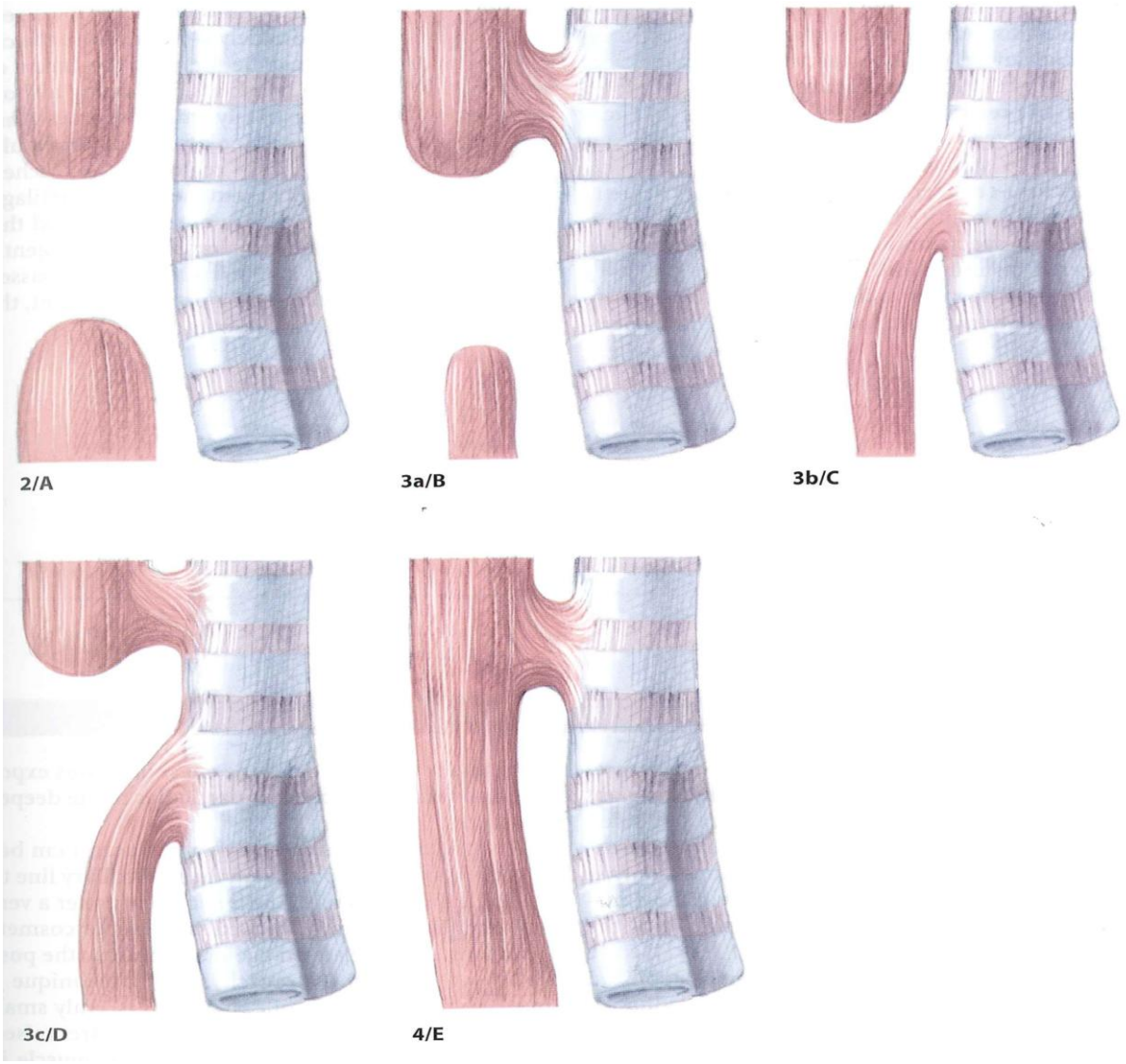

FIGURE 1. Type 2/A (7%) is pure atresia; 3a/ B (2%) has a fistula from the upper pouch; 3b/ C is the far most common variant (>85%), with a blind-ending upper pouch and with a fistula from the lower part of the esophagus to the trachea; 3c/ D (3%) has double fistulas; and 4/ E has an H-shaped fistula. Vogt's type 1 is an extremely rare type, characterized by more or less total abscence of the esophagus, and is not included in the Gross' classification.[6]

DESCRIPTIVE EPIDEMIOLOGY

The incidence of EA has been reported to be around 3 per 10,000 births, without any major changes with time.[7, 8] Infants born with EA are often born prematurely and small for gestational age. A slightly higher percentage of boys than girls are born with the malformation.[9-11] Twins have a higher risk of malformations in general,[12] and they also have an increased risk of EA. Especially monozygotic twins are affected, but mostly only one of the twins has EA.[8]

Aproximately 50% of infants born with EA have at least one associated anomaly,[1, 8, 10] and about 6-10% have been reported to have chromosomal anomalies.[8, 10] The associated anomalies are most often of cardiovascular, musculo-skeletal, anorectal, and genitourinary origin.[7, 13] Some of these malformations occur together more often than by chance alone. This is called an association, of which the VACTERL association is the most well known.[14] The VACTERL association is present in approximately 10% of the cases of EA[15] and is an acronym for vertebral, anal, cardiac, tracheo-esophageal, renal, and limb abnormalities.[16] If three or more of these anomalies occur in one infant, the VACTERL association is said to be present. Only a small proportion of patients have the whole spectrum of six anomalies, thereby presenting the "full" VACTERL association.[17]

In a small percentage of EA patients, the associated anomalies form a syndrome with a known genetic etiology. Single gene conditions where EA might be present include, for example, the rare syndromes CHARGE, Feingold, Anophtalmia-Esophageal-Genital and Pallister-Hall syndrome.[18]

EMBRYOLOGY

During embryogenesis the esophagus, trachea and bronchi derive from a common tube called the foregut. Between 28 and37 days after fertilization the foregut separates into one respiratory and one esophageal component. In recent years the embryology of the foregut in humans has been a subject of much controversy, and currently no agreement exists about the exact mechanisms of normal foregut development. A failure in the process of separation of the ventral respiratory component and the dorsal esophageal component is, however, generally considered to be the mechanism by which EA occurs.[18]

The development of reproducible animal models has facilitated the study of cellular and molecular events underlying abnormal embryogenesis. For example, teratogenic rat and mouse models of EA, with the teratogenic anticancer agent Adriamycin, have been developed. More recently, genetic models with knockout mice with loss-of-function mutations in foregut patterning genes have made it possible to identify key developmental processes that may be disturbed during embryogenesis. In these models, the separation process is associated with a precise temporal and spatial pattern of expression of a number of foregut patterning genes, such as the key developmental gene Sonic hedgehog (Shh) and members of its signaling cascade. Several other factors such as the transcription factor Nkx2.1, Sox2 and members of the Bmp (bone morphogenetic protein) pathway, as well as programmed cell death, have also been suggested as having a role in the tracheo-esophageal separation.[18, 19]

In humans, the characterization of the syndromes resulting from single gene mutations mentioned above, and chromosomal disorders featuring EA, has begun to give new insight into the development of EA on the molecular level.[1, 18] Interestingly, some of the findings in the animal models have their human equivalents. The AEG and Pallister-Hall syndromes are caused by single gene mutations in SOX2 and GLI3 respectively, the latter a member of the SHH pathway.[18] Chromosomal abnormalities such as trisomies (13,18, and 21) and some

duplications and deletions (for example 22q11 and 17q22q23.3) are known to be associated with EA,[1]and the homologue of a Bmp antagonist, for example, is found on 17q.[18]

ETIOLOGY

The etiology of EA, i.e., the causes underlying an incomplete separation of the foregut, is mainly unknown, but is generally considered to be multifactorial.[15]

The occurrence of more than one child with EA in a family is low, of the order of 1%. The twin concordance rate is likewise low, around 2.5 %.[1] As stated above, although twins are slightly overrepresented among infants with EA, mostly only one of the twins is affected.[8] The separation of monozygotic twins is complete within 2 weeks after fertilization, whereas the development of the esophagus does not start until the 4th week of gestation.[20] Taken together, data from family and twin studies suggest that hereditary factors do not play a major role in the etiology of EA. Thus, the majority of EA cases are sporadic and environmental risk factors seem to play an essential part in the etiology of EA. It is reasonable to assume that exposures of the pregnant mother to risk factors during early fetal development, when the esophagus and the trachea are separating, are particularly critical.

In previous studies, only a few environmental exposures of the mother and the child have been investigated in relation to the risk of EA. Several studies have shown that low maternal parity increases the risk of EA in the offspring.[8, 9, 11] Further an increased risk has been found in mothers with more than three previous pregnancies.[9] Very contradictory results have been reported regarding the correlation of maternal age to the risk of EA in the child. No correlation was shown in the largest study,[8] while some studies have pointed to an increased risk with increasing maternal age[11] and in other investigations older mothers have been found to be overrepresented in association with TEF and TEF/EA, but not with isolated EA.[21] Finally, some data suggest an increased risk among mothers younger than 20 years of age.[10] There have been several case reports of mothers who have used the antihyperthyroidism drug methimazole during pregnancy and have had children with EA.[22-26] One study has shown a relation between maternal use of exogenous sex hormones during early pregnancy and risk of EA in the infant.[27] Ethnicity also seems to influence the risk of having an infant with EA, with an increased risk among Caucasian women.[11, 21, 28]

PATHOPHYSIOLOGY

Pathological esophageal motility, with disorders both in the esophageal body and its sphincters, has been shown in patients with EA. This has been documented both clinically, with symptoms of dysphagia and gastroesophageal reflux, and through different objective measurements. Isotopic studies have revealed altered motility. Esophageal pH measurements have shown increased gastroesophageal reflux. Esophageal manometry has provided evidence of abnormally spastic zones, dysfunctional peristaltic waves, incomplete relaxation in the upper esophageal sphincter, and low pressure of the lower esophageal sphincter. In the past, this was thought to be due to partial denervation of the esophagus during surgery, but

disorders of the motor activity of the esophagus have also since been shown to occur preoperatively, suggesting the existence of congenital functional impairment. Immunohistological studies have disclosed alterations both in innervations and in the muscular organization of the esophagus in patients with EA.[29]

The trachea might also be abnormal in patients with EA. Deficiency of tracheal cartilage, and abnormal muscles have been described. These defects will make the tracheal wall less rigid, and if they are severe they will result in tracheomalacia, a condition in which even the physiological narrowing during expiration may result in airway obstruction.[5 16]

DIAGNOSIS

Prenatal

Only in a small minority, fewer than 10 %, of infants with EA the anomly is identified before birth. There are two main prenatal signs of EA that can be seen on ultrasonographic scans, namely polyhydramniosis and absence of or a small stomach bubble. Both of these sonographic criteria are, however, non-specific. The absence of a small stomach bubble also assumes the absence of a TEF from the lower segment, which is present in over 85% of the cases. The combination of polyhydramniosis and absence of a stomach bubble has been shown to have a modest positive predictive value of around 50% in two small case series.[30] Some investigators have also used ultrasound to visualize the dilatation of the blind-ending upper esophageal segment during fetal swallowing. Magnetic resonance imaging (MRI) has been used as a complement to the sonographic investigation.[31]

