

# Alpha-1-Antitrypsin Deficiency Presenting as Neonatal Cholestasis: Predictors of Outcome and Effect of Ursodeoxycholic Acid

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## Abstract

**Background and objective:** Alpha-1-antitrypsin deficiency presenting as neonatal cholestasis occurs in a small percentage of affected individuals. The prognosis is variable, from "healing" to liver cirrhosis and/or severe hepatocellular failure, requiring liver transplantation. We researched for predictors of outcome, including the effect of ursodeoxycholic acid.

**Methods:** Retrospective cohort study of 27 cases of neonatal cholestasis due to alpha-1-antitrypsin deficiency, in the period between 1985 and 2013. Inclusion criteria: patients with neonatal cholestasis and ZZ phenotype. Exclusion criteria: presence of other diagnosis or known risk factors for developing neonatal cholestasis. We analyzed several clinical, biochemical, histological and therapeutic variables. Patients were categorized into two groups: favorable outcome (n=18), unfavorable outcome (n=9). We also divided the patients as treated (n=16), and untreated (n=11) with ursodeoxycholic acid.

**Results:** Splenomegaly at admission ( $P=0.006$ ) and persistent jaundice at 6 months old ( $P=0.007$ ) were associated with unfavorable outcome. The values of conjugated bilirubin ( $P=1.000$ ), aspartate aminotransferase ( $P=1.000$ ), alanine aminotransferase ( $P=0.371$ ) and gamma-glutamyltransferase ( $P=0.667$ ) were not significantly different in both groups of outcome. Early treatment with ursodeoxycholic acid was associated with a favorable outcome ( $P=0.011$ ). Treated patients did not differ significantly from the untreated-ones in biochemical parameters (conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase), and had significantly lower alpha-1-antitrypsin serum levels ( $P=0.015$ ).

**Conclusion:** Splenomegaly at admission and persistence of jaundice at 6 months old were predictive for bad prognosis, and early treatment with ursodeoxycholic acid might have interfered positively in the outcome.

**Keywords:** Alpha-1-antitrypsin deficiency; ZZ Phenotype; Neonatal cholestasis; Neonatal jaundice; Gamma-glutamyltransferase; Ursodeoxycholic acid; Children

## Introduction

Alpha-1-Antitrypsin (AAT) deficiency "Z" variant is a relatively frequent genetic disease (1/2000-3500 live births in most populations) [1-3] in which a point mutation, transmitted in an autosomal co-dominant manner, renders the mutant Z Alpha-1-Antitrypsin Molecule (ZAAT) prone to abnormal folding pathway and aggregation [4]. Pi\*ZZ homozygotes are predisposed both to premature development of pulmonary emphysema and liver disease [5-7].

Predictors of prognosis for liver disease were previously described [1,5,6]. Severe cholestatic liver disease occurred more in certain families, and was associated with male preponderance, suggesting the presence of more than one genetic factor and hormonal influences [1]. Also, duration of jaundice, severity of biochemical abnormalities, and histological features were able to predict outcome at an early stage of the disease [5,6,8].

Although recent advances in the knowledge of the liver disease pathogenesis in AAT deficiency had opened the field for development of new therapy strategies [9-13], currently there are still no prevention options, and liver transplantation [14] is the only therapy when it becomes irreversible. Ursodeoxycholic acid (UDCA) is currently the most widely used therapeutic option for the treatment of cholestatic hepatopathies [15]. Its use has expanded also to other kinds of hepatic

diseases [16], and potentially even to extra hepatic ones [17,18]. Such versatility is the result of its multiple mechanisms of action. Yet, UDCA effect has been scarcely analyzed in AAT deficiency patients, particularly in those presenting with neonatal cholestasis.

We analyzed clinical, biochemical and histological parameters at diagnosis and their impact on the prognosis in a cohort of 27 children with AAT deficiency Z variant presenting as neonatal cholestasis, and we also analyzed the therapeutic effect of UDCA in these patients.

## Patients and methods

A retrospective cohort study was conducted at a single tertiary care hospital involving patients referred from 1985 to 2013. All patients were Caucasian from the same region of our country, where population has deep Celtic roots. Diagnosis and follow-up of all patients was of

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the author's responsibility. This study was based on analysis of clinical data.

