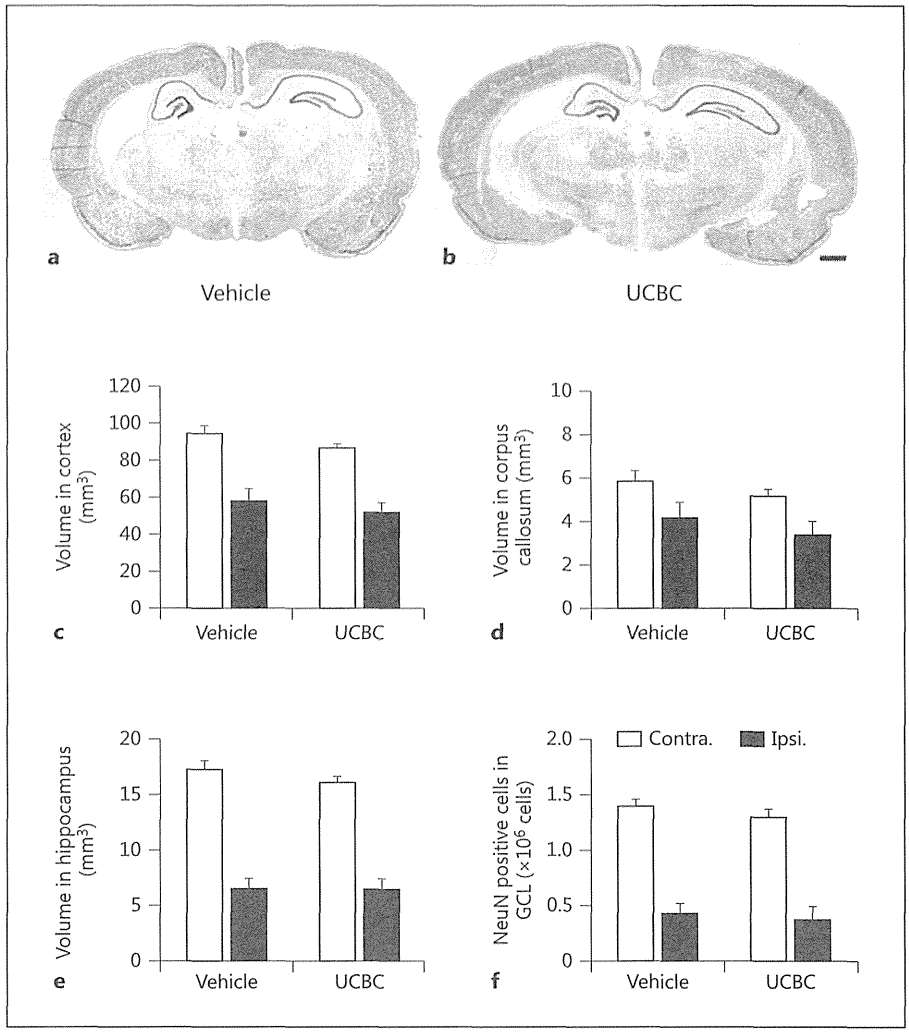

Fig. 5. Impact of the UCBCs on the histological change after HI. The volumes of whole cortex, corpus callosum and hippocampus, and the number of NeuN-positive neurons in whole hippocampus were evaluated using Stereo Investigator version 10 (stereology software) after behavioral tests. **a**, **b** Representative photomicrographs of the brain stained for NeuN from vehicle (**a**) and UCBC-treated rats (**b**) 2 months after HI. Bar = 1,000 μm. There was no significant difference in the volumes of the cortex (**c**), corpus callosum (**d**) or hippocampus (**e**) between the groups. The numbers of NeuN-positive neurons in the GCL were not significantly different between the vehicle and UCBC groups in the hippocampus of ipsilateral hemispheres (**f**). Contra. = Contralateral; Ipsi. = ipsilateral.

cytokine concentrations are increased [24], can induce greater differentiation of grafted cells to astrocytes, decrease the survival rates and/or decrease the beneficial treatment effects in various models such as spinal cord injury [25] and stroke [26] in adult rodents, and irradiation-induced brain injury in young mice [9]. In addition, early administration of mononuclear cells from bone marrow showed fewer treatment effects in a model of adult stroke [27]. However, in neonatal HI, there are two phases of pathological events: primary and secondary energy failure [28, 29]. Primary energy failure occurs within minutes after initial cerebral ischemia; in this phase, acidosis and depletion of oxygen, glucose and adenosine triphosphate lead to acute derangement of intracellular metabolism, resulting in necrosis and cell death. The subsequent secondary energy failure occurs after a variable period following the initial insult. Inflammation, excitatory amino acid release, intracellular calcium inflow, and production of nitric oxide and reactive oxidative species occur in this stage. Therefore, it is reasonable that most therapies, including hypothermia therapy, should be commenced at least before the second phase. The treatment effect induced by UCBC administration for brain injury is considered to be involved in secretion of neurotrophic factors that promote endogenous neurogenesis, prevent loss of neuronal cells and regulate immunity [28], which was shown also in the effect of bone marrow-derived mesenchymal stem cells [30]. Expression levels of various pro-inflammatory cytokines, including interleukin-1 and tumor necrosis factor, are elevated in the early phase of perinatal HI; UCBC administration can decrease these levels, which are also accompanied by decreased expression of cluster of differentiation 68, a biomarker of activated microglia/macrophages in the brain

[31]. Our study showed a decrease in the number of activated microglia by UCBC administration in the early phase of neonatal HI (fig. 1e, 2e). Pimentel-Coelho et al. [13] also reported that intraperitoneal administration of UCBCs 3 h after insult decreased apoptosis and microglial activation, and improved primitive reflexes in a neonatal HI rat model. Therefore, to suppress the elevation of such proinflammatory cytokines, which lead to decreased apoptosis, UCBC administration in the early phase may be a reasonable therapeutic approach, as demonstrated in the present study.

Oxidative stress plays an important role in HI brain damage [32]. Here, we demonstrated a decrease in 4-HNE- and nitrotyrosine-positive cells in the dentate gyrus following UCBC administration (fig. 1c, d, 2c, d). To our knowledge, this study is the first to report antioxidative effects of UCBCs in a neonatal HI rat model. Arien-Zakay et al. [33] reported antioxidative effects of UCBC-derived neural progenitor cells on insulted PC12 cell lines. Further, suppression of oxidative stress after adult transient focal ischemia was observed in an interleukin-1 knockout murine model [34]. As described above, UCBC administration can decrease the elevated expression of proinflammatory cytokines including interleukin-1 [31]. Moreover, the decreased expression of ED1 in the present study (fig. 1e, 2e) indicates that UCBC administration decreased HI-induced inflammation. Thus, the antioxidative effect of UCBC administration might be exerted directly and/or via suppression of inflammation.

Calculations of the immunohistochemically stained cells were focused on the dentate gyrus of the hippocampus, which is one of the most vulnerable areas to hypoxic ischemic insult. Although we calculated the immunohistochemically stained cells with density counts, which is less sensitive than stereological counts, the results revealed that UCBC administration suppressed apoptosis, as indicated by the decrease in the number of cells positive for active caspase-3 and AIF (fig. 1a, b, 2a, b). In the present study, we performed high-resolution analyses of walking patterns using the CatWalk system; however, they were not sensitive enough to detect motor impairment after HI injury. In contrast to human neonates, rat pups after HI injury did not show obvious locomotor abnormalities, as in other studies using the same model [35]; this may have been because of the higher degree of plasticity of the immature rat brain [36]. We also evaluated the learning memory after HI with the shuttle avoidance test and found only a mild tendency to improve the learning memory in the UCBC group; the difference was not significant. This may be a type 2 error, and further studies are required to clarify the potential effects of UCBC therapy on motor impairment and cognitive deficits after HI. In addition, we found no differences between the vehicle and UCBC groups in absolute tissue loss or the number of neurons in the cortex, corpus callosum or hippocampus (fig. 5). Similarly, some former studies failed to show histological improvement following UCBC therapy [12, 16], whereas others did [14, 15]. We have summarized the experimental protocols and results [37]. In many previous reports, 1×10^7 mononuclear cells were administered intraperitoneally 24 h after the insult. We administered the same dose of cells at an earlier time point. Pimentel-Coelho et al. [13] administered UCBCs even earlier (3 h after the insult) using a lower dose (2×10^6) of cells, and showed improvement in morphology and behavior. It is still unclear how the differences between protocols can affect the results. Other possible reasons for the different outcomes can be the severity of the insult and other experimental settings. Considering the fact that the present study failed to show any effect on morphological changes in the chronic phase or improvement of behavioral impairments, despite the fact that several acute injury markers were suppressed, a modified protocol (e.g. repeated administration, combination with some other treatments) should be tried with an aim to achieve sustained neuroprotection.

In the present study, cryopreserved mononuclear cells were used. Even frozen-thawed UCBCs are known to produce various cytokines and chemokines [38], and exert a neuroprotective effect in various animal models [13, 37, 39]. Moreover, from the viewpoint of clinical applications, cryopreservation is essential in the case of allogeneic transplantation, which may be applicable for patients without access to their own cord blood cells.

We used human UCBCs in a rodent injury model. It might have been more suitable to use rat UCBCs. However, it was very difficult to get sufficient numbers of cells from the umbilical cord of rats without expansion in culture. Because the purpose of the present study was to evaluate the treatment effect of mononuclear cells from the umbilical cord without using culture procedures, we used human cells, as in previous publications [13–18].

Another possible limitation in the present study is that we could not monitor/control body temperature in each pup. We placed the pups on/into the temperature-controlled plate/chamber during the HI insult, and returned them to the dam in a temperature-controlled room after the insult. There might be some variation in brain temperature, leading to variation in the degree of brain damage [40].

The choice of injection site is an important issue when using cell infusion for the treatment of brain injury. We administered UCBCs intraperitoneally, as in most previous studies. According to our recent unpublished results, only a small number of cells either injected intraperitoneally or intravenously could be found in the brain, and cells injected intraperitoneally were less seen in the liver, lung or spleen than cells injected intravenously, indicating that many intraperitoneally injected cells might have stayed in the peritoneal cavity. The treatment effect might be through trophic factors secreted by the cells [41]. The extent of brain damage can be influenced by the peripheral inflammatory response [42]. Modulating peripheral inflammation can be a therapeutic target. In a traumatic brain injury model, multipotent adult progenitor cells exerted a neuroprotective effect through interaction with resident splenocytes [43].

In summary, these results indicate that a single intraperitoneal injection of UCBC-derived mononuclear cells 6 h after HI reduced caspase-3, AIF, microglial activations and oxidative stress, but it did not induce morphological or functional protection. Repeated administration or a combination treatment may be required to achieve sustained protection.

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Disclosure Statement

None of the authors have any conflicts of interest associated with this study.

References

1 Lawn JE, Cousens S, Zupan J: 4 million neonatal deaths: when? Where? Why? Lancet 2005;365:891–900.

2 Kurinczuk JJ, White-Koning M, Badawi N: Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev 2010;86:329–338.

3 Askalan R, Wang C, Shi H, Armstrong E, Yager JY: The effect of postischemic hypothermia on apoptotic cell death in the neonatal rat brain. Dev Neurosci 2011;33:320–329.

4 Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, Kapellou O, Levene M, Marlow N, Porter E, Thoresen M, Whitelaw A, Brocklehurst P: Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med 2009;361:1349–1358.

5 Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ: Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet 2005;365:663–670.

6 Sato Y, Oohira A: Chondroitin sulfate, a major niche substance of neural stem cells, and cell transplantation therapy of neurodegeneration combined with niche modification. Curr Stem Cell Res Ther 2009;4:200–209.