Postnatal

The newborn infant with EA classically presents with drooling of saliva, respiratory distress, and feeding difficulties. When attempts are made to introduce a nasogastric tube into the stomach, suspicion of an EA diagnosis is strengthened. A pulmonary X-ray, showing the nasogastric tube curled up in an airdistended proximal esophageal pouch, confirms the diagnosis of EA.[16] A small amount of diluted, non-ionic contrast material may be used to further establish the diagnosis.[32]

TREATMENT

Preoperatively

To prevent aspiration to the airways, a suction catheter should be placed in the proximal esophageal pouch to allow intermittent suction. The infant is transferred to an intensive care unit at a hospital with pediatric surgery facilities.[16]

Screening for associated anomalies is accomplished. A full physical examination is performed. The chest radiograph does not only confirm the diagnosis, but also allows

assessment of the appearance of the heart and lungs. An echocardiogram establishes the presence or absence of structural cardiac anomalies and the location of the aortic arch.[33] A right-sided aortic arch is present in approximately 2.5% of EA patients. [34] Renal ultrasound is performed to determine the existence of any renal anomalies.[33]

Prior to surgical repair of EA, the distance between the proximal and distal esophageal segments, i.e., the length of the gap, is estimated. There is no clear definition of long-gap EA in the literature. The definition has been based on the anatomical type of atresia (no TEF), gap measurements in centimeters (>2)[16] or number of vertebral bodies (>2.5), or simply on an inability to perform a primary anastomosis.[33, 35] If a long-gap EA is suspected, the primary repair is delayed (see under "long-gap EA" below).[33]

A perioperative tracheobronchoscopy is performed in most centers to investigate for the occurrence of one or several fistulas and their location if present. The presence or absence of a trachea-esophageal cleft is also sought for, as well as evidence of vascular compression of the trachea, [33] tracheomalacia, and any other respiratory malformations.[36] The tracheobronchoscopy requires little additional operative time and its complication rate is negligible.[36]

Direct anastomosis

Primary repair should preferably be performed within 48 hours.[33]

Thoracotomy

The typical surgical procedure for reconstructing an EA is described in the following: If a left-sided aortic arch is found on preoperative echocardiography, a right posterolateral thoracotomy is performed. A slightly curved skin incision is placed 1 cm below the tip of the scapula, from the midaxillary line to the angle of the scapula. A muscle-sparing technique is used, in which the latissimus dorsi muscle is retracted posteriorly and the serratus anterior muscle is mobilized and retracted upward and forward. The intercostal muscles are divided at the upper border of the fifth rib. An extra-pleural approach is used to gain access to the EA. The azygos vein is divided. If a TEF is present, a sling is placed behind the distal esophageal segment, the fistula is divided, and the opening into the trachea is closed. The fistula closure is tested for air leakage. To facilitate the identification of the proximal esophageal segment, the anaesthetist is asked to advance a nasogastric tube into the esophagus. Traction sutures are placed to assist mobilization of the proximal esophageal segment. Contrary to the distal segment, the proximal segment has an excellent blood supply and can be dissected up to the thoracic inlet if necessary. The upper pouch is opened and the esophageal ends are anastomosed in a single-layer technique, using an absorbable monofilament 5/0 or 6/0 suture over a transanastomotic silicon tube (5 Ch). A chest tube might be placed if there is risk of leakage.[33] The chest is closed with pericostal sutures, adaptive sutures of the muscles, and the subcutaneous fat and the skin are closed with continuous sutures.[6]

Thoracoscopic repair

The first known thoracoscopic repair in a patient with EA was accomplished in 1999.[37] Proponents of thoracoscopic EA repair claim that this avoids the complications of thoracotomy such as the risk of acute or chronic postoperative pain, rib fusion, scoliosis, and chest wall deformities as well as cosmetic drawbacks with scarring. Uniform compression of the lung and superior anatomical visualization are also mentioned as main advantages. One criticism of the technique is that it utilizes a transpleural approach .[35] The thorascopic EA repair, however, remains a technically challenging operation and several authors have recommended that it only be performed at high-volume centers and by surgeons with an established expertise in minimal-access surgery.[35, 38]

Long-gap EA

Long-gap EA increases the challenge for the pediatric surgeon. The many different repairs that have been used testify to the difficulties in obtaining satisfactory results.[39] There is a consensus that "the best esophagus is the patient's own esophagus" and that it is therefore important to preserve the infant's native esophagus as far as possible. Several intraoperative techniques with the aim of facilitating a primary anastomosis in long-gap EA have been described. An anastomosis under tension is, however, associated with an increased risk of anastomotic leak, severe gastro-esophageal reflux, and esophageal strictures.[35] If the gap between the upper and lower esophageal segments is considered too long for a primary repair, a gastrostomy is usually performed, any TEF is ligated, and a delayed repair is performed after approximately 8-12 weeks.[6] During this time, the segments are thought to grow spontaneously,[6] and growth induction via traction of the esophageal pouches is also described.[39] If the gap is still too wide, a gastric pull-up, or an esophageal replacement with a gastric tube, jejunal graft or colonic interposition might be performed. There is little consensus on which technique is to be preferred, as studies comparing the different strategies are few.[35]

Postoperative management

Feeding through the transanastomotic feeding tube is usually started on day 1 or 2 postoperatively. At day 7, a radiological contrast examination is routinely performed to ascertain the occurrence of any leakage from the anastomosis. If there is no sign of leakage or major stricture, oral feeding is started, and the transanastomotic feeding tube is removed.[33]

OUTCOME

Early morbidity

One of the most severe postoperative complications is anastomotic leak. Factors that may contribute to leaks include failures in the suturing technique (too few or too many sutures, knots tied too tightly or with the mucosa not included in the stitch), or tension in the anastomosis. Most leaks seen at the routine examination produce no obvious clinical

symptoms, and can be treated conservatively. This means that the infant continues with transanastomotic tube feeding, while the leak usually heals within a few days. Some leaks present with saliva in the chest tube, which has been reported to occur in 6-17% of cases. Major leaks are reported in 3-5%, and usually occur within 48-72 hours postoperatively. These infants develop symptoms and signs of mediastinitis, and surgical intervention and systemic broad-spectrum antibiotics are usually required.[33]

Anastomotic stricture is a common early sequel to repair of EA, but is most often noted later in the postoperative period, i.e., weeks to months after surgery. The definition of a stricture varies in different reports and the rates may not be comparable. In the majority of the studies the reported rates are between 37 and 52%, but a need for surgical dilatation has been found to occur in up to 80 % of the patients.[2, 33] Factors that increase the risk of stricture formation include tension in the anastomosis (strictures are more common in patients operated for long-gap EA) [2] and certain surgical techniques. Anastomoses sutured in one layer and end-to-end anastomosis are associated with a decreased risk of strictures, compared to alternative techniques. Moreover, silk sutures may increase the incidence of strictures compared with long-lasting monofilament absorbable sutures. [33] Dilatation of the stricture is preferably performed with balloon dilatation under endoscopic control. Persistent strictures are usually associated with gastro-esophageal reflux, and it is crucial that acid reflux is treated adequately to diminish recurrence of stricture formation.[33]

Recurrent TEF occurs in 3-15% of cases. The fistula typically presents 2 to 18 months after the primary repair.[2] Symptoms of TEF include coughing, choking, cyanosis associated with feeding, and recurrent lower respiratory infections. The diagnosis is confirmed by contrast swallow or, preferably, tracheoscopy. The standard surgical approach involves thoracotomy, but several minimally invasive techniques have been described with varying results.[33]

Late morbidity

Dysphagia is one of the most common symptoms after EA repair. The reported incidence varies between 10 and 60 %, and the differences in results are probably mainly due to differences in the definitions of dysphagia. An anastomotic stricture must be ruled out, but is not present in the majority of patients with dysphagia. The innate esophageal dysmotility that is associated with EA probably plays a role, as also might the surgical dissection (see discussion, pathophysiology).[2]

Another late problem that often occurs after reconstruction of EA is gastro-esophageal reflux (GER). GER is recorded in approximately 50% of all EA cases, although it is universally present to some degree in all patients.[34] The high frequency of GER is thought to be partly due to the esophageal dysmotility mentioned above. Surgical repair causing an anatomical alteration of the gastro-esophageal junction might also contribute.[33] Symptoms of GER in childhood include recurrent vomiting, dysphagia, failure to thrive, growth retardation, recurrent pneumonia, and obstructive respiratory symptoms.[40] It is recommended that antireflux medical treatment is started at a prophylactic stage, and continue for at least 12-18

months after birth.[34] By the end of that time, reflux is outgrown in most patients due to posture and dietary changes.[41] Antacid and prokinetic medications are indicated as first-line therapy if the GER continues. Only a minority of patients require surgical treatment,[42] including a fundoplication.[41] In pre-adolescent children and adults who have been operated on for EA, the reported frequency of reflux symptoms varies between 27 and 75%.[2]

Children born with EA tend to have considerable problems with nutrition and growth, and they are often born premature and small for gestational age (SGA). Feeding problems due to GER, anastomotic strictures or esophageal dysmotility are common in early childhood. The more long-term nutritional outcomes are, however, generally excellent and "catch-up" growth mostly occurs.[34]

Long-lasting functional problems from the respiratory tract are also common after EA surgery.[2] Many factors may contribute to these problems, including aspiration due to esophageal dysmotility and GER, structural instability of the major airways, and abnormal airway epithelium with impaired mucociliary clearance.[33, 34, 43]

Tracheomalacia has been reported to co-exist in 75% of infants born with EA.[44] It often manifests itself with a classic cough, and wheezing is also associated with this condition.[2] Diagnosis is made by tracheo-bronchoscopy. Even though tracheomalacia is frequent in patients operated on for EA, clinically significant tracheomalacia is present in only 10 to 20%, and even fewer require surgical intervention.[33] In general, tracheomalacia improves with age, and surgery is reserved for those with severe cyanotic attacks or recurrent pneumonia. Aortopexy, a surgical procedure where the ascending aorta is sutured to the sternum in order to pull the trachea anteriorly, is then performed.[33 16]

Recurrent episodes of bronchitis and pneumonia are common in the early years of life after EA repair.[2] Respiratory symptoms are, however, more pronounced before the age of 5 years and seem to improve in adolescence.[45] Pulmonary function tests have shown various results. Both restrictive and obstructive respiratory defects, as well as normal lung function, have been reported. Overall, the pulmonary function defects are mild and most patients are reported to have normal exercise tolerance.[2]

Chest wall deformities, including scapular winging, anterior chest wall deformity, and scoliosis, have been reported in up to 25% of all cases of EA.[2] Some females have been found to develop breast asymmetry.[33] Thoracotomy with damage to the innervations, and associated vertebral anomalies, contribute to such problems.