We included patients with AAT deficiency, ZZ phenotype, with presentation of neonatal cholestasis. We excluded patients with the presence of other concomitant diagnosis or known risk factors for developing neonatal cholestasis [19]. Patients with intra-uterine growth retardation were not excluded as this is known to be associated with AAT deficiency [2].

We analyzed demographic data (sex, age at admission and follow-up time) clinical data [gestational age and birth weight, failure to thrive, pale stools, hepatomegaly, splenomegaly, and ascites] at neonatal cholestasis presentation; jaundice duration (< 3 months, 3-6 months, > 6 months old), biochemical data [values of conjugated bilirubin, Aspartate-Aminotransferase (AST), Alanine-Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT) at neonatal cholestasis presentation; serum AAT and phenotype], histological (liver histology performed by 3-6 months old) and therapeutic data (patients treated with UDCA). Histological specimens were randomly evaluated by two Pathologists. Portal inflammation, bile duct proliferation, cholestasis, and giant cell transformation were graded from 0 to 4 according to degree of severity. Bile duct hypoplasia was diagnosed if less than a half of the portal tracts had identifiable bile ducts. The following scoring system was used for fibrosis, as previously described by Francavilla et al. [8]: 1 = portal fibrosis, 2 = portal fibrosis with radiating, non-bridging septa, 3 = bridging fibrosis, 4 = established cirrhosis.

Medical management included enteral nutritional support consisting of milk supplementation with carbohydrates (such as maltodextrin), lipids (medium chain triglycerides) and liposoluble vitamins (A, D, E and K) provided to all patients. UDCA therapy was given to all patients born since 1994 (n = 16). It was started on admission, at a dosage of 15-20 mg/kg/day, divided in two daily doses, and maintained until normalization of both conjugated bilirubin and GGT.

Co-morbidities and outcome were also analyzed. We considered four categories of outcome, as previously reported by Hinds et al. [7]. A – no clinical liver disease after neonatal biochemical dysfunction, B – mild ongoing liver dysfunction, C – moderate chronic liver disease, D – end stage liver disease/liver transplantation/death. Liver histology was available only in some patients between age 3-6 months old. Beyond clinical and biochemical data, we overcome overlap possibilities between B and C categories by analyzing features of liver ultrasound with portal vein Doppler and/or upper endoscopy, available in all patients during follow-up.

Patients were divided into two groups: Group I - favorable outcome (A and B categories) and Group II - unfavorable outcome (C and D categories). We analyzed the mentioned variables and their association with patient outcome.

Then we further divided the sample in another two groups: UDCA untreated patients (corresponding to patients admitted before 1994), and UDCA treated patients (the-ones who were admitted after). We analyzed the same variables and compared both groups, in order to see if they were homogeneous.

### Statistical analysis

Data were analyzed with the computer program IBM SPSS Statistics 22. Categorical variables were evaluated by 2-tailed  $\chi^2$  and statistical significance was defined by using Fisher's exact tests due to small sample sizes. Continuous variables showed a non-parametrically

distribution and were compared by using Mann-Whitney test. Results are expressed as percentages, medians and inter-quartiles (IQ). Differences were considered significant at P value less than 0.05. All reported P values are two-sided.

## Results

### Characterization of whole sample (n=27)

There were 33 patients with AAT deficiency, presenting with neonatal cholestasis. From these we have excluded 6 patients. Three were excluded because they were not ZZ phenotype: one MZ, two SZ (one also had biliary atresia), and one FZ. We also excluded two patients ZZ phenotype with risk factors for neonatal cholestasis: one with *E. coli* septicemia and meningitis in the neonatal period, and another-one with prematurity < 34 weeks and neonatal sepsis without identified agent. Finally, we enrolled 27 patients.

Eighteen patients were males (67%) and nine females. The median follow-up time was 17.50 years (range: 0.54-17.88 and IQ range: 15.21-17.79). Except for the patient who died, only two patients (recently admitted in 2011 and 2012) had follow-up time less than 5 years. All patients had a minimum follow-up period of 2 years. Older patients were transferred for adult care at 18 years old.

The median age at admission was 2.00 months (range: 0.23-32.0 and IQ range: 1.50 – 2.50). More than 90% of patients were admitted at our hospital before 4 months old. Two patients were admitted at 16 and 32 months old with chronic liver disease and a past history of neonatal cholestasis.