7 Lindvall O, Kokaia Z: Stem cells for the treatment of neurological disorders. Nature 2006; 441:1094–1096.

8 Sato Y, Nakanishi K, Hayakawa M, Kakizawa H, Saito A, Kuroda Y, Ida M, Tokita Y, Aono S, Matsui F, Kojima S, Oohira A: Reduction of brain injury in neonatal hypoxic-ischemic rats by intracerebroventricular injection of neural stem/progenitor cells together with chondroitinase ABC. Reprod Sci 2008;15: 613–620.

9 Sato Y, Shinjyo N, Sato M, Osato K, Zhu C, Pekna M, Kuhn HG, Blomgren K: Grafting of neural stem and progenitor cells to the hippocampus of young, irradiated mice causes gliosis and disrupts the granule cell layer. Cell Death Dis 2013;4:e591.

10 Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M: Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 2001;32:2682–2688.

11 Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR: Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: Distribution, migration, and differentiation. J Hematother Stem Cell Res 2003; 12:255–270.

12 Meier C, Middelanis J, Wasielewski B, Neuhoff S, Roth-Haerer A, Gantert M, Dinse HR, Dermietzel R, Jensen A: Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. Pediatr Res 2006;59:244–249.

13 Pimentel-Coelho PM, Magalhaes ES, Lopes LM, de Azevedo LC, Santiago MF, Mendez-Otero R: Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. Stem Cells Dev 2010;19:351–358.

14 Wasielewski B, Jensen A, Roth-Harer A, Dermietzel R, Meier C: Neuroglial activation and CX43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. Brain Res 2012;1487:39–53.

15 De Paula S, Greggio S, Marinowic DR, Machado DC, Da Costa JC: The dose-response effect of acute intravenous transplantation of human umbilical cord blood cells on brain damage and spatial memory deficits in neonatal hypoxia-ischemia. Neuroscience 2012; 210:431–441.

16 Geissler M, Dinse HR, Neuhoff S, Kreikemeier K, Meier C: Human umbilical cord blood cells restore brain damage induced changes in rat somatosensory cortex. PLoS One 2011; 6:e20194.

17 Rosenkranz K, Kumbruch S, Tenbusch M, Marcus K, Marschner K, Dermietzel R, Meier C: Transplantation of human umbilical cord blood cells mediated beneficial effects on apoptosis, angiogenesis and neuronal survival after hypoxic-ischemic brain injury in rats. Cell Tissue Res 2012;348: 429–438.

18 Yasuhara T, Hara K, Maki M, Xu L, Yu G, Ali MM, Masuda T, Yu SJ, Bae EK, Hayashi T, Matsukawa N, Kaneko Y, Kuzmin-Nichols N, Ellovitch S, Cruz EL, Klasko SK, Sanberg CD, Sanberg PR, Borlongan CV: Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic-ischaemic rats with human umbilical cord blood grafts. J Cell Mol Med 2010;14:914–921.

19 Rice JE 3rd, Vannucci RC, Brierley JB: The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol 1981;9:131–141.
20 Osato K, Sato Y, Ochiishi T, Osato A, Zhu C, Sato M, Swanpalmer J, Modjtahedi N, Kroemer G, Kuhn HG, Blomgren K: Apoptosis-inducing factor deficiency decreases the proliferation rate and protects the subventricular zone against ionizing radiation. Cell Death Dis 2010;1:e84.
21 Paxinous G, Watson C: The Rat Brain in Stereotaxic Coordinates, ed 2. San Diego, Academic Press, 1986.
22 Encarnacion A, Horie N, Keren-Gill H, Bliss TM, Steinberg GK, Shamloo M: Long-term behavioral assessment of function in an experimental model for ischemic stroke. J Neurosci Methods 2011;196:247–257.
23 Ichinohashi Y, Sato Y, Saito A, Ito M, Watanabe K, Hayakawa M, Nakanishi K, Wakatsuki A, Oohira A: Dexamethasone administration to the neonatal rat results in neurological dysfunction at the juvenile stage even at low doses. Early Hum Dev 2013;89:283–288.
24 Nakamura M, Houghtling RA, MacArthur L, Bayer BM, Bregman BS: Differences in cytokine gene expression profile between acute and secondary injury in adult rat spinal cord. Exp Neurol 2003;184:313–325.
25 Okada S, Ishii K, Yamane J, Iwanami A, Ikegami T, Katoh H, Iwamoto Y, Nakamura M, Miyoshi H, Okano HJ, Contag CH, Toyama Y, Okano H: In vivo imaging of engrafted neural stem cells: its application in evaluating the optimal timing of transplantation for spinal cord injury. FASEB J 2005;19:1839–1841.
26 Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, Guzman R: Timing of intra-arterial neural stem cell transplantation after hypoxia-ischemia influences cell engraftment, survival, and differentiation. Stroke 2012;43:1624–1631.
27 Uemura M, Kasahara Y, Nagatsuka K, Taguchi A: Cell-based therapy to promote angiogenesis in the brain following ischemic damage. Curr Vasc Pharmacol 2012;10:285–288.
28 Liao Y, Cotten M, Tan S, Kurtzberg J, Cairo MS: Rescuing the neonatal brain from hypoxic injury with autologous cord blood. Bone Marrow Transplant 2013;48:890–900.
29 Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, Peebles D, Wylezinska M, Owen-Reece H, Kirkbride V, et al: Delayed ('secondary') cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. Pediatr Res 1994;36:699–706.
30 Van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ: Repeated mesenchymal stem cell treatment after neonatal hypoxia-ischemia has distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of damage, corticospinal motor tract activity, and sensorimotor function. J Neurosci 2010;30:9603–9611.
31 Rosenkranz K, Tenbusch M, May C, Marcus K, Meier C: Changes in interleukin-1 alpha serum levels after transplantation of umbilical cord blood cells in a model of perinatal hypoxic-ischemic brain damage. Ann Anat 2013;195:122–127.
32 Warner DS, Sheng H, Batinic-Haberle I: Oxidants, antioxidants and the ischemic brain. J Exp Biol 2004;207:3221–3231.
33 Arien-Zakay H, Lecht S, Bercu MM, Tabakman R, Kohen R, Galski H, Nagler A, Lazarovici P: Neuroprotection by cord blood neural progenitors involves antioxidants, neurotrophic and angiogenic factors. Exp Neurol 2009;216:83–94.
34 Ohtaki H, Takaki A, Yin L, Dohi K, Nakamachi T, Matsunaga M, Horai R, Asano M, Iwakura Y, Shioda S: Suppression of oxidative stress after transient focal ischemia in interleukin-1 knock out mice. Acta Neurochir Suppl 2003;86:191–194.
35 De Paula S, Vitola AS, Greggio S, de Paula D, Mello PB, Lubianca JM, Xavier LL, Fiori HH, Dacosta JC: Hemispheric brain injury and behavioral deficits induced by severe neonatal hypoxia-ischemia in rats are not attenuated by intravenous administration of human umbilical cord blood cells. Pediatr Res 2009;65:631–635.
36 Yager JY, Wright S, Armstrong EA, Jahraus CM, Saucier DM: The influence of aging on recovery following ischemic brain damage. Behav Brain Res 2006;173:171–180.
37 Tsuji M, Taguchi A, Ohshima M, Kasahara Y, Sato Y, Tsuda H, Otani K, Yamahara K, Ihara M, Harada-Shiba M, Ikeda T, Matsuyama T: Effects of intravenous administration of umbilical cord blood CD34 cells in a mouse model of neonatal stroke. Neuroscience 2014;263C:148–158.
38 Newman MB, Willing AE, Manresa JJ, Sanberg CD, Sanberg PR: Cytokines produced by cultured human umbilical cord blood (HUCB) cells: implications for brain repair. Exp Neurol 2006;199:201–208.
39 Saporta S, Kim JJ, Willing AE, Fu ES, Davis CD, Sanberg PR: Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. J Hematother Stem Cell Res 2003;12:271–278.
40 DeBow SB, Clark DL, MacLellan CL, Colbourne F: Incomplete assessment of experimental cytoprotectants in rodent ischemia studies. Can J Neurol Sci 2003;30:368–374.
41 Fan CG, Zhang QJ, Tang FW, Han ZB, Wang GS, Han ZC: Human umbilical cord blood cells express neurotrophic factors. Neurosci Lett 2005;380:322–325.
42 Bonestroo HJ, Nijboer CH, van Velthoven CT, Kavelaars A, Hack CE, van Bel F, Heijnen CJ: Cerebral and hepatic inflammatory response after neonatal hypoxia-ischemia in newborn rats. Dev Neurosci 2013;35:197–211.
43 Walker PA, Shah SK, Jimenez F, Gerber MH, Xue H, Cutrone R, Hamilton JA, Mays RW, Deans R, Pati S, Dash PK, Cox CS Jr: Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: preserving the blood brain barrier via an interaction with splenocytes. Exp Neurol 2010;225:341–352.

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ORIGINAL ARTICLE

Surgical complications, especially gastroesophageal reflux disease, intestinal adhesion obstruction, and diaphragmatic hernia recurrence, are major sequelae in survivors of congenital diaphragmatic hernia

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Abstract
Purpose This study aimed to characterize the surgical complications, especially gastroesophageal reflux disease (GERD), intestinal adhesion obstruction (IAO), and diaphragmatic hernia recurrence, in patients with congenital diaphragmatic hernia (CDH).
Methods Between January 1995 and December 2013, we determined the incidence of surgical complications and their predictors in CDH patients. We also examined whether the CDH repair and patch closure were associated with the incidence of IAO and the severity of adhesion.
Results Seventy-four CDH survivors were evaluated. GERD occurred in 28 patients (37.8 %) and recurred in 8 patients (10.8 %). Stomach herniation was a risk factor for GERD, and occurred in 25 patients. IAO occurred in 13 patients (17.6 %). In 240 neonatal laparotomies in the same period, the incidence of IAO was significantly higher in patients who underwent CDH repair than in patients who underwent other neonatal laparotomy ($p = 0.023$). Surgical time and intraoperative bleeding were significantly greater following CDH repair with an artificial patch compared with CDH repair with direct closure.
Conclusion Surgical complications are major sequelae in survivors of CDH repair. CDH repair and artificial patch closure were significantly associated with the incidence of IAO and the severity of adhesion.

Keywords Congenital diaphragmatic hernia · Complication · Hernia recurrence · Gastroesophageal reflux disease · Intestinal adhesion obstruction

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Introduction

Congenital diaphragmatic hernia (CDH) is a life-threatening congenital anomaly that occurs in between 1 in 2,500 to 1 in 4,000 live births. Advances in neonatal intensive care and ventilatory management have led to an improvement in the overall survival rate of CDH patients of up to 90 % in single-institution studies [1–3]. However, the improved survival of patients with CDH has also resulted in an increase in the incidences of early and late postoperative complications [4, 5]. Consequently, clinicians are now focusing on the long-term outcomes of these patients. Common disorders associated with CDH include pulmonary sequelae, neurodevelopmental deficits, chest wall and spinal deformations, hearing loss, and other abnormalities [6]. To date, however, very few studies have described the surgical complications, especially intestinal adhesion obstruction (IAO), in survivors of CDH.

The aim of this study was to describe the incidence and characteristics of surgical complications, focusing on gastroesophageal reflux disease (GERD), IAO, and diaphragmatic hernia recurrence. We also retrospectively examined the surgical data to identify possible risk factors for specific adverse surgical outcomes. Focusing on IAO after CDH repair, we compared the incidence of IAO after CDH repair with that of IAO after neonatal laparotomy. We also compared operative time and the amount of intraoperative

bleeding during ileus surgery between artificial patch repair and direct closure.