Risk of cancer

Long-standing GER is known to cause chronic esophagitis, which in turn may lead to intestinal metaplasia, also called Barrett's esophagus. Barrett´s esophagus is associated with a substantially elevated risk of developing adenocarcinoma of the esophagus. Several studies have shown that the incidence of biopsy-proven esophagitis is markedly more common in

adults operated on for EA than in the corresponding general population, and the incidence of Barrett's esophagus is reported to be four times as common.[2] There is also evidence that other benign esophageal disorders can increase the risk of cancer development in the esophagus. For example there is a highly increased risk of esophageal squamous cell carcinoma among patients with achalasia of the gastric cardia.[46]
Hitherto there have been six case reports of esophageal cancer after repair of esophageal atresia, three patients with squamous cell carcinoma and three patients with adenocarcinoma of the esophagus. Interestingly, all these cases were diagnosed at an exceptionally early age (mean 36 years, range 20 to 46).[2] One recently published cohort study, however, showed no cases of esophageal cancer among 272 patients with a median follow-up of 35 years after EA repair, but further studies are needed.[47]

There is also one case report, recently published, of pulmonary squamous cell carcinoma in a 19-year-old patient operated on in infancy for EA with a TEF.[48]

Mortality

The overall survival rate among EA patients has increased during the last decades, and currently it has been reported to exceed 90%.[2] The mortality from EA is almost exclusively associated with the presence of co-existing major cardiac anomalies and very low birth weight.[49]

The original risk classification for survival of patients with EA was proposed by Waterston in 1962 and was based on birth weight, associated anomalies, and pneumonia. After a steady improvement in the overall survival, an updated risk classification was needed. The currently most commonly used classification was outlined by Spitz in 1994 and is based on associated major congenital cardiac defects and low birth weight, as follows:

Group I: Birth weight over 1500 g with no major cardiac anomaly.
Group II: Birth weight less than 1500 g or major cardiac anomaly.
Group III: Birth weight less than 1500 g plus major cardiac anomaly.

According to Spitz's original data, survival in babies classified as group I was almost 97%, but fell dramatically to only 22% in group III.[50] In one more recent study, based on patients born during the period 1993-2004, survival in babies classified as group I was 98.5%, group II 82% and group III 50%. [51]

Major cardiac and chromosomal defects are the main causes of early death (within 30 days). Various respiratory conditions have been shown to be the most common causes of late deaths (30 days to 2 years), including sudden infant death syndrome, aspiration, tracheomalacia and reactive airway disease.[34, 52]

Quality of life

Only one study has addressed the quality of life in young EA patients, 6-18 years after surgery. These patients reported that reflux symptoms impaired their general health perception. According to their parents, their general health perception was negatively affected by associated anomalies and by older age at follow-up.[53] Most children in this study were at the beginning of their adolescence and the authors speculated that this might have been too early to see any improvement in EA-related symptoms with age, improvement which has been reported in many studies.[53]

Between 15 and 33% of adult EA patients have been found to have impaired quality of life linked with gastrointestinal or respiratory symptoms.[54, 55] Esthetic complaints of the thoracic scar and wing scapula have also been recorded in almost 50 % of the EA patients.[54] There are few studies in which quality of life has been compared after different types of surgery. In one such study it was found that patients who had undergone colonic interposition suffered more from gastrointestinal and respiratory symptoms than those in whom primary anastomosis was performed,[56] while in a more recent study no such difference was found.[54] The impact of associated anomalies has not been shown to influence the general quality of life.[55]

The overall long-term quality of life in adult EA patients has, however, been found to be good. No general differences in overall physical and psychosocial health have been found.[54-56]

AIMS

The principal aim of this thesis is to contribute to the understanding of the etiology of EA. Specific aims are:

- To estimate incidence of EA and the mortality and cancer risk associated with this malformation in a population-based setting.
- To describe an unselected cohort of EA patients with regard to birth weight, length of gestation, SGA, birth (single or multiple), and gender.
- To clarify whether maternal parity, age, or ethnicity influences the risk of EA in the infant.
- To determine whether maternal tobacco smoking, obesity, or low socio-economic status are risk factors for EA in the infant.
- To clarify whether maternal diabetes is a risk factor for delivering an infant with EA.

MATERIAL AND METHODS

The rarity of EA, together with the high risk of selection bias in studies of this disease, makes it difficult to study its etiology. In Sweden, however, there are unique possibilities for studies on large and unselected material through the nationwide registers. The comprehensive national public health care system represents nearly 100 % of all Swedish health care, both with regard to obstetric service from early pregnancy until delivery, and concerning in-hospitalizations. Sweden also has a long tradition of nationwide health care registers and population statistics that are well maintained, validated, and kept continuously updated. Furthermore, the systematic use of personal identity numbers, unique ten-digit numbers introduced in Sweden in 1947,[57] makes it possible to link register data for individual persons and to conduct a complete follow-up.

REGISTERS

1. *The National Patient Register,* held by the National Board of Health and Welfare, was started in 1964 and comprises the diagnoses and surgical procedures of the in-patient care in Sweden. The proportion of the Swedish population covered by this register was 60% in 1969 and 85% in 1983, and since 1987 it has been 100% complete. Reporting is good; less than 2% of all hospitalizations are missing and the main diagnosis was missing in 1% of all registered hospitalizations.[58]
2. *The Medical Birth Register,* held by the National Board of Health and Welfare, contains data on pregnancies and deliveries in Sweden since 1973. Since 1982, when more detailed reporting of data to the register was introduced, the register information has been based on records of: 1) the antenatal care, 2) the delivery, and 3) the medical examination of the infant. Records for only a small percentage of all infants (0.5-3.9%) are missing.[59]
3. *The Register of Congenital Malformations,* held by the National Board of Health and Welfare, was started in 1964 and is formally a part of the Medical Birth Register. Since 1999, when the register was expanded, the register has included the personal identity number of the infant and has contained information on pregnancies from the 28th week of gestation (from the 1st of July 2008 the register has kept information from the 22nd week of gestation), including abortions induced because of malformations or chromosomal abnormalities. The registered data are based on compulsory reporting of chromosomal abnormalities or congenital malformations in infants from maternity wards, pediatric wards, and cytogenetic laboratories. In this register data are missing for approximately 20% of all liveborn infants.[60]
4. *The Swedish Cancer Register,* held by the National Board of Health and Welfare, was established in 1958 and records all new cases of cancer, specified by location and histological type. The register is estimated to be generally 96.3% complete,[61] and 98% complete regarding esophageal cancer.[62]
5. *The Register of the Total Population,* held by Statistics Sweden, has contained highly up-dated (within 14 days) and complete information on dates of death and migration since 1961.

6. *The Swedish Register of Education*, held by Statistics Sweden, was established in 1985 and is a nationwide Swedish population register that contains information on the highest level of formal education of each resident aged 16-74 years in Sweden [63]. Data on highest education are annually matched to the population of ages 16-74 years, living in Sweden on January 1, by linkage with the Total Population Register. Validation studies regarding the data collected in the Swedish Register of Education have shown missing information on education in only 1.9%.[63]

STUDY I

Design

A population-based cohort study of the incidence of EA and the associated mortality and cancer risk was conducted during the period January 1, 1964 through December 31, 2007. The study cohort included all liveborn infants who were delivered with EA according to the ICD codes registered in the National Patient Register, the Medical Birth Register, or the Register of Congenital Malformations. Infants diagnosed after one year of age were excluded. Identification of the cancer risk and mortality was achieved through linkages with the Swedish Cancer Register and the Register of the Total Population, respectively.

Statistical analyses

The incidence (prevalence at birth) of EA was calculated for each calendar year per living births in Sweden. Data on all living births in Sweden, obtained from Statistics Sweden, were used as denominator in the analyses. Associated malformations or chromosomal abnormalities were identified through the ICD codes in the National Patient Register, the Medical Birth Register, or the Register of Congenital Malformations.

The mortality, obtained from the Total Population Register, was assessed by using the Kaplan Meier method. Mortality after birth within 5, 30, 60, 90 days and 1 year was calculated for different calendar time periods. The standardized mortality ratio (SMR) was calculated as the ratio of the observed to the expected number of deaths, based on expected rates derived from the corresponding entire Swedish population. Cox regression analysis was used to calculate the hazard ratio (HR), with 95% confidence interval (CI) of mortality to assess the influence of associated anomalies, very low birth weight, gender, and calendar time periods.

To identify the risk of cancer development, the cohort members were followed-up from the date of birth until the date of diagnosis of cancer, migration, death, or end of study period, whichever occurred first. The relative risk of cancer was calculated through the standardized incidence ratio (SIR) with 95% CI. SIR was calculated by dividing the observed number of cancer cases by the expected number. The expected rates were retrieved through the corresponding entire Swedish general population.

STUDIES II, III AND IV

Design, studies II and III

Nationwide, population-based, and prospective case-control studies were nested within a cohort consisting of all newborn infants entered in the Medical Birth Register during the study period January 1, 1982 through December 31, 2004.

Design, study IV

The design was as in studies II and III, except that the study period was longer, ending in December 31, 2007.

Exposure assessment

Data on maternal parity, age, ethnicity, tobacco smoking, body mass index (BMI), and diabetes, as well as selected covariates were entered prospectively in the Swedish Medical Birth Register. Data on maternal diabetes were also collected prospectively from the National Patient Register. Educational level, which was used as a proxy variable of socioeconomic status, was assessed through the Swedish Register of Education.

Outcome assessment, studies II and III

Eligible as case participants were all liveborn infants of the cohort who were delivered with EA according to the ICD codes recorded in the National Patient Register, the Medical Birth Register, or the Register of Congenital Malformations. Infants diagnosed after the age of one year were excluded. Chosen as control participants were liveborn infants without any recorded congenital malformation, randomly selected from the study cohort. For each case, five control children, who were matched for sex and for calendar year of the delivery of the EA cases, were selected.

Since it is possible that isolated EA and EA associated with other abnormalities might have partly different etiologies, the outcome was stratified into three groups: 1) all infants with EA, 2) infants with isolated EA, and 3) infants with EA associated with other malformations or chromosomal abnormalities. Associated malformations or chromosomal abnormalities were identified by the ICD codes in the National Patient Register, the Medical Birth Register, or the Register of Congenital Malformations.

Outcome assessment, study IV

This procedure was the same as in paper II and III, but the number of controls chosen per case participant was ten instead of five.

Statistical analyses

The association between each of the study exposures and the risk of EA in the infant was analyzed using conditional logistic regression. Relative risks were calculated and expressed as odds ratios (OR) with 95% CI. Adjustments for sex of the child and calendar year of delivery were made through matching. We used two regression models: a crude model without any further adjustments and a multivariable regression model, which also included adjustments for covariates that were deemed to be biologically plausible confounders, i.e., parity, maternal age, maternal ethnicity, tobacco smoking, occurrence of chronic disease, BMI, and years of formal education. Stratified analyses were conducted, using the outcome categories presented above.

RESULTS

STUDY I

Participants

This study comprised 1,126 patients with EA. Associated anomalies were present in 42% (472) of all EA patients and chromosomal abnormalities in 5%.

