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# Amniotic fluid absorption and growth functions in humans: what can we indirectly learn from congenital digestive atresias?

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## Abstract

**Background:** Amniotic fluid (AF) was thought of just as a mechanical cushioning to the foetus. Nowadays, its role during pregnancy is getting more attention, suggesting hitherto unknown aspects. The aim of the study is to speculate on AF nutritional functions and its clinical repercussions based on what digestive tract (DT) atresias seem to suggest.

**Methods:** A retrospective analysis of the patients admitted to our department for DT atresias between 2000 and 2020 was conducted. Patients' birth weight (BW), gestational age (GA) at birth and diagnosis were recorded. The following were excluded from the study: oesophageal atresias (OA) with tracheoesophageal fistula (TOF), colonic and anal atresias and patients with associated major comorbidities. A control group was made of patients admitted to our ward in the same period for congenital pulmonary airway malformations (CPAM). To standardize the BW, it was coupled with birth GA calculating the newborn percentiles. The mean newborn percentiles of OAs, duodenal atresias (DAs), jejunal atresias (JAs), and ileal atresias (IAs) were independently compared with the control group using Student's *t*-test. Lastly, the significance of the frequencies' distribution of newborns born small for gestational age (SGA) between the DT atresias and the control group was evaluated with the $\chi^2$ test, and the OR were calculated. A *p*-value $< 0.05$ was considered statistically significant.

**Results:** A total of 231 patients were eligible for the study: 36 OAs without TOF, mean BW 2488.8 ± 491 g (range 1630–3750 g), mean GA 36.8 ± 2.1 weeks (31–40 weeks), mean newborn percentile 18 ± 22 (1–75); 20 DAs, mean BW 2586.8 ± 577.9 g (1250–3462 g), mean GA 36.2 ± 2.5 weeks (31–40 weeks), mean newborn percentile 31 ± 23 (3–79); 17 JAs, mean BW 2483.5 ± 621.7 g (900–3205 g), mean GA 34.8 ± 2.1 weeks (30–38 weeks), mean newborn percentile 44 ± 28 (4–96); 17 IAs, mean BW 2646.1 ± 769.8 g (1162.0–3888 g), mean GA 35.9 ± 3.2 weeks (30–41 weeks), mean newborn percentile 44 ± 26 (1–82); and 141 CPAMs with mean BW 3287.4 ± 492.0 g (980–4580 g), mean GA 38.7 ± 1.8 weeks (26–41 weeks), mean newborn percentile 43 ± 26 (1–99). The number of SGA neonates was 18 between OA patients (50%), 4 between DAs (20%), 1 between JAs (6%), 2 between IAs (12%) and 11 between CPAMs (8%). The mean percentile of the OAs and DAs was lower than the control group with a *p* of < .01 and .03 while no statistical significance was found in the comparison between DAs, JAs, IAs and CPAMs with a *p* of .06, .86 and .59. The incidence of SGA in the control group resulted lower than the one in the DT atresias where it becomes higher the more proximal the atresia is ($p < .05$). The OR of SGA newborn in the OA group was 11.8, in DA 3.0, in JA 0.7 and in IA 1.6.

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**Conclusion:** AF showed to have a great impact on foetal growth, and its preferred site of absorption seemed to be the stomach and duodenum. Its nutritional role could have an important predictive value in diagnosing DT atresia both prenatally and postnatally.

**Keywords:** Amniotic fluid, Digestive atresia, Prenatal diagnosis, Oesophageal atresia, Duodenal atresia, Jejuno-ileal atresia, Newborns, Growth impairment

## Background

Historically, amniotic fluid (AF) was thought to simply guarantee a mechanical cushion to the foetus during prenatal life.

With the beginning of accurate studies on the production mechanisms and the molecular composition of AF, it became clear that its role during foetal life was largely underestimated. Since then, the number of studies investigating such properties of AF has multiplied [1–9].

Due to ethical reasons, it is difficult to imagine and design a clinical study in which a human embryo is exploited to confirm results obtained with animal tests.

However, analysing the wide spectrum of congenital malformations which can occur in the human species, digestive tract (DT) atresias could be taken as a practical model for speculations on AF's properties and role in the development of foetuses.