Patients and methods

Between January 1995 and December 2013, 83 patients with early symptoms of CDH were managed in Nagoya University Hospital. Seventy-four patients (89 %) survived and were included in this study. The treatment regimen comprised delayed closure, which was preceded by preoperative stabilization with high-frequency oscillatory ventilation, inhaled nitric oxide (iNO), and administration of drugs (e.g., prostaglandin) until pulmonary hypertension was attenuated. The median duration of preoperative stabilization was 4 days. All 74 patients underwent repair of the diaphragmatic defect via subcostal laparotomy. Direct closure was attempted to the best of our ability. Artificial patches (Goretex; W. L. Gore & Associates, Inc., Tokyo, Japan) were only used when it was impossible to perform a primary repair without significant tension. Twenty-four-hour esophageal pH-metry was performed just before discharge and at about 6 months old.

The following information was recorded from the patients' medical records as possible predictors: prenatal diagnosis of CDH, presence of the liver or the stomach in the thoracic cavity, iNO, use of extracorporeal membrane oxygenation (ECMO), and the method used to close the diaphragmatic defect (i.e., direct closure or use of an artificial patch). The data were analyzed to determine which of these factors were significantly associated with GERD, IAO, or hernia recurrence.

In the same period, 240 patients underwent open laparotomy to treat a variety of diseases other than CDH closure, including necrotizing enterocolitis, intestinal atresia, meconium ileus, and malrotation. We, therefore, compared the incidence and characteristics of IAO between patients who underwent CDH repair or other neonatal laparotomies. We also compared the effects of direct closure or closure with an artificial patch on complications after CDH repair.

Univariate analyses were performed using the χ^2 test or Fisher's exact test for categorical variables, and the Mann–Whitney U test for continuous variables. Values of $P < 0.05$ were considered statistically significant.

This retrospective survey was approved by the ethics committee in Nagoya University Hospital.

Results

Seventy-four newborns who were diagnosed with CDH <24 h after birth and who were alive at hospital discharge were included in this study. Their characteristics are shown in Table 1. The median birth weight was 2,760 g (range 982–4,102 g). The median follow-up of the 74 patients was 50 months (range 4–225 months). Four patients had the right side hernia. Of the 74 patients, 47 (63.5 %) were ventilated with iNO. Thirteen (17.6 %) patients were treated with ECMO and 25 (33.8 %) received artificial patch repairs. The most common surgical complications that needed another operation after CDH repair were GERD, IAO, and hernia recurrence. The numbers and timing of the operations after CDH repair are shown in Table 2. The relationships between the clinical variables and the surgical complications are shown in Table 3. Patients with their stomach located in the thoracic cavity were significantly more likely to undergo surgery for CDH recurrence and fundoplication for GERD than patients with the stomach in the correct location. Prenatal diagnosis, liver located in the thoracic cavity, iNO, use of ECMO, and patch repair method were not significantly associated with complications of CDH repair.

Reoperation for IAO was required in 13/74 (17.6 %) patients who underwent CDH repair compared with 16/240 (6.7 %) patients who underwent other neonatal laparotomies. Of these 16 patients with IAO after neonatal laparotomy, 6 had intestinal perforation, 4 had intestinal atresia, 3 underwent Ladd's operation, 1 had

Table 1 Characteristics of the infants

	n	%
Total	74	100
Males	44	59.5
Right side hernia	4	5.4
Prenatal diagnosis	60	81.1
Liver located in the thoracic cavity	29	39.2
Stomach located in the thoracic cavity	50	67.6
Inhaled nitric oxide	47	63.5
Use of ECMO	13	17.6
Patch repair	25	33.8

ECMO extracorporeal membrane oxygenation

Table 2 Surgical complications and timing of operations after CDH repair

	n (%)	Timing of subsequent operation (months)[a]
GERD	28 (37.8 %)	8 (0–83)
IAO	13 (17.6 %)	4 (1–48)
Hernia recurrence	8 (10.8 %)	2.5 (0–10)

CDH congenital diaphragmatic hernia, *GERD* gastroesophageal reflux disease, *IAO* intestinal adhesion obstruction

[a] Median (range)

Table 3 Associations between clinical variables and surgical complications after CDH repair ($n = 74$)

Variables	GERD			IAO			Hernia recurrence		
	Yes ($n = 28$)	No ($n = 56$)	p	Yes ($n = 13$)	No ($n = 61$)	p	Yes ($n = 8$)	No ($n = 66$)	p
Prenatal diagnosis	24 (86 %)	34 (74 %)	0.55	10 (77 %)	50 (82 %)	0.7	8 (100 %)	52 (79 %)	0.34
Liver located in the thoracic cavity	15 (54 %)	14 (30 %)	0.055	7 (54 %)	22 (36 %)	0.35	5 (63 %)	4 (36 %)	0.25
Stomach located in the thoracic cavity	25 (89 %)	25 (54 %)	**0.002**	9 (69 %)	41 (67 %)	>0.99	8 (100 %)	42 (64 %)	**0.048**
Inhaled nitric oxide	20 (80 %)	27 (59 %)	0.33	10 (77 %)	37 (61 %)	0.35	5 (63 %)	42 (64 %)	>0.99
Use of ECMO	4 (12 %)	9 (20 %)	0.76	3 (23 %)	10 (16 %)	0.69	3 (38 %)	10 (15 %)	0.14
Patch repair	12 (43 %)	13 (28 %)	0.22	6 (46 %)	19 (31 %)	0.34	5 (63 %)	20 (30 %)	0.11

CDH congenital diaphragmatic hernia, *GERD* gastroesophageal reflux disease, *IAO* intestinal adhesion obstruction, *ECMO* extracorporeal membrane oxygenation

Bold values are statistically significant at $p < 0.05$

Table 4 Comparative of operative time and blood loss during reoperation for IAO after CDH repair with an artificial patch, CDH repair with direct closure, or neonatal laparotomy other than CDH repair

	Operative time (min)		Blood loss relative to body weight (g/kg)	
CDH repair with direct closure	79.7 ±36.3	§	3.68 ±3.8	§
CDH with an artificial patch	199.4 ± 50.4	§	40.0 ± 48.8	§
Neonatal laparotomy other than CDH repair	125.6 ± 70.0		7.4 ± 10.1	

IAO intestinal adhesion obstruction, *CDH* congenital diaphragmatic hernia

§ $p < 0.05$

retroperitoneal tumor, 1 had torsion of small intestine and 1 had gastroschisis. Median timing of these IAO operations after neonatal laparotomy was 8 months (range 1–190). The incidence of IAO was significantly greater in patients who underwent CDH repair than in patients who underwent other neonatal laparotomies ($p = 0.023$). The mean duration of the reoperation for IAO was 129.6 ± 72.9 min after CDH repair compared with 125.6 ± 70 min after other neonatal laparotomies ($p = 0.98$). The mean intraoperative blood loss during IAO reoperation relative to the patient's body weight was 18.8 ± 36.3 g/kg after CDH repair compared with 7.4 ± 10.1 g/kg after other neonatal laparotomies ($p = 0.26$). The severity of intestinal adhesion in all patients of the CDH repair was not significantly different from that in patients of other neonatal laparotomies.

In terms of CDH repair methods, the operative time and intraoperative blood loss relative to body weight were greater in patients who received an artificial patch compared with patients who underwent direct closure or patients who underwent other neonatal laparotomies (Table 4). These results suggested that ileus operation after CDH repair with an artificial patch is a technically difficult procedure associated with increased blood loss.

Discussion

Surgical complications that require another laparotomy are thought to be common in CDH survivors, but data are limited regarding the long-term incidences of these complications and their risk factors [4, 6–9]. According to prior reports, the most common conditions that require surgical treatment after CDH repair include GERD, IAO, and diaphragmatic hernia recurrence [8–14]. In our study, we examined the relationships between CDH repair and these complications, and evaluated possible risk factors, including prenatal diagnosis, location of the liver or stomach in the thoracic cavity, iNO, use of ECMO, and the use of an artificial patch for hernia repair.

Gastroesophageal reflux disease is a well-known complication of CDH repair, and is thought to occur in 12–81 % of patients with CDH, including 38 % of patients who underwent CDH repair in our study [6]. The mechanisms responsible for GERD have not been fully clarified in CDH survivors. In our study, stomach translocation to the thoracic cavity was the only risk factor for GERD. Liver located in the thoracic cavity or patch repair was not associated with GERD. Three patients with liver herniation and two with patch repair did not have stomach herniation because they had CDH on the right. All of them did not have GERD. So in this study, liver herniation or patch repair was not significantly associated with GERD. Kieffer et al. [15] reported that the presence of an intrathoracic stomach at operation is associated with pathological GERD, and that a shortened abdominal esophagus and obtuse angle of His were implicated in the phenomenon.

Closing a large defect by approximation and direct suture under tension may place excess strain on the crus, increasing the risk of a hiatal hernia [11]. Esophageal and gastric manometry in CDH patients revealed that abnormal peristaltic contractility and propagation could predispose to GERD. Therefore, a combination of these congenital abnormalities may result in GERD after CDH repair.

Hernia recurred in eight CDH survivors (10.8 %). Recurring diaphragmatic hernias have been reported in 8–50 % of patients with CDH [8]. Therefore, a large defect that requires patch repair might increase the risk of recurrence [10]. However, in our study, only the location of the stomach in the thoracic cavity was a significant risk factor for hernia recurrence. In our patients, the hernias recurred soon after CDH repair, but they can recur several months to several years after the CDH repair. Patients may remain asymptomatic and the recurrence is often found incidentally. Therefore, the long-term risk and incidence of recurrence are still unclear.

Intestinal adhesion obstruction was one of the most common reasons for reoperation after CDH repair in our patients. IAO was reported to occur in approximately 10–20 % of CDH patients [11]. Some prior studies have described about GERD or diaphragmatic hernia recurrence, but very few studies have described IAO after CDH repair. So we performed a close examination of the cases of IAO after CDH repair. In our study, IAO was found in 13 patients (17.6 %) after CDH repair; this rate was significantly greater than that in patients who underwent other neonatal laparotomies. Other neonatal laparotomies were performed under many different causes and conditions. In this point of view, our comparative study may include any bias, but it has some informative one. In other reports, the incidence of IAO ranged from 2.2 to 6 % after neonatal laparotomies other than CDH repair [16–18]. Although comparative studies of CDH patients and patients undergoing neonatal laparotomy have not been performed, these earlier data support our findings. Several mechanisms may contribute to the increased susceptibility to IAO in CDH patients. For example, abnormal positioning of the intestine may cause intestinal kinking and increased intra-abdominal pressure may impair peristalsis [13]. Furthermore, prolonged illness and intestinal paralysis could increase the risk of intestinal adhesion and bowel obstruction.

We also showed that the mean operative time and blood loss were significantly greater in CDH patients who received an artificial patch than in CDH patients who underwent direct closure or patients who underwent other neonatal laparotomies. It was reported that patch repair significantly increased the risk of IAO in CDH patients [11]. We did not observe a similar association between IAO and CDH repair, but patch repair did increase the severity of intestinal adhesion and ileal release. Large CDH defects requiring an artificial patch for closure pose a surgical challenge, and may increase the risk of future operations, which will also be technically difficult. It is possible that intraperitoneal placement of a patch may have pathologic effects that promote tissue adhesion. Therefore, operative time and intraoperative blood loss were significantly greater in patients who received an artificial patch compared with patients who underwent direct closure. Adverse surgical outcomes were reported to be more common in patients with a large CDH defect requiring patch repair [7, 10, 19–22], and our results support the validity of this clinical condition.