Incidence

The number of EA patients diagnosed during the period 1987-2007 was 687, on the basis of which a mean incidence was calculated to be 3.13 per 10,000 live births. The corresponding figure for the period 1999-2007 (based on 278 EA patients) was 3.16 per 10,000 live births. No statistically significant trends were seen over time (p for trend=0.94).

Mortality

The absolute survival in different calendar periods improved with time as shown in Table 1 and in Figure 2.

TABLE 1. The absolute survival during the entire study period and survival rates categorized into calendar year periods, in a cohort of 1,126 patients with esophageal atresia, calculated by the Kaplan Meier method.

Calendar year period	30 days survival	90 days survival	1 year survival
1964-2007	86% (967/1126)	85% (953/1126)	80% (896/1126)
1964-1969	72% (21 / 29)	69% (20 / 29)	62% (18 / 29)
1970-1979	77% (177 / 229)	75% (173 / 229)	74% (170 / 229)
1980-1989	87% (249 / 286)	87% (248 / 286)	83% (236 / 286)
1990-1999	90% (297 / 330)	88% (291 / 330)	86% (284 / 330)
2000-2007	88% (223 / 252)	88% (221 / 252)	86% (216 / 252)

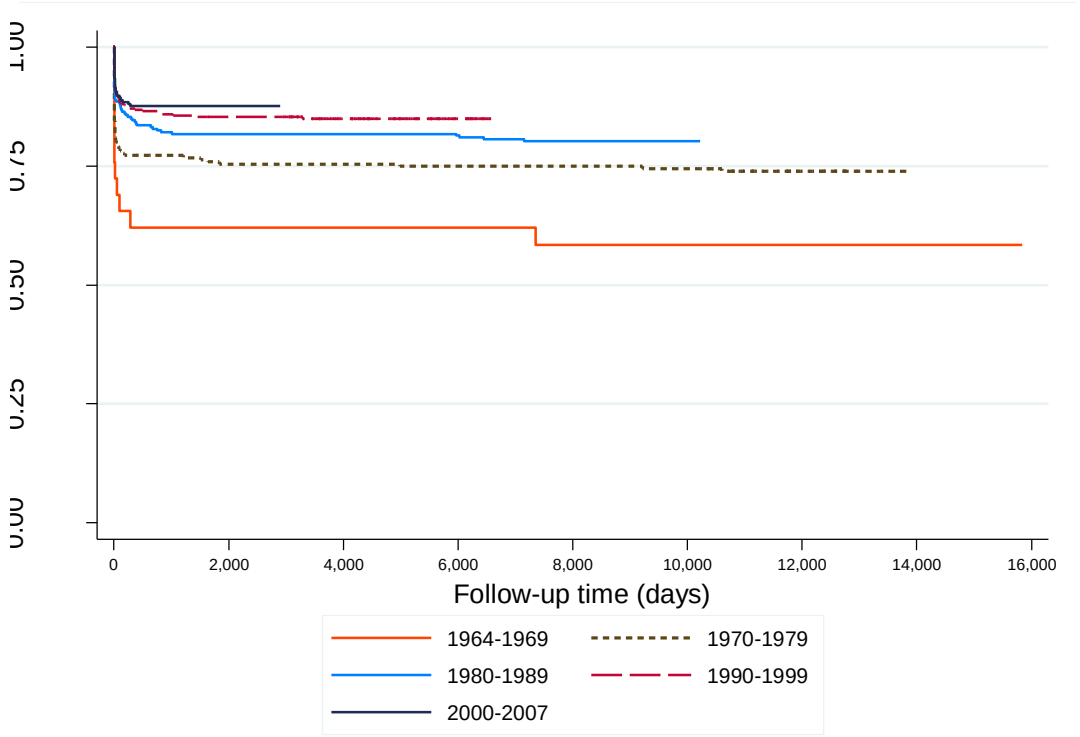

FIGURE 2. Kaplan Meier curves of the survival, categorized into calendar year periods, in a cohort of 1,126 patients with esophageal atresia.

Patients with EA had an almost 12 times higher risk of mortality compared to the background population. The gradually decreased risk of mortality with time is also presented in Table 2, expressed in SMR and in adjusted HR.

TABLE 2. Risk of mortality among patients with esophageal atresia, expressed as standardized mortality ratios (SMR) and hazard ratios (HR) with 95% confidence intervals (CI), in different calendar time periods.

Time period (calendar year)	**SMR (95% CI)**	**HR (95% CI) ***
1964 - 2007	12.0 (10.4-13.7)	-
1964 - 1969	25.4 (12.7-45.4)	4.6 (2.3-9.2)
1970 - 1979	17.2 (13.1- 22.3)	3.1 (2.0-4.7)
1980 - 1989	13.6 (10.4- 17.4)	2.1 (1.4-3.2)
1990 - 1999	10.1 (7.7- 13.1)	1.2 (0.8-1.8)
2000 - 2007	7.7 (5.5- 10.4)	1.0 (reference)

* adjustments were made for gender, associated anomalies, and birth weight

The increased risk of mortality was most pronounced, and the increase was significant, during the first five years after the repair of the EA (Table 3).

TABLE 3. Risk of mortality, expressed as standardized mortality ratios (SMR) with 95% confidence intervals (CI), in different age groups after surgical repair of esophageal atresia.

Age group (years)	**SMR (95% CI)**
All age groups	12.0 (10.4-13.7)
0-1	15.5 (13.5-17.8)
1-2	6.3 (2.3-13.7)
2-3	17.8 (6.5-38.8)
3-5	8.8 (2.4-22.5)
5-10	2.7 (0.3-9.7)
10-15	1.9 (0.1-10.5)
15-20	4.1 (1.1-10.6)
20-25	1.0 (0.0-5.5)
25-30	2.8 (0.3-10.0)
30-35	0.0 (0.0-9.7)
35-40	0.0 (0.0-25.9)

The mortality was higher in females (SMR 17.2; 95% CI 14.1- 20.8) than in males (SMR 9.3; 95% CI 7.7- 11.2).

The mortality was almost five times higher in the group of EA patients with associated anomalies than in the group with isolated EA (HR 4.9, 95% CI 3.7-6.6). This difference seemed to be slightly more pronounced if the associated malformation was circulatory (HR 5.6; 95% CI 4.0-7.8).

EA patients with a very low birth weight (<1500g) showed a seven times higher risk of mortality compared to patients of higher weight (HR 7.0; 95% CI 4.9-10.1). The impact of birth weight on mortality seemed to be greatest in earlier time periods.

Cancer

In this analysis, 870 EA patients with a mean follow-up of 16.9 years (range 1-42 years) were included, together contributing 14,692 person-years at risk. No increased risk of cancer compared to the background population was found (SIR 0.9; 95% CI 0.2-2.6). There were no cancers of the esophagus, larynx, trachea, or lungs in the EA cohort.

STUDIES II, III, AND IV

Participants, studies II and III

These studies comprised 722 patients with EA.

Table 4 summarizes some characteristics of the study participants. The EA children in general had a lower birth weight, were more often prematurely born, and were more often small for gestational age than the children without this malformation. The frequency of twin birth was also higher in the EA group (7%) than among the controls (2%). A slight male predominance (57%) was noted among the children with EA.

TABLE 4. Characteristics of the infants born with esophageal atresia and the control participants, matched for sex of the child and for calendar year of the delivery.

Characteristics	**Controls** (total=3,610) Number (%)	**EA cases** (total=722) Number (%)
Birth weight (in grams)		
<1500	24 (1)	95 (13)
>=1500	116 (3)	203 (28)
>=2500	3457 (96)	396 (55)
Missing data	13 (0)	28 (4)
Gestational age at birth (in weeks)		
<32	29 (1)	81 (11)
33-37	163 (5)	218 (30)
>37	3,409 (94)	411 (57)
Missing data	9 (0)	12 (2)
Small for gestational age*		
Yes	75 (2)	158 (24)
No	3,149 (97)	456 (70)
Missing data	26 (1)	36 (6)
Birth		
Single	3,521 (98)	650 (90)
Twinning	84 (2)	50 (7)
Gender		
Male	2,075 (57)	415 (57)
Female	1,535 (43)	307 (43)

* Data only calculated for single births

Participants, study IV

This study comprised 780 patients with EA.

Different maternal exposures and risk of EA

The results are summarized in Table 5.

TABLE 5. Maternal exposures and risk of esophageal atresia in the child, expressed in odds ratios (OR) with 95% confidence intervals (CI).

Exposure	Number of cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI) *
Parity (number)			
1	351/1489	1.00 (reference)	1.00 (reference)
2	224/1321	0.72 (0.60-0.87)	0.68 (0.56-0.83)
3	94/558	0.72 (0.59-0.91)	0.64 (0.49-0.83)
4+	53/242	0.93 (0.67-1.28)	0.79 (0.55-1.12)
Age (years)			
<20	13/91	1.00 (reference)	1.00 (reference)
20 - 24.9	134/720	1.31 (0.71-2.41)	1.43 (0.77-2.68)
25 - 29.9	246/1303	1.33 (0.74-2.40)	1.56 (0.85-2.88)
30 - 34.9	209/1017	1.45 (0.80-2.63)	1.83 (0.98-3.43)
35 - 39.9	98/413	1.68 (0.92-3.11)	2.09 (1.09-3.99)
40+	22/66	2.37 (1.11-5.04)	3.04 (1.37-6.74)
Ethnicity			
Non-Nordic	57/410	1.00 (reference)	1.00 (reference)
Nordic	664/3199	1.50 (1.11-2.02)	1.43 (1.07-1.93)
Tobacco smoking			
Non-smokers	528/ 2673	1.00 (reference)	1.00 (reference)
1-9 cigarettes/day	74/ 425	0.88 (0.68-1.15)	0.90 (0.68-1.16)
>9 cigarettes/day	43/ 242	0.90 (0.64-1.26)	0.88 (0.62-1.25)
BMI			
<20	73/ 398	1.00 (reference)	1.00 (reference)
20-25	262/ 1463	0.96 (0.72-1.29)	0.94 (0.70-1.25)
25-30	100/ 530	1.01 (0.72-1.41)	0.98 (0.70-1.37)
>30	33/ 175	1.00 (0.64-1.57)	0.99 (0.64-1.55)
SES (years of formal education)	69/ 393	1.00 (reference)	1.00 (reference)
0-9	373/ 1853	1.15 (0.87-1.51)	1.00 (0.75-1.34)
10-12	278/ 1343	1.18 (0.89-1.57)	0.94 (0.69-1.29)
>12	2/ 21		
Maternal diabetes			
No	762 / 7697	1.0 (reference)	1.0 (reference)
Yes	18 / 103	1.8 (1.1-2.9)	1.7 (1.0-2.8)

* Adjustments were made for parity, age, ethnicity, tobacco smoking during early pregnancy, chronic disease, body mass index, and educational level, while matching was made for sex of the child and calendar year of delivery.

Parity

An increased risk for EA in the infant was noted for women having their first child. A more than 30% decrease in the risk of EA was found for mothers delivering their second (OR 0.68; 95% CI 0.56-0.83) or third child (OR 0.64; 95% CI 0.49-0.83), compared to first-time mothers.