The primary objective of this study is to verify the influence of DT atresias on the newborn's weight at birth, as this relation might represent an indirect clue of AF's role in the nutritional support of the foetus.

The secondary objective of the study is to speculate about the potential preferred gastrointestinal site of absorption of the AF.

## Methods

A retrospective analysis of all patients admitted for DT atresias (oesophageal and small bowel atresias) in our Neonatal Surgery Department between 2000 and 2020 was conducted.

Patients' birth weight (BW), gestational age (GA) at birth and diagnosis, based on intraoperative findings, were recorded. Considering the possibility of the passage of AF to the gastrointestinal tract in patients born with oesophageal atresia (OA) with tracheoesophageal fistula (TOF), we included only patients born with type I OA, without TOF.

Due to the rarity and accordingly limited numbers of DT atresias affecting colonic segments, patients born with colonic atresias were excluded from the study. Moreover, considering the negligible absorption function of the rectum, rectal atresias were excluded from the study.

To investigate the effect of DT atresias on the neonatal attributes, we decided to use congenital pulmonary airway malformations (CPAM) as a control group. Such a decision was based on the absence of healthy neonatal patients born from normal pregnancies in our department. Careful attention was paid to excluding patients with severe forms of CPAM or circulatory shunting, which can determine impairments in the amount of AF during foetal life. Therefore, we retrospectively analysed all patients admitted for CPAM in our department, and patients' BW and GA at birth were recorded.

To avoid selection biases, only isolated DT atresias and CPAM were included in the study.

As the collected data were represented by continuous and normally distributed (verified using the Shapiro-Wilk test) variables, they were expressed in mean values ± standard deviation. For the purpose of standardization, BW was coupled with birth GA with respect to the newborn percentiles.

The newborn's percentiles of BW for GA were found to follow a normal distribution, which was verified using the Shapiro-Wilko test. Then, we compared the mean percentiles in OAs, duodenal atresias (DA), jejunal atresias (JA) and ileal atresias (IA) with those of the control group using Student's *t*-test. A *p*-value below 0.05 was considered statistically significant.

Lastly, the statistical significance of the frequencies' distribution of newborns born small for gestational age (SGA), defined as below the 10th percentile newborn, between the DT atresias and the control group was evaluated with the $\chi^2$ test and the odds ratios (OR). The results were calculated to estimate the risk of babies affected by OA, DA, JA or IA to be born SGA compared to the control group. A *p*-value below 0.05 was considered statistically significant.

## Results

A total of 231 patients were deemed eligible for the study:

- Thirty-six OAs without TEF with mean BW 2488.8 ± 491.0 g (range 1630–3750 g), mean GA 36.8 ± 2.1 weeks (range 31–40 weeks), mean newborn percentile 18 ± 22 (range 1–75)
- Twenty DAs with mean BW 2586.8 ± 577.9 g (range 1250–3462 g), mean GA 36.2 ± 2.5 weeks (range 31–40 weeks), mean newborn percentile 31 ± 23 (range 3–79)

- Seventeen JAs with mean BW 2483.5 ± 621.7 g (range 900–3205 g), mean GA 34.8 ± 2.1 weeks (range 30–38 weeks), mean newborn percentile 44 ± 28 (range 4–96)
- Seventeen IAs with mean BW 2646.1 ± 769.8 g (range 1162.0–3888 g), mean GA 35.9 ± 3.2 weeks (range 30–41 weeks), mean newborn percentile 44 ± 26 (range 1–82)
- One hundred forty-one CPAMs with mean BW 3287.4 ± 492.0 g (range 980–4580 g), mean GA 38.7 ± 1.8 weeks (range 26–41 weeks), mean newborn percentile 43 ± 26 (range 1–99).

The number of SGA neonates was 18 among OA patients (50%), 4 among DAs (20%), 1 among JAs (6%), 2 among IAs (12%) and 11 among CPAMs (8%).

The mean percentiles in OAs and DAs were significantly lower when compared with the control group with a *p*-value respectively below 0.01 and 0.03. On the other hand, no statistical significance was found in the comparison between JAs, IAs and CPAMs with a *p* respectively of 0.86 and 0.59.