In conclusion, although advances in the treatment of CDH have remarkably improved its survival rates, CDH survivors frequently develop surgical complications, including GERD, IAO, and hernia recurrence. The location of the stomach in the thoracic cavity at initial surgery was the only predictor of these complications in this study. The incidence of IAO was significantly greater after CDH repair than after other neonatal laparotomies. The operative procedure involved in the release of an IAO after CDH repair with an artificial patch is very difficult and is associated with a high risk of bleeding because of the formation of firm adhesions. Neonates are faced with a long time to experience complications associated with CDH repair. Therefore, it is vital that pediatric surgeons evaluate new strategies to reduce the risk of these surgical complications.

References

1. Boloker J, Bateman DA, Wung JT, Stolar CJ (2002) Congenital diaphragmatic hernia in 120 infants treated consecutively with permissive hypercapnea/spontaneous respiration/elective repair. J Pediatr Surg 37:357–366. doi:10.1053/jpsu.2002.30834
2. Downard CD, Jaksic T, Garza JJ, Dzakovic A, Nemes L, Jennings RW, Wilson JM (2003) Analysis of an improved survival rate for congenital diaphragmatic hernia. J Pediatr Surg 38:729–732. doi:10.1016/jpsu.2003.50194
3. Javid PJ, Jaksic T, Skarsgard ED, Lee S (2004) Survival rate in congenital diaphragmatic hernia: the experience of the Canadian Neonatal Network. J Pediatr Surg 39:657–660. doi:10.1016/j.jpedsurg.2004.01.022
4. Chiu P, Sauer C, Mihailovic A, Adatia I, Bohn D, Coates AL, Langer JC (2006) The price of success in the management of congenital diaphragmatic hernia: is improved survival accompanied by an increase in long-term morbidity. J Pediatr Surg 41:888–892. doi:10.1016/j.jpedsurg.2006.01.026
5. Chiu P, Hedrick HL (2008) Postnatal management and long-term outcome for survivors with congenital diaphragmatic hernia. Prenat Diagn 28:592–603. doi:10.1002/pd.2007
6. Bagolan P, Morini F (2007) Long-term follow up of infants with congenital diaphragmatic hernia. Semin Pediatr Surg 16:134–144
7. Jancelewicz T, Vu LT, Keller RL, Bratton B, Lee H, Farmer D, Harrison M, Miniati D, Mackenzie T, Hirose S, Nobuhara K (2010) Long-term surgical outcomes in congenital diaphragmatic

hernia: observations from a single institution. J Pediatr Surg 45:155–160. doi:10.1016/j.jpedsurg.2009.10.028
8. Lally KP, Engle W (2008) Postdischarge follow-up of infants with congenital diaphragmatic hernia. Pediatrics 121:627–632. doi:10.1542/peds.2007-3282
9. Lund DP, Mitchell J, Kharasch V, Quigley S, Kuehn M, Wilson JM (1994) Congenital diaphragmatic hernia: the hidden morbidity. J Pediatr Surg 29:258–264. doi:10.1016/0022-3468(94)90329-8
10. St Peter SD, Valusek PA, Tsao K, Holcomb GW 3rd, Ostlie DJ, Snyder CL (2007) Abdominal complications related to type of repair for congenital diaphragmatic hernia. J Surg Res 140:234–236. doi:10.1016/j.jss.2007.03.018
11. Peetsold MG, Heij HA, Kneepkens CM, Nagelkerke AF, Huisman J, Gemke RJ (2009) The long-term follow-up patients with a congenital diaphragmatic hernia: a broad spectrum of morbidity. Pediatr Surg Int 25:1–17. doi:10.1007/s00383-008-2257-y
12. Koivusalo AI, Pakarinen MP, Lindahl HG, Rintala RJ (2008) The cumulative incidence of significant gastroesophageal reflux in the patients with congenital diaphragmatic hernia—a systematic clinical, pH-metric, and endoscopic follow-up study. J Pediatr Surg 43:279–282. doi:10.1016/j.jpedsurg.2007.10.014
13. Arena F, Romeo C, Baldari S, Arena S, Antonuccio P, Campennì A, Zuccarello B, Romeo G (2008) Gastrointestinal sequelae in survivors of congenital diaphragmatic hernia. Pediatr Int 50:76–80. doi:10.1111/j.1442-200X.2007.02527.x
14. Vanamo K, Rintala RJ, Lindahl H, Louhimo I (1996) Long-term gastrointestinal morbidity in patients with congenital diaphragmatic defects. J Pediatr Surg 31:551–554. doi:10.1016/S0022-3468(96)90494-7
15. Kieffer J, Sapin E, Berg A, Beaudoin S, Bargy F, Helardot PG (1995) Gastroesophageal reflux after repair of congenital diaphragmatic hernia. J Pediatr Surg 30:1330–1333. doi:10.1016/0022-3468(95)90497-2
16. Festen C (1982) Postoperative small bowel obstruction in infants and children. Ann Surg 196:580–583
17. Janik JS, Ein SH, Filler RM, Shandling B, Simpson JS, Stephens CA (1981) An assessment of the surgical treatment of adhesive small bowel obstruction in infants and children. J Pediatr Surg 16:225–235
18. Choudhry MS, Grant HW (2006) Small bowel obstruction due to adhesions following neonatal laparotomy. Pediatr Surg Int 22:729–732. doi:10.1007/s00383-006-1719-3
19. Koziarkiewicz M, Taczalska A, Piaseczna-Piotrowska A (2013) Long-term follow-up of children with congenital diaphragmatic hernia—observations from a single institution. Eur J Pediatr Surg. doi:10.1055/s-0033-1357751
20. Diamond IR, Mah K, Kim PC, Bohn D, Gerstle JT, Wales PW (2007) Predicting the need for fundoplication at the time of congenital diaphragmatic hernia repair. J Pediatr Surg 42:1066–1070. doi:10.1016/j.jpedsurg.2007.01.046
21. Su W, Berry M, Puligandla PS, Aspirot A, Flageole H, Laberge JM (2007) Predictors of gastroesophageal reflux in neonates with congenital diaphragmatic hernia. J Pediatr Surg 42:1639–1643. doi:10.1016/j.jpedsurg.2007.05.016
22. Kamiyama M, Kawahara H, Okuyama H, Oue T, Kuroda S, Kubota A, Okada A (2002) Gastroesophageal reflux after repair of congenital diaphragmatic hernia. J Pediatr Surg 37:1681–1684. doi:10.1053/jpsu.2002.36693

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Original Articles

The lung to thorax transverse area ratio has a linear correlation with the observed to expected lung area to head circumference ratio in fetuses with congenital diaphragmatic hernias

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ABSTRACT

Background/Purpose: The purpose of this study was to clarify the relationship between the lung to thorax transverse area ratio (L/T ratio) and the observed to expected lung area to head circumference ratio (O/E LHR), based on the results of a nationwide Japanese survey conducted in 2011, and to evaluate the compatibility of these prognostic predictors of fetal CDH.

Methods: Two hundred and forty-two prenatally diagnosed isolated CDH patients born between 2006 and 2010 were included in the present analysis. A regression analysis was conducted to investigate the relationship between the L/T ratio and the O/E LHR based on 191 simultaneous measurements of these parameters in 120 patients.

Results: The linear regression equation between the L/T ratio and the O/E LHR was: L/T ratio = 0.0233 + (0.00222 × O/E LHR), ($R = 0.847$, $p < 0.0001$). According to this equation, 25% of the O/E LHR, the cut-off value used in the fetal intervention for CDH, was equivalent to an L/T ratio of 0.08, a commonly accepted cut-off value for identifying the most severe cases of fetal CDH.

Conclusions: As there is a positive correlation between the L/T ratio and the O/E LHR, these two parameters proved to be used interchangeably according to the linear regression equation.

The mortality and morbidity of infants with congenital diaphragmatic hernia (CDH) mainly depend on the severity of pulmonary hypoplasia. Therefore, an accurate prenatal assessment of pulmonary hypoplasia is necessary to establish an optimal treatment strategy for individuals before birth. Although many prenatal prognostic parameters have previously been proposed by various investigators [1–4], measurement of the residual lung size seems to be one of the most reasonable and realistic methods [5–8].

The lung area to head circumference ratio (LHR) was the most commonly used predictor for CDH in the past [5,9,10]. The observed to expected (O/E) LHR has become a standard parameter used for determining the indications for fetal intervention to treat severe cases of CDH [11]. Of note, the O/E LHR was used in the Tracheal Occlusion To Accelerate Lung growth (TOTAL) trial of left CDH patients with severe pulmonary hypoplasia [12,13]. On the other hand, the lung to thorax transverse area ratio (L/T ratio), which was proposed before the publication of the LHR [5,6,9], has been widely used in Japan for the assessment of pulmonary hypoplasia in fetal CDH patients [6,14–16]. The LHR is no longer considered to be independently predictive of survival [17,18], as it was shown to increase according to the gestational age [11,19–21]. In contrast, the O/E LHR is not influenced by gestational age [22] as is the case with the L/T ratio [6,14,19], because it is standardized by the normal mean value of the LHR corresponding to the specific gestational age [11]. Both of the indicators are similarly based on the measurement of the contralateral lung area by using tracing methods [6,21,23] at the transverse section containing the four-chamber view of the heart.

The relationship between the L/T ratio and the O/E LHR has not been studied, despite their similarities. The purpose of this study was to clarify the relationship between the L/T ratio and the O/E LHR and to evaluate the compatibility of these parameters as prognostic predictors of fetal CDH based on the results of a nationwide Japanese survey.

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1. Materials and methods

1.1. Study population

This retrospective cohort study was performed as part of a nationwide Japanese survey of neonatal CDH conducted in 2011. This study was conducted after being approved by the ethics committee of Osaka University Hospital (approval number 11017) and the independent ethics committees of five other participating institutions: Hyogo College of Medicine, National Center for Child Health and Development, Kyushu University, Nagoya University Hospital and Osaka Medical Center and Research Institute for Maternal and Child Health. The data obtained from 72 institutions that consented to participate in a questionnaire survey targeted to the departments of pediatric surgery and/or tertiary perinatal care centers of 159 educational hospitals were retrospectively evaluated. Data were collected as case report forms requesting further details about the patients by the data center located in Osaka University Graduate School of Medicine. The entered data were crosschecked twice by the data center and then were fixed after data cleansing. A total of 614 neonates with CDH were born between 2006 and 2010; the overall profiles of the patients are described elsewhere [24]. Among those subjects, the present study was conducted using the data of the 364 isolated CDH cases that were prenatally diagnosed.

Isolated CDH was defined as being present in CDH infants who did not have other serious congenital anomalies, such as major cardiac anomalies or unfavorable chromosomal abnormalities. Three cases of bilateral diaphragmatic hernia were excluded from the study. The contralateral lung area accompanied by the thorax area and/or the head circumference was measured at least one time in 242 out of the 364 cases. The initial and final measurements were reported in the case report form if those parameters were measured more than two times. A total of 242 study subjects (400 measurements), which accounted for 39.4% of all 614 CDH patients treated at 45 institutes, were ultimately included in the present analysis. Among those subjects, the thorax area measurement was reported 339 times for 210 patients and the head circumference measurement was reported 251 times for 154 patients. The contralateral lung area, the thorax area and the head circumference were simultaneously measured 191 times in 120 patients.