Age

A statistically significant trend of an increasing risk of EA in the child with increasing maternal age was found. Children of women giving birth when 35-40 years and >40 years old showed a two-fold (OR 2.09; 95% CI 1.09-3.99) and three-fold (OR 3.04; 95% CI 1.37-6.74) increase in the risk of EA, respectively, compared to those of mothers who were <20 years old. This association remained when infants with chromosomal abnormalities were excluded (p=0.004).

Ethnicity

A statistically significant increase, by 43% in the risk of EA in the child was observed in the group of mothers born in the Nordic countries compared to those born outside these countries (OR 1.43; 95% CI 1.07-1.93).

Tobacco smoking

There was no overall association between maternal tobacco smoking during early pregnancy and risk of EA in the infant. Among women smoking at least 10 cigarettes per day during early pregnancy, the adjusted OR of EA was 0.88 (95% CI 0.62-1.25).

Obesity

There was no overall association between maternal BMI and risk of EA. Among women with obesity (BMI>30) at the first visit to the antenatal care center, the adjusted OR for EA was 0.99 (95% CI 0.64-1.55), compared to women with a BMI of less than 20.

Socioeconomic status

The risk of EA was not statistically significantly decreased in infants of women with a higher educational level, used as a proxy variable for socio-economic status. Women with more than 12 years of formal education had an adjusted OR of 0.94 (95% CI 0.69-1.29) for development of EA in the child, compared to those with less than 10 years of education.

Diabetes

A statistically significant 70% increase in the risk of delivering a child with EA was found among women with diabetes (OR 1.7; 95% CI 1.0-2.8). When the exposure was stratified into pre-existing and gestational diabetes, the adjusted point estimate seemed to be higher for gestational diabetes, but the difference was not statistically significant (Table 6).

TABLE 6. Maternal pre-existing or gestational diabetes and risk of esophageal atresia in the infant, expressed in odds ratios (OR) with 95% confidence intervals (CI).

Exposure	**Basic model * OR (95% CI)**	**Adjusted model † OR (95% CI)**
No diabetes	1.0 (reference)	1.0 (reference)
Pre-existing diabetes	1.2 (0.4-3.5)	1.1 (0.4-3.1)
Gestational diabetes	1.8 (0.9-3.7)	1.9 (0.9-3.9)

* Matching for sex of the infant and calendar year of delivery.

† Adjustments were made through matching*, and also by using logistic regression for parity, age, ethnicity, tobacco smoking during early pregnancy, chronic disease, body mass index, and educational level.

Cases with isolated EA versus cases with EA and associated malformations

No materially different results were seen in the stratified analyses of cases with isolated EA and EA cases with associated malformations, except regarding the exposures ethnicity and pre-existing diabetes.

The increased risk of EA in the child identified in the group with mothers born in the Nordic countries compared to those born outside these countries was more pronounced in the stratified analysis comprising isolated EA cases only (OR 1.66; 95% 1.06-2.61), but was attenuated and statistically non-significant in EA cases with associated malformations (OR 1.25; 95% 0.83-1.87).

The diabetes analyses that were stratified for both the exposure and the outcome were hampered by low statistical power, but the adjusted risk estimates nevertheless indicated that pre-existing diabetes was positively related to EA with associated malformations (OR 1.9; 95% CI 0.5- 6.9) rather than to isolated EA (OR 0.5; 95% CI 0.1-3.6) (Table 7).

Table 7. Maternal pre-existing or gestational diabetes and risk of isolated esophageal atresia (EA) or esophageal atresia associated with other malformations, expressed in odds ratios (OR) with 95% confidence intervals (CI)

	Number of cases/controls	Basic model * OR (95% CI)	Adjusted model † OR (95% CI)
Pre-existing diabetes			
Isolated EA	1 / 20	0.5 (0.1- 3.8)	0.5 (0.1- 3.6)
EA with associated malformations	3 / 13	2.4 (0.7- 8.4)	1.9 (0.5- 6.9)
Gestational diabetes			
Isolated EA	6 / 29	2.1 (0.9- 5.1)	1.8 (0.7- 4.6)
EA with associated malformations	3 / 21	1.4 (0.3- 6.0)	1.7 (0.5- 6.1)

* Matching for sex of the infant and calendar year of delivery.

† Adjustments were made through matching*, and also by using logistic regression for parity, age, ethnicity, tobacco smoking during early pregnancy, chronic disease, body mass index, and educational level.

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

In the studies included in this thesis, an epidemiological approach was used in an attempt to answer the proposed hypotheses. Epidemiology has been defined as "the study of the distribution and determinants of disease frequency".[64] There are two main types of epidemiological studies, namely cohort and case-control studies.

Cohort studies

A cohort is defined as a designated group of individuals with a known exposure status who are followed-up over a period of time. The purpose of following-up the cohort is to measure the occurrence of one or more specific outcomes, for example death, during the period of follow-up. Eventually, outcome rates for two or more cohorts, for example a cohort exposed to a disease or a medication and an unexposed cohort, are compared.[64]

A *randomized trial* is a subtype of cohort study where the exposure status of each study participant is assigned randomly and the exposure is actively administered to the exposed cohort. In theory, the best empirical evidence regarding disease causation should come from randomized trials in humans, since the randomization tends to produce comparability between the cohorts with respect to all factors other than the exposure that might affect the outcome rate (see confounding) if the study group is sufficiently large. This study design cannot be used however, when studying potential risk exposure related to the outcome EA. It is obviously unethical, for instance, to actively give women potentially harmful exposures and impossible to randomize to a certain BMI, for example.

When it is not suitable to actively give the exposure to the exposed cohort, i.e., to perform an *experimental study*, it is a better alternative to conduct an *observational study*. In an observational study the exposure status of the study patients is already assigned; for example if the exposure is smoking, some people will have decided to smoke (the exposed cohort), while other people do not smoke (the unexposed cohort). The investigator's role is then to classify the study participants into the exposure categories that form the cohorts.

The main advantages of the cohort study are that temporal relations can be taken into account, incidence figures can be calculated, and many different outcomes can be studied at the same time. When the exposure status is assessed at the beginning of the follow-up, the cohort study can be defined as a prospective cohort study. However, if the incidence of the disease under study is low or the latency between exposure and the manifestation of the disease is long, the follow-up period might have to be very long. This is one of the main disadvantages of this study design, as it might be both time consuming and costly. The cohort study can, however, also be undertaken in a retrospective manner. The cohorts are then identified from recorded

information and the time at which the participants are at risk for the outcome is before the beginning of the study.
In many countries, keeping track of the study participants might also be a problem (risk for misclassification of the outcome, see below). Many participants might be what is called lost to follow-up. In Sweden, however, we have excellent possibilities of tracing our study participants through linkages between different registers.

Study I was a retrospective cohort study, undertaken to determine the incidence of EA and the mortality and risk of cancer after EA surgery.

Case-control studies

If the outcome is rare, as in the case of EA, it might be very inefficient, as mentioned above, to perform a prospective cohort study. A retrospective cohort study or a case-control study might then be the study design of choice. In a case-control study, the patients with a certain outcome, for example EA, are identified in a source population. These cases are retrospectively classified into exposed or unexposed. The distribution of exposed/ unexposed individuals in the source population, not having the outcome in question, is then estimated by calculating the distribution of exposed/ unexposed individuals in a randomly selected control group, which should mirror the source population. This selection of controls is based on what is referred to as "the rare disease assumption"; if there is a positive exposure-disease relationship the OR will overestimate the risk ratio, as the proportion of exposed individuals among those remaining free of disease at the end of the follow-up will be lower than among those starting their follow-up. If the disease is rare, however, the OR after using this sampling of controls strategy will be a reasonably good estimate of the risk ratio. The selection of controls is one of the main concerns when carrying out a case-control study, as it is of utmost importance that the controls are sampled independently of their exposure status.

A *nested case-control study* is a study in which the source population is a well-defined cohort from which the controls are sampled randomly. It is then possible to select controls in an unbiased fashion. Studies II, III, and IV were all nested case-control studies.

One other advantage of a high quality case-control study, besides the possibility of reflecting the results of a cohort study in a considerably shorter time and at lower cost, is that different exposures can be studied in respect to the same outcome. This has been utilized in both studies II and III. Possible drawbacks may be doubt about the reliance of exposure data if information on such data has to be obtained retrospectively, i.e., when the outcome has already taken place (see recall bias). This can be avoided, however, if the exposure data has been documented before the outcome has occurred, i.e. collected prospectively, which is the case in all the studies included in this thesis. When depending on already documented data, the investigator is of course dependent on good documentation, and some information might be unavailable.

Validity and precision

There are two main type of errors in epidemiological studies: *systematic errors* (often called bias) and *random errors*.

The level of *systematic errors* affects the internal validity, i.e., the extent to which the results actually measure what the study is aimed to measure. Internal validity is a prerequisite for external validity, i.e., the generalizability of the findings to other populations. The generalizability of the studies in this thesis should be satisfactory, in view of the nationwide, population-based study designs. Systematic errors are often classified into three categories: *selection bias, information bias,* and *confounding*.

Selection bias

This error stems from the procedure of selecting subjects and from factors that influence study participation. Selection bias occurs if the association between the exposure and outcome differs between those who participate in the study and those who do not.

If all cases are recruited from a precisely defined and identified population and the controls are sampled directly from this population, the study is said to be *population-based*. Sweden provides unique possibilities for population-based studies, with the nationwide registers and population statistics, as well as the national public health care system, representing close to 100% of all Swedish health care. A population-based design allays concern of selection bias, since there is theoretically no selection if the participation frequency is 100%. All the four studies included in the present thesis were population-based (study I only in the later timeperiod, when the coverage of the registers was complete).

In study III some exposure data were missing, to an extent that could have introduced selection bias. However, we tested whether the group of mothers with missing or without data were different with regard to tobacco smoking, BMI, or socioeconomic status, and found no significant differences in the risk of EA (data not shown), indicating a lack of strong selection bias.

Selection bias might have been introduced by the fact that the studies were limited to liveborn cases. Differences in exposure may, for example, lead to a different probability of prenatal diagnosis of a congenital malformation, and thereby termination of the pregnancy. For example the exposure maternal age is associated with a higher rate of amniocentesis, which may lead to a higher rate of termination of pregnancy with an infant afflicted with a congenital anomaly among older women. Different exposures may also lead to different rates of spontaneous abortion, and the study may then address the risk factors for survival or for severity of a congenital anomaly rather than factors for the congenital anomaly itself.[69] Some congenital anomalies associated with EA might be identified prenatally, thereby leading to a falsely low frequency of infants with EA with associated anomalies among liveborn infants if these pregnancies are terminated. As a group, infants with isolated EA are not usually detected prenatally, however, and it is reasonable to assume that this isolated congenital

malformation will not lead to a much higher rate of spontaneous abortion either. The group of infants with isolated EA should therefore not be as vulnerable to this kind of bias and this group might also be the most interesting when studying the etiology of esophageal atresia.