The incidence of SGA in the control group, tested with the $\chi^2$ test, resulted significantly lower compared to the DT atresias patients, in which it becomes higher the more proximal the atresia is ($p < .05$).

The OR to be born SGA of patients with OA was 11.8, with DA 3.0, with JA 0.7 and with IA 1.6.

The main data of the study are resumed in Table 1.

## Discussion

Historically, AF was thought to simply represent a mechanical cushion in which the foetus could develop. Nowadays multiple studies have highlighted the complexity not only in terms of function but also in the composition of the AF [10–21].

During foetal life, from the eleventh week of gestation, the baby starts swallowing AF in an amount of about 200–250 ml/kg/day; even if it is true that by breathing and swallowing the amniotic fluid the baby practices using the muscles to be ready for breastfeeding, AF ingestion hides more important effects [22].

After all, the particular composition of the AF highlights how the cushioning function and oral muscle training cannot be the only ones.

AF was found to contain a series of hormones and growth factors such as epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), transforming growth factor beta 1 (TGF-β1), insulin-like growth factor 1 (IGF-1), erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF). These factors play a relevant role not only in enhancing bowel trophism, but also in the immunological defence linked to the presence of various broad-spectrum antimicrobial agents such as antibodies, α-defensine, lactoferrin, lysozyme and secretory leukocyte protease inhibitor [22].

Moreover, AF contains carbohydrates, proteins, lipids and electrolytes which diffuse freely from the placenta to the foetal circulation through the skin before the production of keratin begins and which are then swallowed after this ability is acquired.

Ligation of the oesophagus in foetal rabbits to prevent swallowing followed by the infusion of various solutions into the gut distally to the ligature has been performed to demonstrate the nutritive value of foetal swallowing. Animals infused with lactated Ringer's solution had poor gut development whereas those infused with bovine AF showed normal gut maturation [23].

**Table 1** Main data of the study

| | **Birth weight (g)** | **Gestational age (weeks)** | **Newborn percentile** |
|---|---|---|---|
| *Control group (CPAMS), n = 141* | 3287.4 ± 492.0 | 38.7 ± 1.8 | 43 ± 26 |
| *Oesophageal atresia, n = 36* | 2488.8 ± 491.0 | 36.8 ± 2.1 | 18 ± 22 |
| *Duodenal atresia, n = 20* | 2586.8 ± 577.9 | 36.2 ± 2.5 | 31 ± 23 |
| *Jejunal atresia, n = 17* | 2483.5 ± 621.7 | 34.8 ± 2.1 | 44 ± 28 |
| *Ileal atresia, n = 17* | 2646.1 ± 769.8 | 35.9 ± 3.2 | 43 ± 26 |
| **Newborn percentiles comparison (*p*-values)** | | | |
| *Control vs. OA* | <0.05 | *Control vs. DA* | <0.05 |
| *Control vs. JA* | 0.86 | *Control vs. IA* | 0.59 |
| **Newborns small for gestational age (SGA) rate** | | | |
| *Oesophageal atresia* | 18/36 (50%) | *Duodenal atresia* | 4/20 (20%) |
| *Jejunal atresia* | 1/17 (6%) | *Ileal atresia* | 2/17 (12%) |
| *Control group (CPAM)* | 11/141 (8%) | | |

Data are reported as mean ± standard deviation

*n* number of patients, *OA* oesophageal atresia, *DA* duodenal atresia, *JA* jejunal atresia, *IA* ileal atresia

Lopez de Torre et al. demonstrated growth impairment in chick embryos in which intestinal atresia was surgically reproduced [24].

In birds, the only mean of embryo's nutrition is AF due to the absence of the placenta. Considering that embryogenesis recalls phylogenesis, it is plausible to suppose that the AF maintained nutritional and bowel trophism function throughout the evolution of the species until the human species appeared.

Studies like the aforementioned are not applicable to human foetus for ethical reasons. Therefore, we investigated the effects of atresias on various levels of the digestive system, as they can be seen as a faithful representation of the bowel ligations previously discussed.