1.2. Collected data

The primary outcome measure was the survival to discharge, which was defined as surviving at the time of discharge from the hospital. The secondary outcome measure was the "intact discharge", which is a new concept for prognostic evaluation, defined as being discharged from the hospital without any major morbidity that requires home treatment, including ventilatory support, oxygen administration, tracheostomy, tube feeding, parenteral nutrition or vasodilator administration [4]. The patient demographics, including the gestational age, birth weight, Apgar score at 1 minute, presence of liver and stomach herniation, mode of delivery, gender and side of hernia, were reviewed. Whether a surgery could be performed, the size of the diaphragmatic defect, the surgical procedure performed, the use of high-frequency oscillatory ventilation (HFOV), nitric oxide inhalation (iNO), prostaglandin E_1 or extracorporeal membrane oxygenation (ECMO) were also reviewed. As the indication criteria for surgery were not defined prospectively, the operability of each case was determined according to the clinical decisions of each institution. The highest preductal PaO_2, best oxygenation index and the right to left shunting at the ductus which were determined within 24 h after birth, were reviewed. The contralateral lung area (in square millimeters) and the thorax area (in square millimeters) were measured by manual tracing of the limit of the lung and thorax at the transverse section containing the four-chamber view of the heart in ultrasonography. The head circumference (in millimeters) was measured in the standard biparietal view of ultrasonography. The L/T ratio was defined as the area of the contralateral lung divided by the area of the thorax [19]. The observed LHR, which was the ratio of the contralateral lung to the head circumference, was divided by the appropriate normal mean for gestational age and multiplied by 100 to derive the O/E LHR and expressed as a percentage [21]. The expected LHRs were determined by the published formulas, which are freely available to all by the official calculator in the Tracheal Occlusion To Accelerate Lung Growth (TOTAL) trial website (access http://www.totaltrial.eu/) [12].

1.3. Analysis of the relationship between the L/T ratio and the O/E LHR

A simple regression analysis was conducted to investigate the relationship between the L/T ratio and the O/E LHR based on the simultaneous measurements in 120 cases. Although the initial and final simultaneous measurements were available in 71 cases, only a single simultaneous measurement was available in 49 cases. We decided to use all simultaneous measurements in order to obtain more accurate relationships between the two parameters. The linear regression equation between the L/T ratio and the O/E LHR was derived from the regression analysis. The L/T ratio values which corresponded to the cut-off values of the O/E LHR used in the TOTAL trial entry criteria were calculated according to the linear regression equation.

1.4. Patient outcome according to the prenatal prediction of the disease severity

In the 226 cases of left isolated CDH whose liver herniation was evaluated, the survival to discharge rate was reviewed according to the classification of the disease severity used in the TOTAL trial, which was defined by the combination of the O/E LHR and the presence of liver herniation, as proposed by Deprest et al. [25]. In the cases whose O/E LHR was not measured, the O/E LHR was estimated from the L/T ratio using the linear regression equation. The patient demographics, prenatal and postnatal profiles, including parameters indicating the respiratory status, circulatory status, surgical findings and outcome, were compared among the prenatal risk-stratified classifications defined by the combination of the L/T ratio and the presence of liver herniation, as proposed by Usui et al. [16]. In the cases whose L/T ratio was not measured, the L/T ratio was estimated from the O/E LHR using the linear regression equation. The values of the O/E LHR and L/T ratio were represented by the initial values of two measurements in principle, and the final values were substituted for the patients whose initial value was not available in the case report form.

1.5. Statistical analysis

The statistical analyses were performed using the JMP software program (version 9.02; SAS Institute, Inc, Cary, NC, USA). The frequencies and percentages were used to describe categorical data. The means and standard deviation were used to describe continuous variables. The median and interquartile ranges were used to describe Apgar scores. The chi-square test and Fisher's exact test were used to analyze categorical data. The one-way analysis of variance with Tukey's post-hoc honestly significant difference test was used to compare continuous variables. The Kruskal–Wallis test was used for the comparison of the Apgar scores. The log-rank test and Kaplan–Meier method were used to compare the survival times. Values of $P < 0.05$ were considered to indicate statistical significance.

2. Results

An outline of the patient demographics is shown in Table 1. Of the 242 neonates with prenatally diagnosed isolated CDH, 177 (73.1%)

Table 1
The patient demographics.

Number of patients	242
Gestational age (days)[a]	264.3 ± 8.6
Birth weight (g)[a]	2746 ± 386
Apgar score at 1 min[b]	4 (2–6)
Liver-up[c]	68/239 (28.5%)
Contralateral stomach herniation[d]	35/236 (14.8%)
Caesarean section delivery	177 (73.1%)
Gender (male)	138 (57.0%)
Side of hernia (left)	229 (94.6%)
Surgery performed for diaphragmatic hernia	224 (92.6%)
Time of surgery after birth (h)[b]	56, (30–95)
Patch closure	81/224 (36.2%)
Use of HFOV	212/233 (91.0%)
Use of iNO	166/241 (68.9%)
Use of ECMO	19 (7.9%)
Survival to discharge	200 (82.6%)
Intact discharge	177 (73.1%)

HFOV: high-frequency oscillatory ventilation, iNO: inhaled nitric oxide, ECMO: extracorporeal membrane oxygenation.
[a] Mean ± standard deviation.
[b] Median (interquartile range).
[c] Liver-up, liver occupying more than one-third of the thoracic space.
[d] Contralateral stomach herniation, more than half of the stomach was herniating into the contralateral thoracic cavity.

were delivered by Caesarean section and 224 (92.6%) underwent surgical repair for diaphragmatic hernia at a median age of 56 h after birth. Surgery could not be performed in 18 cases (7.4%) based on the clinical decisions of each institution. It was therefore assumed that these cases were extremely unstable and were considered to be in too serious of a condition to undergo a surgical repair. Two hundred patients (82.6%) survived until discharge, 177 (73.1%) of whom were discharged from the hospital without any major morbidity that required home treatment (Table 1).

2.1. Relationship between the L/T ratio and the O/E LHR

Eighteen of the 120 infants whose L/T ratio and O/E LHR were simultaneously determined died, resulting in an 85.0% survival rate. We found a strong positive correlation between the L/T ratio and the O/E LHR. The linear regression equation between the L/T ratio and the O/E LHR was: L/T ratio = 0.0233 + (0.00222 × O/E LHR), where the regression coefficient was 0.00222, correlation coefficient was 0.847 and coefficient of determination was 0.717 ($p < 0.0001$) (Fig. 1). According to this equation, 15%, 25%, 35% and 45% of the O/E LHRs, the cut-off values used in the TOTAL trial of left CDH patients, were found to be equivalent to 0.06, 0.08, 0.10 and 0.12 L/T ratios, respectively.

2.2. Patient outcome according to the prenatal prediction of the disease severity

In the 226 cases of left isolated CDH, the survival to discharge rate was reviewed according to the four-step stratification proposed by Deprest et al. [25]. The survival rate exhibited a trend toward a decrease as the severity of the disease increased. However, the effect of the liver herniation seemed to be stronger in our series compared to those in the series described by Deprest et al. (Fig. 2). In the prenatal risk-stratified classification [16], there were no significant differences in the patient demographics except for the side of hernia. There were unsurprisingly significant differences in the rate of liver-up and the L/T ratio based on how the each group was defined (Table 2). The highest preductal PaO_2 decreased, and the best oxygenation index increased, as the severity of the disease increased. The right to left shunting at ductus evaluated within 24 h after birth, which suggests the severity of pulmonary hypertension, differed significantly among the three groups, which resulted in the differences in the numbers of patients who used iNO, prostaglandin E_1 and ECMO. Although surgical repair

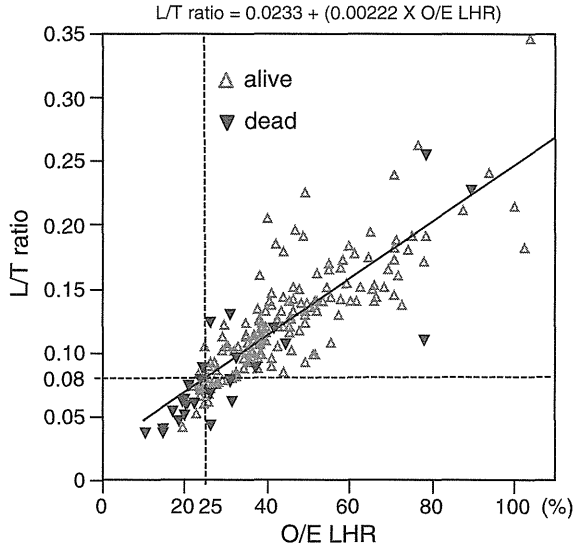

Fig. 1. The relationship between the O/E LHR and the L/T ratio. There was a linear positive correlation between the L/T ratio and the O/E LHR. The linear regression line was: L/T ratio = 0.0223 + (0.00222 × O/E LHR), where the regression coefficient was 0.00222, the correlation coefficient was 0.847 and the coefficient of determination was 0.717 ($p < 0.0001$). The open triangles represent the survivors and the closed triangles represent the non-survivors. The 25% O/E LHR was equivalent to an L/T ratio of 0.08 according to this equation, as indicated by broken lines.

could not be performed in only two (1.3%) cases in group A, surgery was not possible in six out of 16 (35.3%) cases in group C due to their unstable conditions. There were also significant differences in the proportions of patients with diaphragmatic defects exceeding 75%, as rated by the surgical record, as well as the need for patch repair. There were significant differences in the morbidity and mortality among the three groups. The rate of survival to discharge was 93% and the intact discharge rate was 87% in group A, whereas the corresponding rates were 72% and 58% in group B and 35% and 18% in group C, respectively (Table 3). There were also statistically significant differences in the survival curves among the three groups (Fig. 3).

3. Discussion

Since the mortality and morbidity of neonates with CDH primarily depend on the severity of pulmonary hypoplasia, an accurate prenatal assessment of pulmonary hypoplasia is necessary for making a decision about the optimal treatment. Although many prenatal prognostic parameters have been reported previously [1–4], the assessment of the residual lung size seems to be one of the most reasonable and

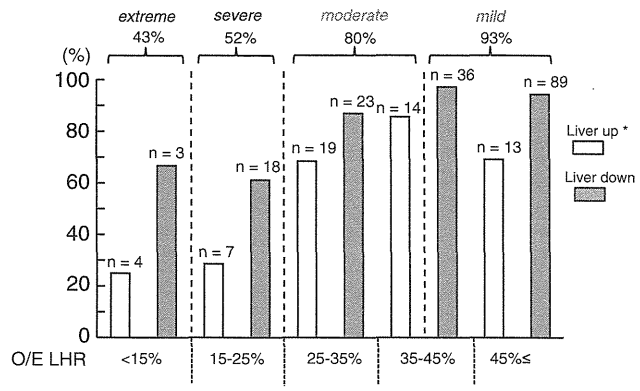

Fig. 2. The survival rates depending on the O/E LHR measurements and presence of liver herniation. *Liver-up, liver occupying more than one-third of the thoracic space.

Table 2
The patient demographics and prenatal findings according to the prenatal risk-stratified classification [16].