**Clinical data:** One patient had a past history of prematurity (36 weeks gestation) and 6 of intra-uterine growth retardation. The majority had hepatomegaly (n=26), 8 had splenomegaly, 7 had failure to thrive, 6 pale stools and 1 had ascites.

Jaundice solved before 3 months old in 10 patients (37%), between 3 and 6 months old in 13 patients (48%), and persisted beyond 6 months old in 4 patients (15%).

**a. Biochemical data:** The median serum conjugated bilirubin was 5.00 mg/dl (range: 1.40-9.03 and IQ range: 3.50-6.80). The median values of serum liver enzymes were as follows: AST 115.5 (range: 52.0-233 and IQ range: 88.0-151.25), ALT 75.5 (range: 20.0-136.0 and IQ range: 49.25-101.50) and GGT 772.0 (range: 119.0-1742.0 and IQ range: 386.0-1067.0). The median serum AAT level was 37.0 (range: 22.0-78.0 and IQ range: 29.0-52.0).

**b. Histological data:** Liver histology was performed between 3 and 6 months-old in 18 patients (67%), and was available in 14 (52%). Periodic Acid-Schiff Stain positive and diastase resistant AAT granules were reported in 12 of 14 specimens. All 14 presented with various degrees of portal fibrosis, including septa and bridging fibrosis (F2 e F3), and established cirrhosis in 5 (F4). None presented the bile duct paucity pattern.

**c. Therapeutic data:** UDCA treatment was started in all patients born since 1994 (n = 16), at a median age of 1.55 months (range: 0.23-3.50 and IQ range: 1.12-2.50), and was maintained until normalization of both conjugated bilirubin and GGT which occurred after a median duration time of 2.70 years (range: 2.0-18.0 and IQ range: 2.14-5.83). The longest therapies durations were verified in two patients (13.5 and 18.0 years), with no observed side effects.

**d. Infectious complications and co-morbidities:** One patient had

acute *Cytomegalovirus* infection and died at 6.5 months-old of acute-on-chronic liver failure. Another patient was diagnosed with celiac disease at 11 years old. In this patient neonatal cholestasis evolved to end-stage liver disease requiring OLT at the age of 14 months old. He progressed to graft dysfunction, iron-therapy refractory anemia, and growth impairment, and was submitted to a second OLT at the age of 10 years.

**e. Outcome:** Outcome was favorable in 18 patients (13 in A category and five in B category of outcome) and unfavorable in nine (one in C category, and eight in D category including seven alive with OLT and one death at 6.5 months old of acute-on-chronic liver failure). OLT (n=7) was performed at a median age of 7.5 years (range: 0.70-11.00 and IQ range: 1.17-10.50).

**Characterization and comparison of both groups: Group I - favorable (n=18) and Group II - unfavorable (n=9) outcome – (Table 1)**

Although there was a male gender dominance in group I (72% in group I versus 55% in group II), the difference between the distribution of genders of the two groups is not statistically significant ( $P=0.423$ ). Median age at admission was not significantly different in both groups, despite the presence of the two outliers in the unfavorable group (one patient admitted at 16 months and another at 32 months old, with chronic liver disease and a past history of neonatal cholestasis). Also the median follow-up time was not significantly different in both groups.

**a. Clinical data:** The clinical parameters significantly associated with unfavorable outcome were splenomegaly at presentation ( $P=0.006$ ) and persistence of jaundice beyond 6 months-old ( $P=0.007$ ).

**b. Biochemical data:** Conjugated bilirubin, AST, ALT and GGT values were higher in group II, but the difference was not significant. Also serum AAT was not significantly different in both groups ( $P=1.000$ ).

**c. Histological data:** Liver histology was performed in 18 patients, in all of them at the age of 3-6 months-old. In group I it was performed in 12 and available in 10; in group II it was performed in 6 and available in 4. AAT granules were reported in 8 out of 10 exams in group I and in 4 out of 4 in group II. Various degrees of portal fibrosis were reported in all specimens of both groups; established cirrhosis (F4) was present in 3 of group I and in 2 of group II.

**d. Therapeutic data:** Therapy with UDCA was significantly associated with a favorable outcome ( $P=0.011$ , and coefficient association +0.533).