Our results showed that OAs and DAs have a mean BW percentile statistically inferior to the control group. Contrarily, no statistically relevant differences were found between the mean percentile of the other atresias with the control group (Fig. 1A).

Moreover, the incidence of SGA newborns decreases the more distal the level of the atresia, having the

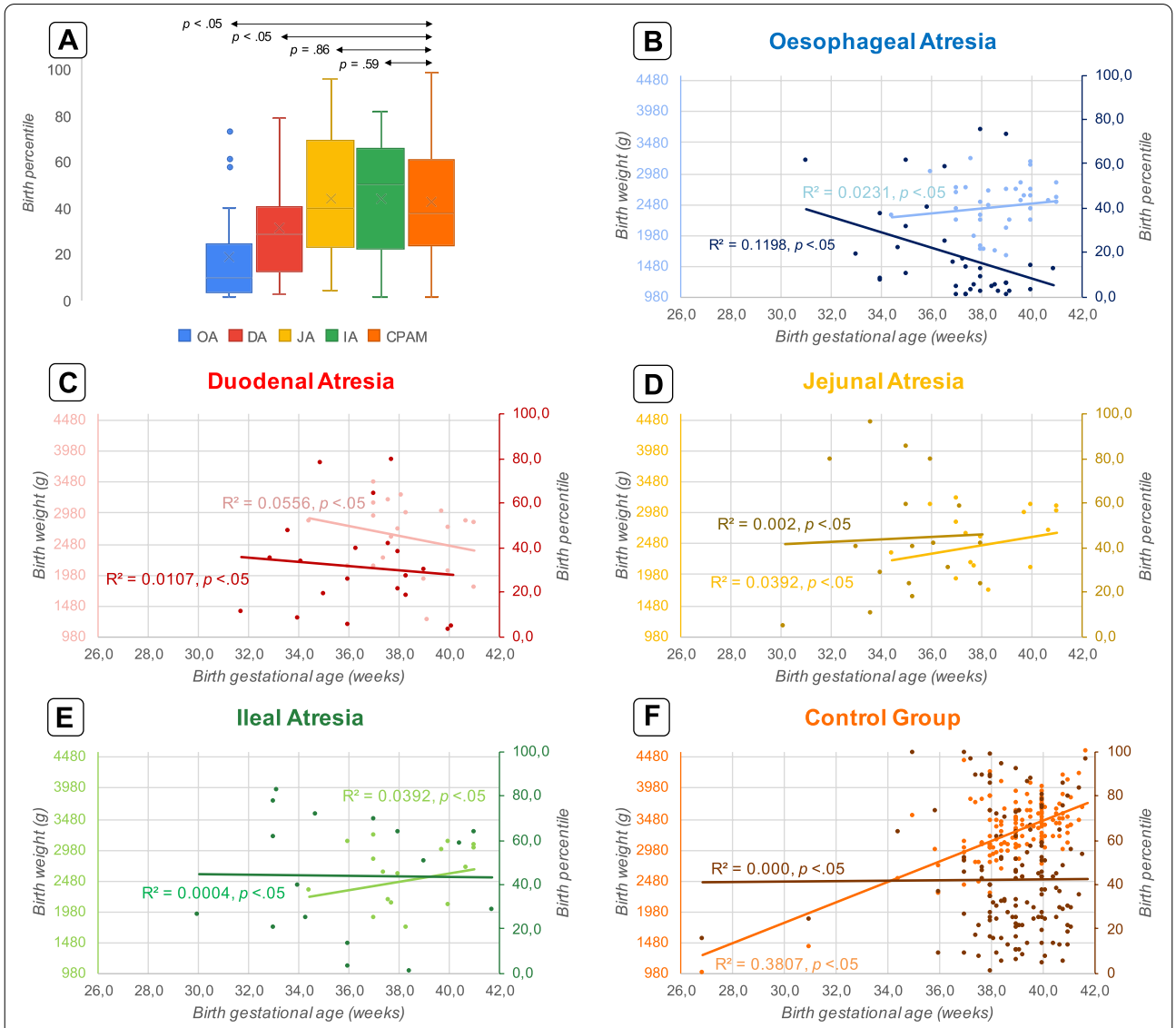


**Fig. 1** **A** Weight at birth standardized using birth percentile: the figure shows how the mean percentile of OA and DA is significantly inferior to the control group ($p < .05$) while the ones of IA and JA do not show a statistically significant difference with the control group; the interquartile ranges, expressing the 50% of the patients of every group, present lower birth percentile values in OA and DA while the ones of JA and IA, and the control group assume higher values and are similar to each other. **B**–**F** Relation between birth weight, birth percentile, and gestational age, see the text for the description