Definition of the group [16]	Group A	Group B	Group C	P
	L/T ratio ≥ 0.08 with liver-down	L/T ratio ≥ 0.08 with liver-up[a] or L/T ratio < 0.08 with liver-down	L/T ratio < 0.08 with liver-up[a]	
Number of patients	151	71	17	
Gender (male)	89 (58.9%)	36 (50.7%)	12 (70.6%)	0.265
Side of hernia (left)	149 (98.7%)	64 (90.1%)	13 (76.5%)	< 0.001
Gestational age at birth (days)	265 ± 7.7	263 ± 10.6	264 ± 6.9	0.313
Birth weight (kg)	2.76 ± 0.37	2.68 ± 0.45	2.87 ± 0.26	0.141
Caesarian section delivery	109 (72.2%)	53 (74.7%)	12 (70.6%)	0.908
Liver-up[a]	0 (0.0%)	51 (71.8%)	17 (100%)	< 0.001
Contralateral stomach herniation	5/148 (3.4%)	20/71 (28.2%)	10/17 (58.8%)	< 0.001
L/T ratio	0.148 ± 0.053*	0.106 ± 0.039**	0. 059 ± 0.020***	< 0.001

[a] Liver-up, liver occupying more than one-third of the thoracic space; Contralateral stomach herniation, more than half of the stomach was herniating into the contralateral thoracic cavity; L/T ratio, contralateral lung to thorax transverse area ratio.
* $P < .05$ A vs B.
** $P < .05$ B vs C.
*** $P < .05$ C vs A.

realistic methods. It has previously been reported that the LHR, which was first described in 1996 [5], was increased according to the gestational age in normal fetuses [21] and also in the fetuses with CDH [11,19]. The reason for this increase in the LHR with the gestational age is due to the difference in the rate of the increase of the lung area and head circumference. Peralta et al. reported that there was a four-fold increase in the LHR between 12 and 32 weeks of gestation in normal fetuses because of these differences [21]. Approaches to standardize the LHR by using the normal mean value of the LHR have been proposed to provide a constant value throughout the gestational period [11]. The LHR was originally defined as the contralateral lung area determined using a two-dimensional perpendicular linear measurement, divided by the head circumference [5]. However, two other methods to determine the lung area were subsequently proposed [9,21], and the tracing method was finally found to be the most reproducible method to measure the lung area [21,23].

The L/T ratio has been widely used in Japan, because it was first described in 1990 for the assessment of pulmonary hypoplasia in CDH [6], and has been applied for the assessment of pulmonary hypoplasia in CDH neonates since then [15,16,26]. The L/T ratio was originally reported to be constant throughout the gestational period in normal fetuses [6]. This parameter was redefined as the contralateral lung area, to make it more consistent with the LHR, divided by the area of the thorax as measured by the tracing method [19], although the original definition was determined by using the area of both lungs. Thus, there are several similarities between these two parameters. First, both parameters exhibit constant values throughout the gestational period, and the other is that only the contralateral lung area is measured by using the tracing method. However, the relationship between these two parameters has not been studied, despite their similarities.

A strong positive correlation between the L/T ratio and the O/E LHR was found, and a linear regression equation between the L/T ratio and the O/E LHR was obtained. According to this linear regression equation, several important cut-off values of both parameters can be interchanged. Interestingly, a 25% O/E LHR, the cut-off value for the most severe cases as used in the TOAL trial for fetal CDH, was found to be equivalent to an L/T ratio of 0.08, a commonly accepted cut-off

Table 3
The respiratory status, circulatory status, intraoperative findings and outcomes according to the prenatal risk-stratified classification [16].

Definition of the group [16]	Group A	Group B	Group C	P
	L/T ratio ≥ 0.08 with liver-down	L/T ratio ≥ 0.08 with liver-up[a] or L/T ratio < 0.08 with liver-down	L/T ratio < 0.08 with liver-up[a]	
Number of patients	151	71	17	
Apgar score at 1 min	5 (3–7) (n = 143)	4 (2–5) (n = 66)	2.5 (1.25–4) (n = 16)	<0.001
Highest preductal PaO_2 (Torr)[b]	257 ± 134* (n = 145)	199 ± 135** (n = 69)	75 ± 70*** (n = 17)	<0.001
Best oxygenation index[b]	5.7 ± 5.9* (n = 143)	14.3 ± 17.5** (n = 68)	32.0 ± 24.5*** (n = 17)	<0.001
Right to left shunting at ductus[b]	55/143 (38.5%)	40/68 (58.8%)	13/17 (76.5%)	0.001
Use of HFOV	130/145 (89.7%)	64/69 (92.8%)	16/17 (94.1%)	0.680
Use of iNO	85/151 (56.3%)	63/71 (88.7%)	15/16 (93.8%)	<0.001
Use of prostaglandin E_1	45/149 (30.2%)	35/71 (49.3%)	14/17 (82.4%)	<0.001
Use of ECMO	4 (2.7%)	9 (12.7)	5 (29.4%)	<0.001
Inoperable cases	2 (1.3%)	10 (14.1%)	6 (35.3%)	<0.001
Diaphragmatic defects ≥ 75%[c]	27/149 (18.1%)	38/61 (62.3%)	8/11(72.7%)	<0.001
Patch closure	31/149 (20.8%)	40/61 (65.6%)	8/11 (72.7%)	<0.001
Survival to discharge	141 (93.4%)	51 (71.8%)	6 (35.3%)	<0.001
Intact discharge	131 (86.8%)	41 (57.8%)	3 (17.7%)	<0.001

HFOV, high-frequency oscillatory ventilation, iNO, nitric oxide inhalation; ECMO, extracorporeal membrane oxygenation.
[a] Liver-up, liver occupying more than one-third of the thoracic space.
[b] The highest pre PaO_2, best oxygenation index and the right to left shunting at ductus were determined within 24 h after birth.
[c] The size of the diaphragmatic defect was rated by a surgeon according to the surgical record.
* $P < .05$ A vs B.
** $P < .05$ B vs C.
*** $P < .05$ C vs A.

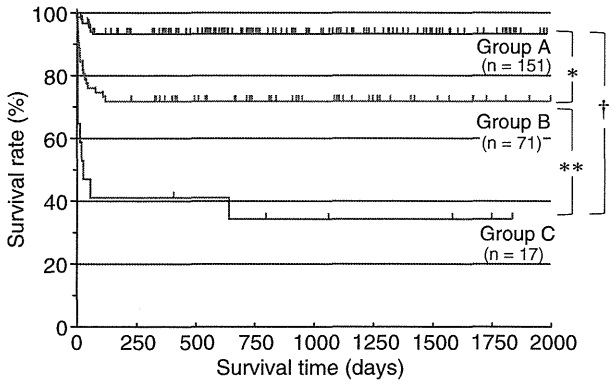

Fig. 3. The survival curves for patients with isolated CDH, compared using the prenatal risk-stratified classification [16]. *$P < .001$; **$P < .001$; †$P < .001$.

value for identifying the most severe cases of fetal CDH in Japan. These results suggested that the patients considered to be the most severe cases in Japan also met the criteria for fetal intervention for left CDH patients with severe pulmonary hypoplasia in the TOTAL trial protocol, which was the first international prospective randomized controlled trial for fetoscopic tracheal occlusion [12,13]. In the nationwide Japanese survey for fetal CDH, 57.7% of the patients were measured for the L/T ratio, and only 42.3% of the patients were measured for the O/E LHR. However, owing to this conversion equation, both of the parameters can be generated for the evaluation of the patient CDH severity if either of the parameters was measured.

To verify the accuracy and the universal applicability of the prenatal risk-stratified classification, which was proposed by Usui et al., and was defined as the combination of the L/T ratio and the presence of liver herniation [16], we applied the classification to this cohort as a different population from the original cohort using the conversion equation. Although the patient demographics except for the side of the hernia, were similar between the three groups classified using this system, the prenatal and postnatal profiles, including the stomach position, parameters indicating the respiratory status, circulatory status, surgical findings and outcome were significantly different between the three groups, suggesting that the prenatal risk-stratified classification is also valid in other cohorts, such as that in the nationwide Japanese questionnaire survey. The indication for a fetal intervention of the patients proposed by Deprest et al. [25] can be estimated by using the conversion eq. in the patients whom the L/T ratio was solely measured without measurement of LHR. The rate of survival to discharge was 93% in the mild group, 80% in the moderate group, 52% in the severe group and 43% in the extreme group (Fig. 2). Compared to this four-step stratification used in the TOTAL trial, our prenatal risk-stratified classification therefore seems to have better discrimination of disease severity. It is possible to describe the prenatal risk-stratified classification as shown in Table 4 using the O/E LHR instead of the L/T ratio according to the linear regression equation (Table 4).

When the characteristics of both parameters were compared, the gestational variation and the procedure of the lung area measurements were similar. However, there were concerns that the individual fetal growth variation is not considered when determining the O/E LHR. There may be a possibility for an overestimation in a small-for-date fetus, as the O/E LHR of these fetuses, which should have a lower LHR compared to an appropriate-for-date fetus, would be evaluated based on the normal mean value. The L/T ratio includes, by nature, individual fetal growth variation, and it can be determined with standard values for gestational age or with for a relevant population. More importantly, calculating the L/T ratio is a simple task to perform.

A major limitation of this study is that it was conducted in a retrospective manner using a questionnaire. Many of the institutions had a small number of cases, and the treatment strategies, including the indication criteria for surgery, were determined by each institution. There may have been inaccurate measurement of both parameters due to the limited experience of the physicians with such infants. More accurate prospective studies and an analysis of the correlation based on the timing of the measurement are therefore needed to confirm the present findings. Despite these limitations, an excellent positive correlation was observed between the L/T ratio and O/E LHR in the present study, and these two parameters proved to be compatible according to a linear regression equation. These results suggested that the linear regression equation may become a useful tool for all populations.

Table 4
The prenatal risk-stratified classification described using the O/E LHR instead of the L/T ratio.

Group A	O/E LHR ≥ 25% with liver-down
Group B	O/E LHR ≥ 25% with liver-up [a], or O/E LHR < 25% with liver-down
Group C	O/E LHR < 25% with liver-up [a]

[a] Liver-up, liver occupying more than one-third of the thoracic space.

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References

[1] Albanese CT, Lopoo J, Goldstein RB, et al. Fetal liver position and prenatal outcome for congenital diaphragmatic hernia. Prenat Diagn 1998;18:1138–42.
[2] Kitano Y, Nakagawa S, Kuroda T, et al. Liver position in fetal congenital diaphragmatic hernia retains a prognostic value in the era of lung-protective strategy. J Pediatr Surg 2005;40:1827–32.
[3] Hatch EI, Kendall J, Blumhagen J. Stomach position as an in utero predictor of neonatal outcome in left-sided diaphragmatic hernia. J Pediatr Surg 1992;27:778–9.
[4] Kitano Y, Okuyama H, Saito M, et al. Re-evaluation of stomach position as a simple prognostic factor in fetal left congenital diaphragmatic hernia: a multicenter survey in Japan. Ultrasound Obstet Gynecol 2011;37:277–82.
[5] Metkus AP, Filly RA, Stringer MD, et al. Sonographic predictors of survival in fetal diaphragmatic hernia. J Pediatr Surg 1996;31:148–52.
[6] Hasegawa T, Kamata S, Imura K, et al. Use of lung–thorax transverse area ratio in the antenatal evaluation of lung hypoplasia in congenital diaphragmatic hernia. J Clin Ultrasound 1990;18:705–9.
[7] Barnewolt CE, Kunisaki SM, Fauza DO, et al. Percent predicted lung volumes as measured on fetal magnetic resonance imaging: a useful biometric parameter for risk stratification in congenital diaphragmatic hernia. J Pediatr Surg 2007;42:193–7.
[8] Cannie M, Jani J, Meersschaert J, et al. Prenatal prediction of survival in isolated diaphragmatic hernia using observed to expected total fetal lung volume determined by magnetic resonance imaging based on either gestational age or fetal body volume. Ultrasound Obstet Gynecol 2008;32:633–9.
[9] Lipshutz GS, Albanese CT, Feldstein VA, et al. Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. J Pediatr Surg 1997;32:1634–6.
[10] Harrison MR, Keller RL, Hawgood SB, et al. A randomized trial of fetal endoscopic tracheal occlusion for sever fetal congenital diaphragmatic hernia. N Engl J Med 2003;349:1916–24.
[11] Jani J, Nocolaides KH, Keller RL, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. Ultrasound Obstet Gynecol 2007;30:67–71.
[12] DeKoninck P, Gratacos E, Van Mieghem T, et al. Results of fetal endoscopic tracheal occlusion for congenital diaphragmatic hernia and the set up of the randomized controlled TOTAL trial. Early Hum Dev 2011;87:619–24.
[13] Deprest J, De Coppi P. Antenatal management of isolated congenital diaphragmatic hernia today and tomorrow: ongoing collaborative research and development. J Pediatr Surg 2012;47:282–90.
[14] Tsukimori K, Masumoto K, Morokuma S, et al. The lung-to-thorax transverse area ratio at term and near term correlates with survival in isolated congenital diaphragmatic hernia. J Ultrasound Med 2008;27:707–13.
[15] Masumoto K, Teshiba R, Esumi G, et al. Improvement in the outcome of patients with antenatally diagnosed congenital diaphragmatic hernia using gentle ventilation and circulatory stabilization. Pediatr Surg Int 2008;25:487–92.