**e. Infectious complications and co-morbidities:** In group I there were neither major infectious complications, nor co-morbidities in the first year of life. In group II there was the patient with acute *Cytomegalovirus* infection (no UDCA treatment; dead), and the patient with celiac disease (treated with UDCA; alive, submitted to OLT) ( $P=0.103$ , and coefficient association -0.400). There were no records for minor ailments, with no need of hospitalization.

	Favorable Outcome (N=18)	Unfavorable Outcome (N=9)	P Value
<b>Clinical Parameters</b>			
Sex (M:F)	13:05	05:04	0.423*
Follow-up duration (months) Median (Range) IQ Range	16.81(2.25-17.88) 14.31-17.79	17.50 (0.54-17.88)9.05-17.86	0.420**
Age at admission (months) Median (Range) IQ Range	1.55 ( 0.23-4.00) 1.18-2.50	2.00 (1.50-32.00) 1.65-10.0	1.000**
Intra-uterine growth retardation (n=6)	4	2	1.000*
Failure to thrive (n=7)	3	4	0.175*
Pale stools (n=6)	3	3	0.330*
Hepatomegaly (n=26)	17	9	1.000*
Splenomegaly (n=8)	2	6	0.006*
Jaundice persisting at 6 months old (n=4)	0	4	0.007*
Infectious complications and Co-morbidities	0	2	P = 0,103* $\Phi$ = - 0,400
<b>Biochemical Parameters (at presentation of neonatal cholestasis)</b>			
Conjugated bilirubin (mg/dl) Median (Range) IQ Range	4.80 (1.40-9.03) 3.10-6.42	5.40 (3.50-7.90) 3.90-7.50	1.000**
Aspartate aminotransferase (vr: <56 UI/L) Median (Range) IQ Range	109.0 (57.0-178.0) 97.0-142.5	137.0 (52.0-233.0) 75.0-216.0	1.000**
Alanine aminotransferase (vr: <39 UI/L) Median (Range) IQ Range	64.0 (20.0-180.0) 49.5-88.0	102.0 (28.0-136.0) 60.0-116.0	0.371**
Gamma-glutamyltransferase (vr: <45 UI/L) Median (Range) IQ Range	630.0 (119.0-1600.0) 335.0-986.8	938.0 (462.0-1742) 755.0-1207	0.667**
Serum alfa-1-antitrypsin (vr: 93-220 mg/dl) Median (Range) IQ Range	33.0 ( 22.0-61.0) 25.9-52.0	37.0 (25.0-78.0) 33.0-60.0	1.000**
<b>UDCA Therapy</b>			
Yes	14	2	P = 0.011* $\Phi$ = + 0,533
No	4	7	

(\* Fisher's Exact Test, \*\*) Mann-Whitney test

**Table 1:** Clinical, biochemical and therapeutic parameters in both groups of patients, with favourable and unfavourable outcome.

**Characterization and comparison of both groups: untreated (n=11) and treated (n=16) patients with UDCA – (Table 2):** Both of these groups of patients did not differ significantly in the evaluated clinical or biochemical parameters.

AAT serum level was significantly lower ( $P=0.022$ ) in the treated group.

## Discussion

Neonatal cholestasis is a rare entity with a wide spectrum of even more rare underlying diseases [20]. Among them, AAT deficiency “Z” variant is the most common inherited cause [21]. Yet, only 10% of homozygote ZZ individuals will present neonatal cholestasis [2]. This indicates that other factors (environmental and/or genetic modifiers) are involved in liver disease pathogenesis [4,9-11], influencing clinical presentation and prognosis.

Previous studies showed that a significant percentage of ZZ patients presenting by neonatal cholestasis develop chronic liver disease and its associated complications. In Sveger et al study [2], 14 of 122 PiZZ patients suffered from neonatal cholestasis; nine of these had severe liver disease (64%) and five had mild disease at 6 months old. In the study by Nemeth et al. [5], 10 patients were followed to the ages of 4-20 years, and 40% progressed to liver cirrhosis. Nebbia et al. [6] reported 56% of cirrhosis (25/45 patients) by 8 years old, and Francavilla et al. [8] reported 25% (21/61 patients) progressing to end-stage liver disease requiring OLT during a 10-year observation period.