Information bias

When the information regarding the exposure or outcome is wrong, information bias can arise. Such misclassification can be either *non-differential* or *differential*. With non-differential misclassification, the misclassification of one of the variables (for example exposure) does not depend on the person's status for the other (in this example, the outcome). The potential difference in risk rate between the different groups is then diluted, and the risk estimates are erroneously driven against a lack of association. If the misclassification is differential, on the other hand, the result might either show a false weaker or false stronger association than the true risk.

There could have been some misclassification of the occurrence of EA (the exposure in study I, the outcome in studies II, III and IV) in each of the registers used to ascertain the diagnosis. This information, however, was obtained from a combination of three different registers, and the level of missing cases of EA resulting from use of this strategy has been found to be negligible.[59] When working with the cohorts of EA patients based on these three registers, we have become aware of one problem due to misclassification, our so called "twinning problem". In the two first conducted studies, studies II and III, we received a cohort of EA patients from the National Board of Welfare that included patients from the Register of Congenital Malformations registered before 1999. Before 1999, only the birth date, not the personal identity number of the child, was registered. To make the national registers more complete, data entered in one of the registers had also been documented in the other registers. In this case, the diagnosis of EA had been entered in the Register of Congenital Malformations for one of the twins in some twin pairs. When the data were carried on to the Medical Birth Register, both of the twins in 22 twin pairs were registered as having the malformation, misclassifying 22 patients as cases. We became aware of this error after papers II and III had been published, but the results about twinning presented in Table 4 are corrected. In studies I and IV, the problem was dealt with before the data were analyzed.

Recall bias is a type of information bias that might occur if information on exposure data has to be assessed in a retrospective manner, i.e., when the outcome has already taken place. In all the studies included in the present thesis, the exposure data were documented in the registers in a prospective manner, before the outcome had occurred. Thus, recall bias should not have taken place. Misclassification of the exposure data is of course possible anyway. In study II, when approaching maternal ethnicity, we used women born in the Nordic countries as a proxy variable for white or Caucasian ethnicity. We assumed that women born in the Nordic countries are predominantly of white or Caucasian ethnicity. This assumption should most probably lead to some misclassification of this exposure, since today the populations of these countries are multicultural, i.e., a mix of people from various ethnicities. Such error would

most likely be non-differential, however, i.e., similar between cases and controls, and would thus not explain the identified associations, but dilute the effects against the null.

Another concern regarding ethnicity is that the terminology used in paper II and III was incorrect. Ethnicity was in fact categorized into Nordic (Swedish, Norwegian, Danish, Finnish, or Icelandic) or non-Nordic, while in study II and III the Nordic countries were incorrectly referred to as Scandinavian countries, which only include Sweden, Norway and Denmark. This error has been corrected in this thesis frame, in which the term Nordic is used instead of Scandinavian.

In study III, exposure data on tobacco smoking during pregnancy might have been misclassified if women had under-reported such use. However, the validity of the smoking information has previously been assessed by analyzing the relation between the mean birth weight of infants and reported maternal smoking, and good correlation was found.[59] In study IV, also, methodological drawbacks include possible misclassification of the exposure, i.e., maternal diabetes, but any such misclassification should be non-differential and thus not explain the seemingly positive association.

When conducting studies on the etiology of birth defects, misclassification of some exposures might also be related to inability to pinpoint the relevant dose and timing of the exposure. It is reasonable to assume that risk exposures of the pregnant mother during early fetal development, just when the esophagus and the trachea are separating, are particularly critical for the development of EA. In study IV, where the exposure is diabetes, this might be the case. If we assume that high blood glucose levels are teratogenic, there is misclassification of exposure if the blood glucose level was well regulated during the critical time period of embryogenesis, even though the pregnant women was correctly diagnosed as having diabetes. Such an error should, however, have a diluting effect on positive results.

There might also be etiologic heterogenicity of EA, which could dilute positive associations if the outcome is misclassified into subgroups that are not embryologically or pathogenetically meaningful. In all the papers included in the present thesis, we tried to evaluate possible etiological differences by stratifying the outcome into three groups: 1) all infants with EA, 2) infants with isolated EA, and 3) infants with EA associated with other malformations or chromosomal abnormalities.

Confounding

Confounding could be explained as confusion or mixing of effects. A confounder is a factor that might have an effect on the outcome, is imbalanced between the exposure groups, but is not an effect of the exposure, i.e., it does not lie in the causal chain. In observational studies, confounding is a general source of error. There are many ways, however, to try to avoid it. Taking gender as an example, restriction could adjust for a possible confounding effect by only including one of the sexes in the study. Another possibility is matching. The gender of the controls is then chosen in the same frequency as that of the cases. The possible

confounders could also be dealt with in the analytical phase, i.e., by stratification or multivariable regression analyses. The limited knowledge about the etiology of EA reduces the ability to select relevant potential confounders. In all the papers included in this thesis, however, we tried to control for all most biologically plausible confounders by matching, stratification, and adjustments through multivariable regression analyses.

Random errors

Random errors (also called chance errors) are the errors that remain after systematic errors have been eliminated. They represent the variability of the data. The amount of random error is related to the sample size of the study. Random errors affect the precision, which is reflected statistically by the *confidence interval* and *p value*.

When an estimate is presented as a single value, it is referred to as a point estimate. To indicate the precision of the point estimate, we use the *confidence interval*. A small study has a wider confidence interval, indicating less precision, and the point estimate is subject to a higher risk of random error. If the confidence interval is set to 95%, it means that if the study could be replicated many times, the correct point estimate should lie within this interval 95% of the times.

The null hypothesis states that there is no association between the exposure and the outcome, i.e., the relative risk (RR)=1.0. The *p value* is the probability of observing an association seen in the specific results if the null hypothesis is true. A high p value indicates that the results are highly consistent with the null hypothesis, while a low p value indicates that the null hypothesis should be rejected.

There are two types of random errors, type I (α) and type II (β).

A type I (α) error occurs when the null hypothesis is rejected even though the null hypothesis is in fact true, i.e., when a statistically significant association between an exposure and an outcome is found in a study when actually no causal relationship exists (chance). This is a threat to all studies, including those in which several analyses are conducted, i.e., where there is multiple testing. In the studies included in this thesis attempts have been made to avoid or reduce the risk of such chance findings by thoroughly writing and following the study protocols for each study, with formulation of clear hypotheses and with categorization done in advance, as well as by making decisions as to which models are to be used before carrying out the analyses.

A type II (β) error occurs when the study fails to reject the null hypothesis when the null hypothesis is in fact false, i.e., when no statistically significant association is found even though a causal relationship does exist. This could be a result of misclassification of disease or exposure data or insufficient statistical power. Despite the low incidence of EA, the number of EA patients included in the studies in this thesis is large in comparison with that in previous studies, which should provide a good basis to avoid random errors. In study I,

however, the low expected occurrence of cancer in our cohort revealed a clearly limited statistical power in the evaluation of cancer risk. Moreover, in study IV the low prevalence of diabetes among fertile women resulted in limited statistical power, particularly in the stratified analyses.

FINDINGS AND IMPLICATIONS

Characteristics of EA patients

Our unselected assessment of EA in study II has provided evidence that children born with EA have a lower birth weight and more often are prematurely born, of male gender and twins compared to children born without this malformation. These findings are well in line with previously published data.[8, 10, 11, 21, 28]

The prevalence of associated anomalies among infants with EA is high. The frequency of associated anomalies reported in previous studies varies between 43% and 61%.[7, 8, 21] The studies included in the present thesis confirm this high prevalence of associated anomalies in patients with EA, with figures ranging from 42% in study I, over 49% in studies II & III, to 50% in study IV. The small differences seen between the studies might be due to different study periods.

Approximately 6-10% of infants born with EA have been reported to have chromosomal anomalies in previous literature.[8, 10] We found figures of 5% in study I and 7% in studies II and III. These figures are in the lower interval, which might imply some level of misclassification of the cases with chromosomal anomalies. In study I, the study period started in 1964, and the ICD codes defining chromosomal anomalies in the first ICD period, 1964-1968, were restricted to a few syndromes. Much progress in the field of genetics has taken place since the 1960s and for each new ICD version, the coding has become increasingly detailed, probably thereby resulting in more robust estimates in later years.

Incidence

Study I indicated a stable incidence of EA of about 3 cases per 10,000 live births in Sweden since the year 1987. The mean incidence of 3.13 cases per 10,000 livebirths is well in line with previous reports. Strengths of this study are that it is both large and population-based. There is one much larger study addressing the incidence of EA, based on 2,693 EA cases, in which an incidence of 2.55 per 10,000 births was reported.[8] That study, however, was not population-based, but relied on several registers of congenital malformations with varying ascertainment and coverage. In one population-based study an incidence of 3.4 per 10,000 births was found, based on 227 cases in the south-west of England,[7] while another population-based study, based on 292 cases registered in the California Birth Defects Monitoring Program, the reported incidence was 2.82 per 10,000 births.[21] Our results are in between the results of those studies. As our study is based on a larger sample, it might represent a more robust estimate. When comparing our study with these other population-based studies it

should be kept in mind, however, that the incidence in study I is the number of EA patients per 10,000 livebirths, prevalence rate at birth of EA. In both previous population-based studies intrauterine fetal deaths were also included. Only live births were considered in our study, since it represents a more robust estimate. If intrauterine fetal deaths had been included, it could have introduced detection bias due to a limited rate of autopsies. Further, at the time when the study was undertaken, only intrauterine fetal deaths after the 28th gestational week were recorded in the Swedish registers, in contrast to most other countries, where this limit was set to the 22nd gestational week. This would have made a fair comparison impossible anyhow.

Among the total of 2,305,858 newborn children constituting the study cohort in studies II and III, 821 had a recorded diagnosis of EA, corresponding to an incidence of 3.56 per 10,000 live births. The "twin problem", with 22 patients misclassified as EA cases, makes the estimate too high, but should not have had any major impact on the results. The use of different study periods might also explain the slightly higher incidence estimate compared to that in study I.

Mortality

In study I, we showed that the overall survival in EA has improved substantially during the last decades in Sweden. This probably reflects advances in neonatal intensive care, and pediatric cardiology, as well as pediatric surgery. The overall survival rate among patients with EA has been reported to exceed 90% in case series.[13, 52] The survival rates obtained in study I were slightly lower than these previously reported rates, but there are several differences between our study and previously published data that indicate a higher validity of our study.

Compared to our approach, previously published reports were hospital-based case reports, which usually have a higher level of accuracy and contain more detailed information on the cases, but might comprise selected groups of patients. Our study, with a nationwide, population-based cohort design, would be less prone to selection bias. Moreover, our sample size was higher.

Another major difference is that in previously published studies, the follow-up period is not clearly stated, and the frequency of cases lost to follow-up is unknown, which makes it difficult to evaluate the overall survival. In most countries, keeping track of the study participants is a problem, leading to a risk for misclassification of the outcome or loss to follow-up. In Sweden, however, there are excellent opportunities for follow-up of patients regarding mortality through the nationwide registers.

In our study, EA patients had an almost 12 times higher risk of mortality compared to the background population. This increased risk was most pronounced during the first 5 years after the repair of the EA. To our knowledge, all previously published papers on mortality are based on case series, which in contrast to a cohort study design have no reference group. The Swedish registers used in our study not only give information about the patients with EA, but

also provide excellent possibilities of obtaining data on the general population to use as reference data.