[16] Usui N, Kitano Y, Okuyama H, et al. Prenatal risk stratification for isolated congenital diaphragmatic hernia: results of a Japanese multicenter study. J Pediatr Surg 2011;46:1873–80.
[17] Heling KS, Wauer RR, Hammer H, et al. Reliability of the lung-to-head ratio in predicting outcome and neonatal ventilation parameters in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2005;25:112–8.
[18] Ba'ath ME, Jesudason EC, Losty PD, et al. How useful is the lung-to-head ratio in predicting outcome in the fetuses with congenital diaphragmatic hernia? A systematic review and meta-analysis. Ultrasound Obstet Gynecol 2007;30: 897–906.
[19] Usui N, Okuyama H, Sawai T, et al. Relationship between L/T ratio and LHR in the prenatal assessment of pulmonary hypoplasia in congenital diaphragmatic hernia. Pediatr Surg Int 2007;23:971–6.
[20] Usui N, Kitano Y, Okuyama H, et al. Reliability of the lung to thorax transverse area ratio as a predictive parameter in fetuses with congenital diaphragmatic hernia. Pediatr Surg Int 2011;27:39–45.
[21] Peralta CFA, Cavoretto P, Csapo B, et al. Assessment of lung area in normal fetuses at 12–32 weeks. Ultrasound Obstet Gynecol 2005;26:718–24.
[22] Jani J, Nocolaides KH, Benachi A, et al. Timing of lung size assessment in the prediction of survival in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2008;31:37–40.
[23] Jani J, Peralta CFA, Benachi A, et al. Assessment of lung area in fetuses with congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2007;30:72–6.
[24] Nagata K, Usui N, Kanamori Y, et al. The current profile and outcome of congenital diaphragmatic hernia: a nationwide survey in Japan. J Pediatr Surg 2013;48: 738–44.
[25] Deprest JA, Flemmer AW, Gratacos E, et al. Antenatal prediction of lung volume and in-utero treatment by fetal endoscopic tracheal occlusion in severe isolated congenital diaphragmatic hernia. Semin Fetal Neonatal Med 2009;14:8–13.
[26] Kamata S, Hasewaga T, Ishikawa S, et al. Prenatal diagnosis of congenital diaphragmatic hernia and perinatal care: assessment of lung hypoplasia. Early Hum Dev 1992;29:375–9.

Pneumothoraces As a Fatal Complication of Congenital Diaphragmatic Hernia in the Era of Gentle Ventilation

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Abstract

Introduction Pneumothorax remains a life-threatening complication that occurs in congenital diaphragmatic hernia (CDH), even under respiratory management with gentle ventilation. The aim of this study was to evaluate the prevalence of pneumothoraces as a fatal complication during the management of CDH based on the results of a nationwide Japanese survey conducted in the era of gentle ventilation.

Materials and Methods A retrospective cohort study was performed as part of a nationwide Japanese survey of CDH. A total of 510 neonates with isolated CDH born between 2006 and 2010 were included in this study. The patients were divided into four groups according to operative findings related to the diaphragmatic defect size and operability, which represents the disease severity: defects less than 25%, defects more than 25% but less than 75%, defects more than 75%, and a patient group that was unable to undergo surgery. The prevalence of pneumothorax and the survival rate were compared with respect to each disease severity group. Each case was evaluated to determine whether the development of a pneumothorax was the primary cause of death.

Results Of the 510 neonates with isolated CDH, 69 patients developed a pneumothorax before and/or after surgical intervention. Of the 69 patients 38 patients died, and only 26 patients were discharged from the hospital without any major morbidity that requires home treatment ("intact discharge"). The prevalence of pneumothorax increased and the survival rate and intact discharge rate decreased as the severity of the disease worsened. The number of patients whose pneumothorax was presumed to

Keywords
- congenital diaphragmatic hernia
- pneumothorax
- gentle ventilation
- lung injury
- pulmonary hypoplasia

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be the primary cause of death also increased as the severity of the disease worsened. The survival rate of the patients with pneumothorax was significantly lower than that of the patients without pneumothorax among the groups with 25 to 75% defects and 75% or more defects.

Conclusions Pneumothoraces was found to more likely occur in neonates with CDH associated with a large defect of the diaphragm. The survival rate and intact discharge rate decreased as the severity of the disease worsened, especially among the patients who developed pneumothorax accompanied by large diaphragmatic defects. No other risk factors related to pneumothorax occurrence were found, except for the severity of the disease itself, thus suggesting that pneumothorax was associated with a lethal outcome in neonates with CDH associated with a large defect of the diaphragm.

Introduction

Congenital diaphragmatic hernia (CDH) remains one of the most challenging neonatal diseases facing neonatologists and pediatric surgeons, as it continues to be associated with a high mortality and morbidity due to pulmonary hypertension and pulmonary hypoplasia. Over the past several decades, there have been advances in treatment strategies including extracorporeal membrane oxygenation (ECMO), high-frequency oscillatory ventilation (HFOV), inhaled nitric oxide (iNO), and fetal interventions that have improved the outcomes of CDH patients. However, CDH patients exhibit a broad range of disease severity depending on the components of pulmonary hypertension and pulmonary hypoplasia, which directly affects mortality and morbidity.

Accumulating evidence has shown that ventilator-induced lung injury caused by hyperventilation can have a significant negative impact on short-term outcomes[1,2] and long-term pulmonary sequelae[3,4] in neonates with CDH. To prevent the negative effects of hyperventilation, "gentle ventilation" strategies based on the concept of permissive hypercapnia and permissive hypoxia have been adopted as standard protocol for the treatment of neonatal CDH in many institutions.[5–9] However, even under treatment with a gentle ventilation strategy, the incidence of pneumothorax occurrence, a life-threatening preoperative and/or postoperative complication, reportedly remains high, with rates ranging from 18 to 36%.[10–13] The aim of this study was to evaluate the prevalence of pneumothorax as a fatal complication during the management of CDH and to analyze factors contributing to the development of pneumothoraces based on the results of a nationwide Japanese survey conducted in the era of gentle ventilation strategies.

Materials and Methods

Patient Selection

This retrospective cohort study was performed as part of a nationwide survey of neonatal CDH conducted in 2011 with the support of the Ministry of Health, Labor and Welfare of Japan.[14] The study was performed after being approved by the ethics committee of Osaka University Hospital (approval number of 11017) and the independent ethics committees of five other participating institutions: Hyogo College of Medicine, National Center for Child Health and Development, Kyushu University, Nagoya University Hospital, and Osaka Medical Center and Research Institute for Maternal and Child Health. Data obtained from 72 institutions that consented to participate in a questionnaire survey targeted to the departments of pediatric surgery and/or tertiary perinatal care centers of 159 educational hospitals were retrospectively evaluated. Data were collected as case report forms requesting further details about the patients by the data center that was located in Osaka University Graduate School of Medicine. The entered data were cross-checked twice by the data center and then were fixed after data cleansing. A total of 614 neonates with CDH were born between 2006 and 2010; the overall profiles of the patients are described elsewhere.[14] This study was conducted using only the data of 520 isolated CDH cases defined as CDH infants who did not have serious congenital anomalies, such as major cardiac anomalies or unfavorable chromosomal abnormalities. Four patients with no description of the development of a pneumothorax and six patients whose diaphragmatic defect size was not rated by a surgeon were excluded from this study. Therefore, 510 patients with isolated neonatal CDH were ultimately included in the following analysis.

Data Collection

The primary outcome measure was the occurrence of pneumothorax independent of surgical repair of the diaphragm. We defined pneumothorax as an air leakage from the lungs which was diagnosed by chest X-rays. The cases of preoperative and/or postoperative pneumothorax and of ipsilateral, contralateral, and bilateral pneumothoraces were all included in the primary outcome measures. The secondary outcome measures were survival to discharge, defined as surviving until the time of discharge from the hospital, and "intact discharge," which is a new concept for prognostic evaluation, defined as being discharged from the hospital without any major morbidity that requires home treatment including ventilatory support, oxygen administration, tracheotomy, tube feeding, parenteral nutrition, or vasodilator administration.[15] The patient demographics, including gestational age,

birth weight, Apgar score at 1 minute, prenatal diagnosis with findings of liver herniation, and lung-to-thorax transverse area ratio,[16] mode of delivery, gender, and side of hernia, were reviewed. Whether a surgery could be performed, the incidence of right-to-left shunting at the ductus within 24 hours after birth and the use of HFOV, iNO, or ECMO were also reviewed. As arterial blood gas data, the highest Pao_2 within 24 hours after birth, including the ventilator settings and the lowest $Paco_2$ within 24 hours after birth were analyzed. Although the blood gas data were in principle obtained from the preductal artery, data for the postductal artery were substituted in patients whose preductal arterial data were not available.

Stratification According to Disease Severity

Among the patients who underwent surgical intervention for a diaphragmatic hernia, the time of surgery after birth, diaphragmatic defect size, and operative method were reviewed. The diaphragmatic defect size was rated by a surgeon to evaluate and classify the severity of disease based on the three levels. The patients were divided into four groups according to operative findings related to the diaphragmatic defect size and operability: defects less than 25%, defects more than 25% but less than 75%, defects more than 75%, and a patient group that was unable to undergo surgery. The prevalence of pneumothorax was compared between the disease severity groups. Each case was evaluated to determine whether the development of pneumothorax was the primary cause of death using a questionnaire. In each subgroup having the same severity of disease, several parameters related to pneumothorax occurrence were compared between the patients who developed pneumothorax and those who did not.

Statistical Analysis

The statistical analyses were performed using the JMP software program (version 9.02; SAS Institute, Inc, Cary, North Carolina, United States). The mean and standard deviation or median and interquartile range were used to describe continuous variables. The frequency and percentages were used to describe categorical data. Student *t*-test and an analysis of variance were used to compare continuous variables. The χ^2 test and Fisher exact test were used to analyze categorical data. *p* values of less than 0.05 were considered to indicate statistical significance.

Results

Prevalence of Pneumothorax and the Survival Rate

An outline of the patient demographics is shown in ►Table 1. Of the 510 neonates with isolated CDH, 361 (70.8%) neonates were diagnosed prenatally and 471 (92.4%) neonates underwent surgical repair for diaphragmatic hernia at a median age of 56 hours after birth. A total of 429 patients (84.1%) survived until discharge, 380 (74.5%) patients of whom were discharged from the hospital without any major morbidity that requires home treatment. (►Table 1).