In our study, 9/27 patients (33%) progressed unfavorably, during the observational period median follow-up time of 17.50 years, (IQ range: 15.21-17.79); the outcome was better than in Nebbia et al. [6] study, and slightly worse than in Francavilla et al [8], but within a significantly longer observational period (the double) than in these

two studies (although in a smaller sample). As previously described [6,8], and although in a smaller sample, the persistence of jaundice beyond 6 months old, and the early development of splenomegaly (early sign of cirrhosis), were associated with unfavorable prognosis. On the contrary, biochemical markers like conjugated bilirubin, liver enzymes, and AAT serum levels at presentation had no influence on outcome. In what concerns to the presence of AAT granules there was no statistically significant differences; yet, again we notice the scarcity of the sample that turns impossible any conclusion on this subject, as other sort of comparison of the histological features.

Possible environmental modifiers (infectious complications and co-morbidities) were registered only in the group with unfavorable outcome, but the difference was not significant when compared with the favorable outcome group. Yet, we must remind that there were no records for minor ailments. These may have been more rapidly diagnosed and treated in the more recent patients, since health care have significantly improved through the years. On this matter, we note that 64% of patients of the control group, and only 13 % of the UDCA-treated group, progressed unfavorably. Once there is a decade of difference between these two cohorts, there is a bias in favor of UDCA-treated group, in regards to health care quality, that can be assumed as significantly better after 1994. Despite this, we also tried to study the influence of UDCA therapy on the outcome of these patients. In our study, only 2/16 (13%) of patients treated with UDCA progressed unfavorably, against 7/11 (64%) of patients not treated with UDCA. This raises the possibility that UDCA treatment may have positively influenced outcome.

The recent advances in the knowledge of the liver disease pathogenesis in AAT deficiency opened the field for development of new therapy strategies [11,12]. In summary, AAT deficiency is characterized by accumulation of misfolded ZAAT within hepatocytes

	UDCA Therapy YES (n=16)		UDCA Therapy NO (n=11)		P value
<b>Clinical Parameters</b>					
Sex (M:F)	12:04		06:05		0.411*
Follow-up duration (months) Median (Range) IQ Range	15.85 (2.25-17.88) 9.15-17.79		17.79 (0.54-17.88) 16.67-17.88		0.252**
Age at admission (months) Median (Range) IQ Range	1,55 (0,23-3,50)	1.13-2.50	2,50 (1,50-32,00)	1.50-4.0	0.452**
Intra-uterine growth retardation (n=6)	3		3		0.662*
Failure to thrive (n=7)	4		3		1.000*
Pale stools (n=6)	4		2		1.000*
Hepatomegaly (n=26)	15		11		1.000*
Splenomegaly (n=8)	3		5		0.206*
Jaundice persisting at 6 months old (n=4)	2		2		1.000*
<b>Biochemical Parameters (at presentation of neonatal cholestasis)</b>					
Conjugated bilirubin (mg/dl) Median (Range) IQ Range	5,20 (2,80-9,03) 3.53-6.90		4.25 (1,40-7,90) 2.90-6.75		0.667**
Aspartate aminotransferase (vr: <56 UI/L) Median (Range) IQ Range	130,5 (52,0-233,0) 88.0-151.25		107.0 (75.0-216.0) 86.75-151.25		0.667**
Alanine aminotransferase (vr: <39 UI/L) Median (Range) IQ Range	61.75 (20.0-100.0) 49.25-81.0		112.0 (28.0-136.0) 57.0-120.0		0.193**
Gamma-glutamyltransferase (vr: <45 UI/L) Median (Range) IQ Range	718.0 (119.0-1742.0) 345.5-986.8		938.0 (334.0-1207.0) 462.0-1110.0		0.667**
Serum alfa-1-antitrypsin (vr: 93-220 mg/dl) Median (Range)	30.5 (22.0-61.0) 25.6-40.0		52.0 (25.0-78.0) 37.0-60.0		0.015**
(*) Fisher's Exact Test, (**) Mann-Whitney test					

**Table 2:** Clinical and biochemical parameters, and outcome, of both groups of patients, treated and non-treated with Ursodesoxycholic Acid (UDCA).