The finding of a strong correlation between circulatory malformations associated with EA and an increased risk of mortality was expected. In previous literature, addressing the impact of circulatory malformations on EA mortality, a circulatory malformation has been defined as "a major cyanotic or non-cyanotic heart lesion (patent ductus arteriosus excluded, unless operation was required)".[52] This stringent definition was not possible in our study since such detailed information was not available in the registers. If we had been able to use this definition, the impact of circulatory malformations on EA mortality in our study would probably have been greater. The inclusion of less severe circulatory malformations, reducing the effect of circulatory malformations on mortality, is most probably also the answer to why very low birth weight seemed to influence the mortality more than did circulatory anomalies in our study, a finding that is not in line with previous literature.[13, 49, 65]

In previous studies, birth weight seemed to have less impact on mortality in more recent years,[13, 49, 65] an observation also made in study I. It has even been questioned whether birth weight still is a risk factor for mortality in infants born with EA.[65] In the present study it was found that very low birth weight was still an important risk factor for mortality after repair of EA, which is in line with findings in most other recent studies.[13, 49]

Cancer

In study I, we found no support for any strongly increased risk of cancer development, including esophageal and respiratory tumors. This is in agreement with the only previously published cohort study on cancer risk after EA surgery.[47] Both these studies were, however, severely hampered by a low statistical power. Our study was larger and contributed with more person-years at risk than the other study. On the other hand, our cohort members were on average younger and were followed-up for a shorter mean time period. Our intention was, however, to study the cancer risk at an early age since according to case reports, all EA patients who had later developed cancer had been given their cancer diagnosis at an exceptionally early age (mean age 36 years, range 20 to 46 years).[2] In contrast to the other cohort study, we included patients from the age of one year. We did not expect to find cases of cancer as early as in childhood, but on the other hand, if there had been such a case it would have been devastating to miss it. We therefore decided to include all the patients in the cohort with more than one year of follow-up.

We have a virtually complete follow-up of the EA patients, which should imply a valid measurement of cancer as an outcome, but the low expected occurrence of cancer in our cohort revealed the limited statistical power concerning the evaluation of the cancer risk and prohibited any firm conclusions from being drawn regarding this issue. There is a need for more research with very long follow-up of large cohorts of EA patients before an association between EA and a risk of cancer can be ruled out.

Different maternal exposures and risk of EA

Parity

Study II indicated an increased risk of EA in newborn infants of first time mothers, both of isolated EA and EA associated with other anomalies. Interestingly, adjustments for maternal age did not attenuate this association. This result is in line with a finding of a 50% decrease in the risk of having a child with EA among women with at least two childbirths, compared to mothers with one birth only.[11] Similarly, a 35% and 25% increase in the risk of having a child with isolated EA and EA with associated malformations, respectively, has been reported in first time mothers compared to women with higher parity.[8]

It has been speculated whether an increased risk of EA with low parity might be linked with fertility problems,[8] a theory supported by the finding of an increased frequency of EA in several studies investigating the risk of congenital malformations in the infant after in vitro fertilization (IVF).[66, 67] Mothers who achieve a pregnancy after IVF generally have a lower parity, and are of older age, than mothers who conceive their children naturally.[66] The increased rate of multiple births has been shown to be one reason for the increase in risk of congenital malformations in infants from pregnancies after IVF. Various studies have shown effects of parental sub-fertility on the neonatal outcome also in the absence of IVF, suggesting that parental and notably maternal characteristics, and not the IVF procedure in itself, are the main causes of this increased risk.[68]

Maternal age

Partly contradictory results have previously been obtained regarding the relation between maternal age and risk of EA in the child. While no such association was found in one study,[8] other studies have shown an increased risk with increasing maternal age,[11] particularly if the EA was combined with other malformations.[21] It has been suggested that this potential link between EA and maternal age is due only to confounding by chromosomal abnormalities, as older mothers are at increased risk of having a child with chromosomal abnormalities and such abnormalities are over-represented among infants with EA.[1] Study II adds support to the hypothesis that higher maternal age is dose-dependently associated with risk of EA in the child, but we found no reason to implicate chromosomal abnormalities in this effect, since the association remained when the analysis was restricted to cases without such abnormalities.

Both age and parity may be indicators of a role of endocrine or other biological mechanisms in the etiology of EA.[69] There might, for example, be a higher genetic vulnerability to environmental risk factors among older mothers and those with lower parity, possibly as a result of accumulated DNA damage or less efficient DNA repair mechanisms.

Ethnicity

It has previously been suggested that ethnicity may influence the risk of having a child with EA, with an increased risk among Caucasians compared to other ethnic groups. [11, 21, 28] Since

most people from the Nordic countries are of Caucasian ethnicity, the increased risk of EA in the children of mothers born in the Nordic countries, compared to those born outside these countries (study II) adds evidence that Caucasian ethnicity is a risk factor. The increased risk among Caucasians of having an infant with isolated EA has also been supported in previous studies.[11, 21] An increased risk in this ethnic group might reflect differences in environmental risk exposures, as well as differences in genetic predispositions, triggered by environmental risk factors.

Tobacco smoking

At the time when study III was accepted for publication there were no published studies addressing the role of maternal tobacco smoking in the etiology of EA. Tobacco smoking during pregnancy had been shown to increase the prevalence of orofacial clefts in several studies and an association had also been found with a few other specific congenital malformations.[70-74] No strong teratogenic effects of smoking during early pregnancy with regard to congenital malformations in general had been seen.[75, 76] Among the studies addressing the role of maternal tobacco smoking in the etiology of malformations of the gastrointestinal system, one study showed a slightly increased association,[75] while another had shown no association.[76]

There are now two other case-control studies addressing the role of maternal tobacco smoking in the risk of EA.[77, 78] In the first of those studies no increased risk of EA with maternal smoking was found.[78] The other study showed no increased risk of the total group of EA. In the analyses stratified into isolated EA or EA with associated anomalies, no consistent association was observed, but a statistically significant increase in the risk of EA with associated anomalies was found in a subgroup of smokers. This association with tobacco smoking was more pronounced in EA cases with the VATER association. Combined exposure to maternal tobacco smoking and alcohol further elevated some of the ORs to some extent.[77] Both of these studies were, however, smaller than study III, addressing the same issue. Further, the other two studies were also based on retrospectively collected data, gathered through questionnaires. The response rates of the questionnaires were 73% and 76% in the respective case group of these two studies, which might imply some selection bias. The most important difference, however, is that the other studies were based on retrospectively collected data, in contrast to our prospectively collected data on exposure. The recall of past exposure during early pregnancy in parents with an infant afflicted with a major malformation, compared to parents with a healthy infant, might be substantially biased. This is in fact a classic example of recall information bias. Our larger study with prospectively collected data on smoking, validated through comparison with data on birth weight, should therefore provide more robust estimates of tobacco smoking in relation to the risk of EA compared to the other studies. No association between maternal tobacco smoking during early pregnancy and EA in the infant was seen in our study.

Obesity

At the time when study III was accepted for publication, maternal obesity had been reported to be associated with an increased risk of neural tube defects in the child,[79] but few studies had examined obesity in relation to the risk of other congenital malformations. In the available studies, in which only a very limited number of EA cases had been included, there were, however, indications of an increased risk of EA among infants of obese mothers.[79, 80]

More recently, one case-control study describing the relation between maternal obesity and different congenital anomalies, including 98 cases of EA, showed no increase in the risk of either isolated EA or EA with associated malformations among overweight or obese women.[81] A systematic review and meta-analysis have also been published on the same issue, showing an increased risk for a range of birth defects with maternal obesity. Regarding EA, however, no association was found in an analysis based on 222 EA cases.[82] Our large study supports the lack of any association between maternal obesity and risk of EA.

Socioeconomic status

Knowledge of socioeconomic inequalities can give clues to proximate causal agents. For examle early studies of neural tube defects in which socioeconomic status gradients were observed led to the finding of a protective effect of folic acid on such defects.[69, 83] Most non-chromosomal anomalies have been shown to increase in prevalence with increasing socioeconomic disadvantage.[84] The literature describing socioeconomic status inequalities with respect to EA is sparse and hampered by limited statistical power.[84] Our findings, using educational level as a marker of socioeconomic status, contradict an increased risk of EA in women of low socioeconomic status. Sweden might not, however, be an ideal country for such studies, as the socioeconomic differences might not be as pronounced as in other countries.

Diabetes

In study IV we found a seemingly 70 % higher risk of EA in infants of women with any type of diabetes during pregnancy compared to those of women without diabetes.

Maternal diabetes types 1 and 2, diagnosed before pregnancy, i.e. pre-existing diabetes, constitute a well known risk factor for many congenital malformations.[85] Studies investigating the effect on the gastrointestinal system are, however, sparse and the results are conflicting. Associations have been seen in some studies,[86, 87] but not in others.[88, 89] None of these previous studies have focused on EA, and the number of EA cases has been limited. Results of a recent, larger, case-control study suggested that women with pre-existing diabetes had an increased risk of delivering a child with EA associated with other malformations, but not isolated EA.[85] Our analyses in study IV were hampered by low statistical power, only speculations can be made since no significant differences were seen, but the adjusted point estimates are in line with the possibility that pre-existing diabetes might be positively related to EA with associated malformations rather than to isolated EA. Support for a role of maternal

diabetes in the etiology of EA with associated anomalies is also provided by a study showing that maternal diabetes is strongly associated with early cardiovascular malformations, especially among infants with a multisystem, predominantly VACTERL association, in which EA is one of the features.[90]

Infants of mothers with gestational diabetes have been shown to be at an increased risk of congenital anomalies overall, similar in nature to those associated with pre-existing diabetes, although the relationship has seemed to be weaker.[85] One study has shown an increased risk of EA in women with gestational diabetes,[87] otherwise no such association has been found with EA[33] or when EA was included in a group of gastrointestinal malformations.[85, 86] Although our results were not statistically significant after stratifying the exposure into pre-existing and gestational diabetes, the association with EA seemed to be stronger for gestational than for pre-existing diabetes. In gestational diabetes, the blood-glucose levels are often increased in the second trimester.[91] Biologically, the teratogenic effects, however, ought to be exerted during an earlier period of pregnancy. It has been shown previously that undiagnosed type 2 diabetes is often mistakenly diagnosed as gestational diabetes.[92] It is therefore possible that our results regarding gestational diabetes at least partly reflect undiagnosed type 2 diabetes. Alternatively, factors other than the elevated blood glucose level might explain an association between gestational diabetes and EA. We were able to adjust for many possible confounders, except family history, and the tendency toward a stronger association between gestational diabetes and EA remained in the adjusted analyses, indicating that no such confounding explains the results.

A larger study is needed to evaluate the role of diabetes in the etiology of EA and to determine whether there are any differences between pre-existing and gestational diabetes of the mother and the risk of EA in the infant.