lowest percentage in the control group and high percentages in OAs and DAs (Fig. 2).

Considering such results, we can indirectly infer that AF plays a role in the nutritional support of the foetus.

The fact that only OA and DA seem to be significantly affected by AF deprivation in terms of foetus growth could suggest mainly the stomach and marginally the duodenum as the principal sites of absorption of the AF.

Indeed, the mean BW percentiles between JAs and IAs are not statistically different when compared with the control group hence confirming that it is sufficient for the AF to pass the oesophagus and to arrive to the stomach and duodenum to carry out its nutritional function.

Based on these explanations, we are not surprised that OAs and DAs together account for more than 60% of the total SGA newborns enrolled in the present study, the control group included.

The effects of AF as gestation progresses are depicted in Fig. 1B–F. If we consider the trend of the relation between BW and GA at birth, we can observe how it assumes a positive trend with a more rapid increase the lower the level of the atresia. The control group exhibits the steepest increase. In fact, such results could be predicted: the foetus counts on the placenta as the main important vehicle of nutritional agents. Therefore, unless a placental disease is present, we expect that a foetus affected by DT atresia can continue to gain weight. The

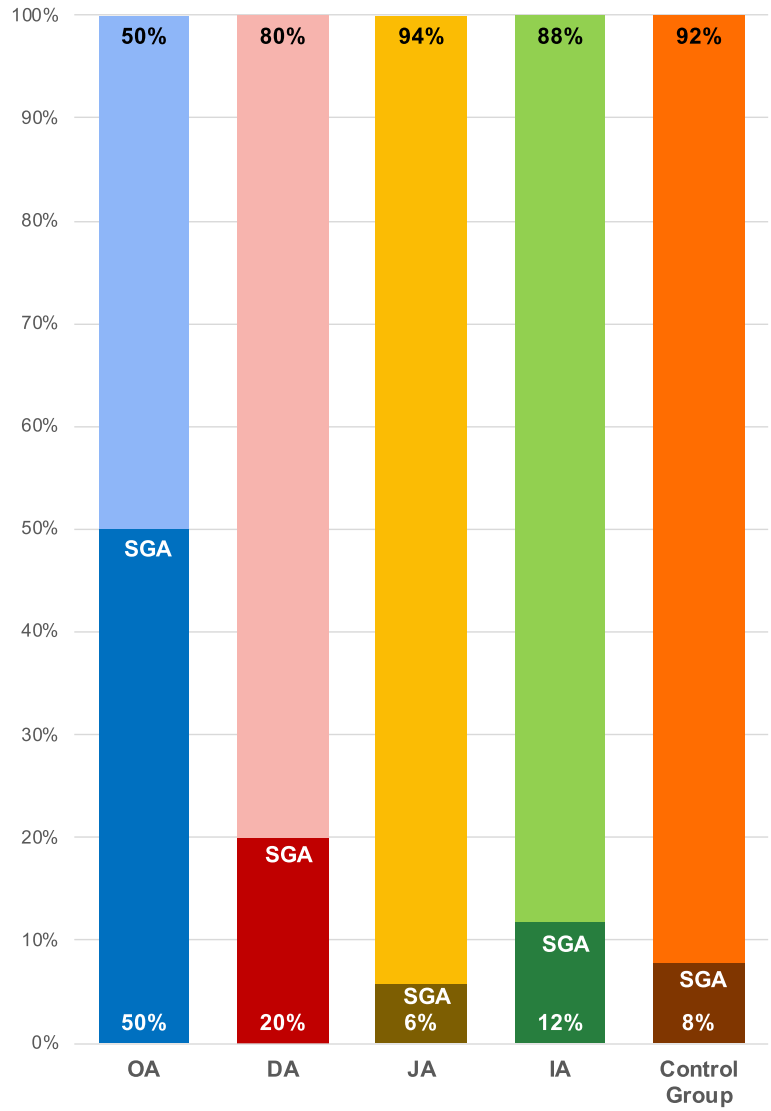


**Fig. 2** Percentage of small for gestational age (SGA) newborn vs adequate for gestational age ones in the OA, DA, JA, IA, and control groups