and cholangiocytes. The mutant protein (Z variant) differs from the normal (M variant) by a single amino acid substitution (Glu342 Lys). The abnormal protein polymerizes and is being kept held in the ER. There are two major pathways for its degradation: the proteasomal and the autophagic. Because liver damage is mediated by a gain-of-toxic function mechanism, the degradative pathways theoretically represent a protection mechanism, and therefore are candidates for polymorphisms that constitute genetic modifiers of the liver disease phenotype, but until now none has been described [22]. ER accumulation of ZAAAT is known to induce ER stress and activate a number of intracellular signaling pathways. Three major ER stress response pathways include the ER overload response, which is characterized by nuclear factor kappa B activation, the unfolded protein response that involves up-regulation of chaperones, foldases and degradation factors, and apoptosis. The use of a pharmacological chaperone therapy has already been tried in AAT deficiency patients but with not much success [23], and actually other drugs are under investigation [24,25].

Meanwhile, the efficacy of UDCA in AAT deficiency patients (a widely used drug in liver cholestatic diseases such as biliary atresia [26]) has never been adequately tested, especially in patients presenting with neonatal cholestasis. The only report [27] is a retrospective study with 42 children concluding that UDCA may normalize or significantly improve clinical and biochemical status in children with liver disease associated with ZZ AAT deficiency, and that the initial values of serum GGT and total serum bilirubin may have a prognostic value as to the efficacy of treatment. In this study, although patients are all ZZ homozygous, the sample includes not only patients with neonatal cholestasis but also patients with other liver disease phenotypes (hepatomegaly, fortuitous finding of elevated transaminases, and screening in siblings). This study also lacks a control group of non-treated patients (with a clinical presentation similar to the group of the treated-ones), and treatment with UDCA had a very erratic age of initiation and duration time.

Our study, although also retrospective, enrolled only ZZ homozygous patients with the same liver disease phenotype. Treatment was started very early (at admission) in all of them and in the same daily dosages and with the same criteria for cessation. We also used a control group with the same inclusion and exclusion criteria, and both groups (treated *versus* untreated patients) were homogenous in terms of clinical and biochemical parameters; serum level of AAT in the treated-ones was significantly lower. Our study showed an association of better outcome with early treatment with UDCA in AAT deficiency homozygous ZZ patients presenting with neonatal cholestasis ( $P=0.017$ ). Despite the small sample-size and the two cohorts not being contemporaneous, we speculate this may not be a fortuitous association. In effect, if we consider the most current knowledge on the liver disease pathogenesis in AAT deficiency we will find some possible points of interference of UDCA. Among its mechanisms of action, UDCA inhibits the hepatocytes and cholangiocytes apoptosis by interfering in the three main apoptotic pathways [18]. One of the apoptosis pathways involves the ER stress, occurring when protein production and trafficking systems in the organelle break down, leading to accumulation of misfolded protein, as it happens in AAT deficiency. UDCA inhibits the Reactive Oxygen Species (ROS) formation, thus inhibiting ER stress. In addition, tauro-ursodeoxycholic acid, the UDCA metabolite, acts as a chemical chaperone that reduces ER stress, and it also inhibits apoptosis induced by ZAAAT via inhibition of Bad [28]. Beyond its effects on apoptosis other mechanisms of action of UDCA explain its potential benefit in cholestasis such as modulation of the expression of liver transporters and enzyme systems [18].

These UDCA actions are particularly interesting in an AAT deficiency group of patients presenting with neonatal cholestasis, since beyond a disease caused by a defect in a protein folding and aggregation [29], liver enzymatic systems are immature at this age. A relevant percentage of these patients spontaneously evolve well, but it may be possible to rise it up with the help of UDCA, in an appropriate dose and duration time, which needs to be determined.

The advances in the understanding of both the AAT deficiency liver disease pathogenesis and the UDCA mechanisms of action allow us to speculate that the results of our small series may not be casual. Yet, it would also be very interesting to characterize this whole sample of patients with whole-genome sequencing, to better evaluate if differences in outcome are correlated with molecular variants in AAT gene, or other genes associated with the pathways of polymerization and degradation of AAT granules in the liver (genes not yet known).

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#### Ethical Approval

This article was approved by Local Ethics Committee. It does not contain any studies with human participants or animals performed by any of the authors. The published data respect the anonymity and do not contain sensitive data.

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