CONCLUSIONS

- The incidence of EA has been stable during recent decades, at least in Sweden.
- Associated malformations and chromosomal abnormalities are common in children with EA.
- The survival among EA patients has improved substantially during recent decades in Sweden.
- Occurrence of associated anomalies and a very low birth weight are linked with a worse prognosis.
- The risk of cancer might not be increased during the first decades of life in patients operated on for EA.
- Children with EA have a lower birth weight, and are more often prematurely born, of male gender and twins, compared to children without this malformation.
- There seems to be an increased risk of EA among children of mothers having their first delivery, of older age, and of Caucasian ethnicity.
- There does not seem to be any increase in the risk of EA among children of mothers who smoke, are obese, or have a low socioeconomic status during early pregnancy.
- Maternal diabetes during pregnancy might increase the risk of EA in the child.

FUTURE PERSPECTIVES

Epidemiological studies of exposures during pregnancy and the risk of birth defects in the infant often reveal weak to moderate associations. Weak relative risks recurring in well-designed studies may, however, reflect true causal mechanisms. One example is folic acid supplementation and a reduced risk of neural tube defects (ORs from 1/3 to 1).

Weak relative risks could be the result of a combination of errors (or factors) that might dilute positive associations. Some of these errors have already been mentioned in this thesis, e.g., misclassification of some exposures due to inability to pinpoint the relevant dose and timing of the exposure, and possible etiologic heterogenicity of the malformation, which might dilute positive associations if the outcome is misclassified into subgroups that are not embryologically or pathogenetically meaningful.[93]

Another factor that may contribute to a weak relative risk for a specific exposure is the occurrence of biological interactions. These could be environmental-environmental interactions, environmental-gene interactions, or a combination of different genes. The genetics include both fetal genetics, giving a genetic susceptibility to certain environmental exposures, and maternal genetics giving an impaired intrauterine environment. All birth defects can be presumed to be caused by a combination or an interaction of environmental and genetic factors, i.e., to have a multi-factorial etiology. Many environmental exposures significantly increase the risk of birth defects, but only a minority of exposed individuals are affected.[69] Let me exemplify with the exposure to thalidomide. Although this drug is one of the most dangerous teratogenic exposures known, among the pregnant women who were exposed to thalidomide during the most vulnerable period of embryogenesis, only about one half of the infants had thalidomide embryopathy.[94]

It is a logical sequence in etiological research first to identify exposures that increase the risk and subsequently identify other factors that explain why only some of those exposed are affected. There is still much to be known about environmental risk exposures affecting the risk of EA in the infant. Studies of interactions between different environmental exposures might be a second step. Gene-environment interaction studies for birth defects are evolving, but are still at an early stage.[95] I believe, however, that collaboration between geneticists and epidemiologists with an interest in environmental risk factors could be the future that could make true progress in our knowledge in the etiology of EA.

The Swedish registers, some of them used in the studies included in this thesis, are unexploited gold mines. Sweden has unique opportunities for register-based research. I consider, for example, that the Swedish Twin Registry, the Swedish Prescribed Drug Register, in a few years, and possibly some of the National Quality Registers, might contain relevant information that could promote further investigations of the etiology of EA.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Esofagusatresi (EA) är en allvarlig medfödd missbildning av matstrupen som innebär att matstrupen inte är förbunden med magsäcken. I de flesta fall av EA är den övre delen av matstrupen en sluten ficka och den nedre delen av matstrupen förbunden med luftstrupen. Ungefär hälften av de barn som föds med EA har också andra missbildningar och/ eller kromosomavvikelser. Behandlingen av EA är kirurgisk, med en omfattande operation redan under något av barnets första levnadsdygn. Överlevnaden har förbättrats genom åren. Besvär från magtarmkanalen och från luftvägarna är dock fortfarande vanliga hos barn och vuxna som opererats för EA, vilket kan medföra försämrad livskvalitet.

För att kunna hitta förebyggande åtgärder är det viktigt att förstå varför missbildningen uppkommer. I nuläget finns väldigt lite kunskap om vad som kan öka risken för den gravida kvinnan att föda ett barn med EA. Resultat från tvilling- och familjestudier har visat att ärftliga faktorer spelar en underordnad roll, varför omgivningsfaktorer borde vara viktiga. Det huvudsakliga syftet med min avhandling är att bidra till en ökad kunskap om orsaker till att EA uppkommer. Avhandlingen belyser även förekomst, dödlighet och risk för cancerutveckling förenat med EA samt beskriver karakteristika hos barn födda med EA.

I Sverige föds cirka 30 barn med EA varje år. För att studera orsaker till att missbildningen uppkommer behövs studier med många patienter. Sverige har unika möjligheter att samla ihop uppgifter om ett stort antal patienter genom våra nationella register och denna avhandling bygger på uppgifter ur dessa register.

Delarbete I belyser förekomst av EA samt dödlighet och risk för cancerutveckling associerat med EA. Förekomst och dödlighet har studerats tidigare, men inte i en större och komplett nationsomfattande studie, som är mindre känslig för felkällor som exempelvis skevt urval. Det finns flera publicerade fallbeskrivningar där personer som opererats för EA i barndomen utvecklat cancer i matstrupen i anmärkningsvärt unga år. En mindre studie visade dock ingen ökad risk för cancer hos en grupp personer som opererats för EA. Vår studie omfattade 1126 patienter födda med EA i Sverige under perioden 1964-2007. Studien visade att förekomsten i genomsnitt var drygt tre fall per 10000 födda barn och att förekomsten var relativt konstant över tiden. Vi kunde också konstatera att barn födda med EA hade en 12 gånger ökad dödlighet jämfört med barn i allmänhet. Dödligheten var störst under de första fem åren. Andelen barn med EA som dog minskade avsevärt under de senare decennierna av den studerade perioden. Förekomst av andra associerade missbildningar och mycket låg födelsevikt var förenat med en sämre prognos hos barnen med EA. Ingen ökad risk för cancerutveckling kunde påvisas.

Delarbete II, III och **IV** är alla rikstäckande studier av över 700 barn som fötts med EA under perioderna 1982-2004 samt 1982-2007. De belyser ett antal utvalda faktorer hos den gravida kvinnan som vi trodde skulle kunna öka risken att föda ett barn med EA. Uppgifter

om dessa faktorer är hämtade ur register som bygger på journaler från mödravård, förlossning och barnkliniker. Kvinnor som fött barn med EA har i statistiska analyser jämförts med kvinnor som fött barn födda utan missbildningen med avseende på möjliga riskfaktorer.

I **delarbete II** studerade vi om risken för en gravid kvinna att föda ett barn med EA var kopplad till antal tidigare födda barn, moderns ålder och etnicitet (definierat som mödrar födda i eller utanför Norden). Enligt tidigare studier var dessa faktorer möjligen förknippade med risk att föda ett barn med EA, men resultaten var inte samstämmiga. Vi genomförde därför en större studie. Den visade att förstföderskor hade en ökad risk att föda barn med EA, risken var 30 procent lägre hos omföderskor. Risken att föda barn med EA ökade med stigande ålder hos modern. Kvinnor mellan 35 och 40 år hade en dubbelt så hög risk och kvinnor över 40 år hade en tre gånger högre risk att föda ett barn med EA jämfört med mödrar under 20 år. Mödrar födda i de nordiska länderna hade en 66 procent ökad risk att föda ett barn med EA utan andra associerade missbildningar än de mödrar som var födda utanför Norden. Vi fann också att barn med EA generellt sett hade lägre födelsevikt, oftare var för tidigt födda, oftare var pojkar och oftare tvillingar än andra barn.

I **delarbete III** undersökte vi rökning under tidig graviditet, övervikt och sämre socioekonomiska förhållanden (definierat utifrån utbildningsnivå) som möjliga riskfaktorer hos modern. Dessa faktorer hade i vissa tidigare studier varit förknippade med andra typer av missbildningar, men de hade inte tidigare studerats i förhållande till risk för att föda ett barn med EA. Vår studie visade att rökning under tidig graviditet, övervikt eller sämre socioekonomiska förhållanden inte medförde någon ökad risk för EA hos barnet.

I **delarbete IV** analyserade vi risken att föda ett barn med EA hos mödrar med diabetes. Diabetes är en känd riskfaktor för en rad olika missbildningar, men resultaten var motstridiga med avseende på risken att föda ett barn med EA. Vår studie visade att diabetes under graviditeten verkade öka risken att föda ett barn med EA. Vi fann en 70 procent högre risk hos mödrar med någon form av diabetes under graviditeten jämfört med mödrar utan sjukdomen.

Sammantaget visade delarbetena som ingår i denna avhandling att förekomsten av missbildningen EA har varit stabil och överlevnaden förbättrats avsevärt de senaste decennierna. Risken för cancerutveckling i unga år verkar inte vara ökad. Barn födda med EA har en lägre födelsevikt, är oftare för tidigt födda, är oftare pojkar och oftare tvillingar än andra barn. Risken för en gravid kvinna att föda ett barn med EA verkar öka om modern är förstföderska, äldre, född i Norden eller har diabetes. Rökning under graviditet, övervikt och socioekonomiska förhållanden verkar däremot inte påverka risken för EA.

ACKNOWLEDGMENTS

Mitt stora tack till alla som på olika sätt har bidragit till denna avhandling, ett särskilt varmt tack till:

Jesper Lagergren, min huvudhandledare, för att jag har fått förmånen att vara doktorand under bästa möjliga omständligheter! Tack bland annat för snabb och klok feedback och för inspiration i stunder av tvivel. Du är en klippa!

Bengt Karpe, min bihandledare, för ditt engagemang och för att du delat med dig av dina omfattande kliniska erfarenheter.

Yunxia Lu, my co-supervisor, for sharing your expert statistitical knowledge, for discussions and support.

Agneta Nordenskjöld, min forskarutbildnings mentor, för trevliga luncher med samtal om forskning, men också om att vara forskare och samtidigt vara kliniker och ha familj.

Weimin Ye and Chongqi Jia, for introducing me to the world of biostatistics.

Emma Nilsson, medförfattare, för att du delat med dig av din stora registerkunskap.

Gruppen för övre gastrointestinal forskning, före detta och nuvarande medlemmar, för att jag fått förmånen att dela doktorandtiden med er.

Johan Wallander, verksamhetschef på barnkirurgen, och **Björn Frenckner,** ST-chef, för engagemang, stöd och uppmuntran i min forskarutbildning.

Alla kollegor på barnkirurgiska klinken, för att ni har fått mig att känna mig som en del av kliniken trots att jag inte har varit i tjänst på barnkirurgen på länge.

KIs forskarskola för kliniker inom epidemiologi, för en fantastisk doktorand utbildning.

Maud Marsden, för utmärkt och engagerad språkgranskning.

Vänner, för att jag får dela stort och smått med er och för att ni finns där trots att jag inte alltid är den bästa på att höra av mig.

Släktingar, inte minst **Hans och Lisa,** för glädjen att ha er som familj.

Mamma Gunilla och Pappa Sivert, för kärleksfullt stöd, ert engagemang i mina studier och för all praktisk uppbackning.

Wille och Märta, för den stora lyckan att vara er mamma.

Niclas, min älskade man och bäste vän, för all kärlek. Du gör mig till en bättre människa.

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