Table 1 Patient demographics

Patient number	510
Gestational age (d), mean ± SD	265.4 ± 13.8
Birth weight (g), mean ± SD	2,645 ± 452
Apgar score at 1 min, median (interquartile range)	5 (3–7.3)
Prenatal diagnosis, (%)	361 (70.8)
Liver-up, (%)	86/339 (25.4)
L/T ratio < 0.08 (equivalent of o/e LHR < 25%), (%)	57/200 (28.5)
Caesarean section at delivery, (%)	309 (60.6)
Gender (male), (%)	287 (56.3%)
Side of hernia (left/right/both)	463/44/3
Surgery performed for diaphragmatic hernia, (%)	471 (92.4)
Time at surgery after birth (h), median (interquartile range)	56 (28–96)
Patch closure, (%)	118/471 (25.1)
Use of HFOV, (%)	367 (78.9)
Use of iNO, (%)	282 (55.4)
Use of ECMO, (%)	37 (7.3)
Survival to discharge, (%)	429 (84.1)
Intact discharge, (%)	380 (74.5)

Abbreviations: ECMO, extracorporeal membrane oxygenation; HFOV, high-frequency oscillatory ventilation; iNO, inhaled nitric oxide; L/T ratio, lung-to-thorax transverse area ratio; o/e LHR, observed-to-expected lung area-to-head circumference ratio; SD, standard deviation.

Of the 510 neonates with isolated CDH, 69 (13.5%) neonates developed a pneumothorax before and/or after surgical intervention. Of the 69 patients, 38 (55.1%) patients died and only 26 (37.7%) patients were discharged from the hospital without any major morbidity that requires home treatment. The prevalence of pneumothorax increased as the severity of disease, represented by the operative findings, worsened. Indeed, the prevalence of pneumothorax in the inoperable group was 46%, whereas that observed in the less than 25% defects group was only 2%. The number of patients whose pneumothorax was presumed to be the primary cause of death increased as the severity of the disease increased. Approximately 40% of the patients died of pneumothorax in both the 75% or more defects group and the inoperable group (►Table 2). The survival to discharge rate and intact discharge rate decreased in association with the size of the diaphragmatic defects, and all patients in the inoperable group died. The survival rate was significantly lower in the patients who developed pneumothorax compared with that observed in the patients who did not develop a pneumothorax among the patients with 25 to 75% defects. In the 75% or more defects group, the survival-to-discharge rate as well as the intact discharge rate was significantly decreased among the patients who developed pneumothorax (►Table 2).

Table 2 Incidence and outcomes of pneumothorax occurrence according to the disease severity evaluated based on the operative findings

	< 25% defects	25–75% defects	≥ 75% defects	Inoperable cases	*p*
Patient number, *n*	87	259	125	39	
Pneumothorax, *n* (%)	2 (2)	23 (9)	26 (21)	18 (46)	< 0.001
Pneumothorax as primary cause of death, *n* (%)	0 (0)	2 (9)	10 (38)	8 (44)	0.035
Survival to discharge, *n* (%)	87 (100)	251 (97)	91 (73)	0 (0)	< 0.001
Survival without pneumothorax, *n* (%)	85 (100)	232 (98)[a]	81 (82)[a]	0 (0)	< 0.001
Survival with pneumothorax, *n* (%)	2 (100)	19 (83)[a]	10 (38)[a]	0 (0)	< 0.001
Intact discharge, *n* (%)	87 (100)	228 (88)	67 (54)	0 (0)	< 0.001
Intact discharge without pneumothorax, *n* (%)	85 (100)	210 (89)	61 (62)[a]	0 (0)	< 0.001
Intact discharge with pneumothorax, *n* (%)	2 (100)	18 (78)	6 (23)[a]	0 (0)	< 0.001

[a] $p < 0.01$, with pneumothorax versus without pneumothorax.

Analysis of Risk Factors for the Development of Pneumothorax

In the 25 to 75% defects group, the best oxygenation index within 24 hours after birth was higher and a higher mean airway pressure was required in the patients who developed a pneumothorax compared with that observed in the patients who did not. This may be related to the higher incidence of persistent pulmonary hypertension of the neonate (PPHN), determined based on the incidence of right-to-left shunting at the ductus, in the patients with pneumothorax compared with that observed in patients without pneumothorax. The higher incidence of PPHN resulted in higher rates of iNO and ECMO use in the patients with a pneumothorax compared with those observed in the patients without a pneumothorax (►Table 3).

Patients in the 75% or more defect group showed a higher best oxygenation index and a higher rate of patch closure compared with the patients in the 25 to 75% defects group regardless of pneumothorax occurrence (►Table 3 and ►Table 4). In the 75% or more defects group, there were no significant differences in the parameters, except for a slight difference in the rate of iNO use between the patients who developed pneumothorax and the patients who did not (►Table 4). Inoperable patients clearly showed a lower highest Pao_2, a higher lowest $Paco_2$, and a higher best oxygenation index, thus suggesting a more severe condition of the disease, compared with the patients in the 75% or more defect group without reference to pneumothorax occurrence (►Table 4 and ►Table 5). No significant differences were observed in any parameter between the patients with a pneumothorax and the patients without pneumothorax among the inoperable patients. These patients died at a median age of 2 days after birth regardless of pneumothorax occurrence (►Table 5).

Discussion

Before the mid-1990s, the use of hyperventilation to induce alkalosis was the mainstay of respiratory management for the treatment of PPHN in neonates with CDH, as it was believed that alkalosis could reverse or eliminate ductal shunting by decreasing pulmonary vascular resistance and pulmonary artery pressure.[17] However, subsequent evidence demonstrated that ventilator-induced lung injury caused by hyperventilation could have a significant impact on the survival statistics,[1,2] as well as the long-term pulmonary function, in infants with CDH.[3,4] A large single-center retrospective series of nonsurvivors with CDH treated with a hyperventilation strategy showed that 62 of 68 cases (91%) exhibited evidence of diffuse alveolar damage with hyaline membrane formation, 44 of 62 cases (65%) exhibited evidence of pneumothorax, and 35 of 68 (51%) cases exhibited evidence of parenchymal hemorrhage. Sakurai et al suggested that a significant degree of lung injury was related to a high peak inspiratory pressure and that ventilator-induced lung injury plays an important role in the mortality of patients with CDH.[2]

A permissive hypercapnia strategy was advocated for ventilation in infants with PPHN more than 25 years ago.[18] To prevent the negative effect of hyperventilation in the respiratory management of CDH, a permissive hypercapnia strategy was adopted for neonates with CDH at several institutions in the late 1990s. Case series of CDH have shown that airway pressure limitation and tolerance of hypercapnia, with a focus on preductal oxygen saturation, are the most important factors favorably influencing outcomes.[5–9] The widespread use of iNO therapy from the mid-1990s, which had a selective effect on decreasing pulmonary vascular resistance and pulmonary artery pressure, may have contributed to the switch from hyperventilation strategies to gentle ventilation strategies. Therefore, gentle ventilation strategies based on the concept of permissive hypercapnia and permissive hypoxia have become the mainstay of respiratory management for neonates with CDH. Although the survival rate has improved under the use of gentle ventilation strategies, the incidence of pneumothorax occurrence, a life-threatening preoperative and/or postoperative complication, reportedly

Table 3 Comparison of the parameters in the patients with 25–75% defects in the diaphragm

Development of pneumothorax	No ($n = 236$)	Yes ($n = 23$)	p
Gestational age (d), mean ± SD	267 ± 11	265 ± 9	0.521
Birth weight (g) , mean ± SD	2,804 ± 404	2,757 ± 399	0.589
Apgar score at 1 min, median (interquartile range)	6 (3–8)	4 (3–6)	0.054
Caesarean section at delivery, *n* (%)	141 (60)	16 (70)	0.503
Prenatal diagnosis, *n* (%)	162 (69)	20 (87)	0.093
Liver-up, *n*/*N* (%)	15/149 (10)	2/20 (10)	1.000
L/T ratio < 0.08, *n*/*N* (%)	15/85 (18)	3/11 (27)	0.427
Use of HFOV, *n*/*N* (%)	156/215 (73)	22/23 (96)	0.053
Highest Pao_2 within 24 h after birth (Torr), mean ± SD	235 ± 142	194 ± 122	0.183
Lowest $Paco_2$ within 24 h after birth (Torr), mean ± SD	35 ± 23	38 ± 16	0.548
Best oxygenation index within 24 h after birth, mean ± SD	5.5 ± 5.4	8.4 ± 7.6	0.022[a]
Mean airway pressure (cmH_2O), mean ± SD	12.6 ± 2.9	14.2 ± 2.6	0.015[a]
Right-to-left shunting at ductus within 24 h after birth, *n*/*N* (%)	62/216 (29)	15/23 (65)	0.001[b]
Use of iNO, *n*/*N* (%)	107/235 (46)	18/23 (78)	0.004[b]
Use of ECMO, *n*/*N* (%)	5/236 (2)	3/23 (13)	0.026[a]
Time of surgery after birth (h), median (interquartile range)	52 (28–85)	76 (39–141)	0.293
Patch closure, *n*/*N* (%)	26/236 (11)	4/23 (17)	0.319

Abbreviations: ECMO, extracorporeal membrane oxygenation; HFOV, high-frequency oscillatory ventilation; iNO, inhaled nitric oxide L/T ratio, lung-to-thorax transverse area ratio.
[a] $p < 0.05$.
[b] $p < 0.01$.

Table 4 Comparison of the parameters in the patients with ≥ 75% defects in the diaphragm

Development of pneumothorax	No ($n = 99$)	Yes ($n = 26$)	p
Gestational age (d), mean ± SD	262 ± 14	263 ± 14	0.828
Birth weight (g), mean ± SD	2625 ± 463	2779 ± 453	0.131
Apgar score at 1 min, median (interquartile range)	3 (2–5)	3 (2–4)	0.493
Caesarean section at delivery, *n*/*N* (%)	66 (67)	18 (69)	1.000
Prenatal diagnosis, *n*/*N* (%)	83 (84)	23 (88)	0.762
Liver-up, *n*/*N* (%)	38/77 (49)	9/23 (39)	0.478
L/T ratio < 0.08, *n*/*N* (%)	20/44 (45)	8/16 (50)	0.778
Use of HFOV, *n*/*N* (%)	82/95 (86)	24/26 (92)	0.521
Highest Pao_2 within 24 h after birth (Torr), mean ± SD	184 ± 128	195 ± 146	0.698
Lowest $Paco_2$ within 24 h after birth (Torr), mean ± SD	38 ± 18	36 ± 11	0.700
Best oxygenation index within 24 h after birth, mean ± SD	12.8 ± 13.3	15.6 ± 16.9	0.411
Mean airway pressure (cm H_2O), mean ± SD	14.3 ± 3.5	14.1 ± 2.6	0.834
Right-to-left shunting at ductus within 24 h after birth, *n*/*N* (%)	53/92 (58)	19/25 (76)	0.109
Use of iNO, *n*/*N* (%)	78/99 (79)	25/26 (96)	0.043[a]
Use of ECMO, *n*/*N* (%)	15/99 (15)	5/26 (19)	0.563
Time of surgery after birth (h), median (interquartile range)	69 (28–123)	100 (26–129)	0.893
Patch closure, *n*/*N* (%)	67/98 (68)	19/26 (73)	0.847

Abbreviations: ECMO, extracorporeal membrane oxygenation; HFOV, high-frequency oscillatory ventilation; iNO, inhaled nitric oxide; L/T ratio, lung-to-thorax transverse area ratio; SD, standard deviation
[a] $p < 0.05$.