deceleration in the increase of the positive trend in the DT atresias, when compared with the control group, suggests the existence of another factor taking part in the foetus growth, which fails in this category of patients: it is presumably the absorption of the AF, strengthening once more our theory.

DAs represent the only exception, presumably due to statistical bias resulting from the limited number of patients enrolled in the study.

Concerning the relation between BW percentiles, standardized by the match with GA and birth GA, expressed in Fig. 1B–F too, we observe how the trends in the control group and the distal atresias (JA and IA) are slightly positive or null. From a statistic viewpoint, inference can be made that the GA is not sufficient to explain the variability of the birth percentiles. The explanation might be that, in normal conditions, the foetus weight increases under the influence of a variety of individual biological elements that intervene constantly and independently from the gestational age. OAs and DAs behave differently: the slope of the trendline decreases, drastically in the case of OAs, revealing that in these groups of newborns, an unknown factor determined a growth impairment that worsens as the pregnancy progresses. Such evidence is not only another indirect clue of the effect of AF on foetus growth but also represents further evidence of the stomach as a probable major site of absorption of the fluid and suggests that AF's nutritional function becomes more important as gestation proceeds.

The data suggested by our work certainly harbour important clinical implications.

The first is that our data stresses the attention on how patients with proximal DT atresias are extremely fragile: their low percentile at birth requires a major grade of care from physicians due to their growth restriction. Moreover, surgeons and anaesthesiologists are well conscious of how low weight and poor nutritional state could have a negative impact in terms of perioperative and postoperative complications.

Furthermore, in case of prenatal findings of bowel dilation in a SGA foetus, the obstetrician must suspect a DT atresia.

In addition, in the case of newborn suspected of neonatal bowel obstruction, the birth percentile can help the surgeon not only in considering the possibility of a DT atresia but coupled with the imaging, it is also possible to predict the defect site in the DT before intraoperative findings are available.

We are aware of the limitations of this study. First is the limited number of patients: to further confirm our ideas, multicentre studies are paramount.

A further limitation is the purely speculative nature of the present study; nevertheless, as previously discussed, our conclusions are reported in similar studies with animal foetuses [23, 24].

## Conclusions

Our study confirms that AF has an important role in foetal nutrition and growth and the main site of absorption of the AF appears to be the stomach and the duodenum.

Specifically, newborns with OA or DA present with a more severe growth impairment during gestation.

In case of SGA foetus with a prenatal ultrasound finding of bowel distension, obstetricians should always consider the possibility of a DT atresia.

Newborns affected by proximal DT atresia, in addition to the surgical correction of the congenital anomaly, need a more watchful care to overcome their nutritional and growth deficit.

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#### Authors' contributions

Conceptualization: GP, MaL, MiL, and TG. Methodology: GP and TG. Software: SD, FP, and CC. Validation: TG, MiL, and MaL. Formal analysis: GP. Investigation: GP, SD, FP, and CC. Resources: TG, MiL, and MaL. Data curation: GP, SD, FP, and CC. Writing—original draft preparation: GP. Writing—review and editing: ET, TG, and MaL. Supervision: TG. Project administration: GP. All authors have read and agreed to the published final version of the manuscript.

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#### Availability of data and materials

Please contact the author for data requests.

## Declarations

#### Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of IRCCS Sant'Orsola Malpighi University Hospital (CHPED-01-19-IA). Informed consent was obtained from all subjects involved in the study.

#### Consent for publication

Written informed consent has been obtained from the patients to publish this paper.

#### Competing interests

The authors declare that they have no competing interests.

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## Publisher's Note