Anesthesia management in pediatric patients with and without congenital anomaly: A retrospective study

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Abstract
Aim: In this study, we aimed to compare perioperative anesthesia management in pediatric patients with and without congenital anomaly.
Material and Methods: The records of patients aged 0-16 who were operated between 2012-2017 in the pediatric surgery clinic were retrospectively evaluated. The patients were divided into two groups with a congenital anomaly (Group I) and without an anomaly (Group II). The patients were evaluated in terms of demographic characteristics, ASA risk, current surgery, anesthesia induction method-technique, difficult mask ventilation, complications related to anesthesia, and 30-day mortality rates in the postoperative intensive care unit (ICU) were evaluated.
Results: The study included 102 patients. A statistically significant difference was found between the groups in terms of the type of surgery and ASA risk (p<0.05). It was determined that 77.6% of the patients in Group I had easy mask ventilation, and the frequency of minor complications associated with intraoperative anesthesia was 42.9% (p<0.05). It was determined that the number of patients taken to the postoperative ICU was higher in Group I (p<0.05). It was determined that 10 patients with anomaly died, and 30-day mortality in ICU was 59%.
Discussion: Complication of surgical procedures in patients with anomalies affects the anesthetic management of these patients in many ways. A detailed evaluation of these patients will increase the quality of life and comfort.

Keywords
Pediatric anesthesia; Congenital anomaly; Mortality
Introduction

Pediatric patients’ physiological, anatomical and pharmacological characteristics are different from adults and each other [1]. Anesthesia management of pediatric patients with congenital anomalies requires a multidisciplinary approach because of prolonged surgery, excessive loss of blood and the need for transfusion, airway problems such as difficult mask ventilation and intubation, etc. [2, 3]. There are many reports of complications related to anesthesia management in newborn, neonatal period patients [1, 4-13].

Recent technological developments in anesthesia and surgical applications and equipment cause us to encounter such patients in anesthesia applications. This study aims to evaluate perioperative anesthesia management of patients with and without congenital anomalies, operated on for various reasons in the pediatrics clinic of our hospital.

Material and Methods

Permission for this retrospective observational study was granted by the local ethics committee (Date:19/04/2017, Meeting No: 2017/08). The records of patients aged 0-16 years who were operated on by the same physician between January 2012 and April 2017 were evaluated. Patients over 16 y/o, premature, and intubated outside the operating room for multiple trauma were excluded. Data were obtained from the hospital automation database and anesthesia records. While all patients with anomalies were included in the study, patients without congenital anomalies who had the same age, gender, and weight distribution and match compatibility were randomly selected.

A total of 102 patients were divided into two groups as those with a congenital anomaly (Group I, n=49) and those without (Group II, n=53). The gender, age (0-28 days, 29 days-1 year, 1.1-3 years, 3.1-6 years, and 6.1-16 years), weight, American Society of Anesthesiologists (ASA) risk category, current surgery (thorax+gastrointestinal, urogenital, other [minor surgeries such as burn debridement, swallowing a foreign substance, tongue-tie, etc.], previous surgery [yes/no], surgical procedure [urgent/elective], premedication method, anesthesia-surgery-recovery durations, anesthesia induction method (iv, inhalation, iv+inhalation), induction agent (propofol, ketamine, fentanyl, propofol+fentanyl), anesthesia technique (laryngeal mask airway [LMA], endotracheal intubation, mask ventilation, sedation), use of neuromuscular blocker [yes/no], difficult mask ventilation (easy/difficult), and regional anesthesia (RA) applications [yes/no] data were retrieved from archives.

The need for invasive monitoring, use of steroids, antiemetic, atropine, neostigmine and anesthesia-related complications (difficult airway [difficult mask, difficult intubation], cardiovascular instability, laryngo-bronchospasm, aspiration, other [minor complications: difficult vascular access, hypothermia, allergy, pain etc.]) were recorded from intraoperative records. From postoperative records, analgesic applications (tramadol, parol, local infiltration), presence of nausea/vomiting, intubated/extubated status of the patients leaving the theatre, postoperative follow-up location (intensive care unit [ICU], service) and 30-day mortality rates in ICU were evaluated.

Statistical Analysis

The study sample was determined as 70 patients, with 95% confidence (1-α), 80.7% test power (1-β), and d=0.621 impact size.

The data were analyzed using SPSS v.23. Kolmogorov-Smirnov was used to test the normal distribution. The normally distributed data were tested using t-test and presented as mean ± standard deviation, others were tested using the Mann Whitney-U test and presented as median (min-max). A p-value <0.05 was determined to be significant.

Results

The mean age of the participants included in the study was 4±4.13 years, and their mean weight was 14239.98 ±13550.47 grams. There was no significant difference between the groups in terms of demographics or surgical procedures (p>0.05) (Table 1); 46.9% in Group I were in ASA-2 risk category, and 79.2% in Group II were in ASA-1 (p<0.001) (Table1). There was a significant difference between the groups in terms of surgical procedures; 42.9% of the patients underwent thorax + gastrointestinal surgery (anal-ileal atresia, tracheoesophageal fistula (TEF), appendectomy, ileus), and 62.3% underwent urogenital surgery (circumcision, hypospadias, undescended testicle, hydrocele, hernia) (p=0.006). A total of 59.2% in Group I had no previous surgery, and the same was true for 92.5% in Group II (p<0.001) (Table 1). None of the patients were premedicated or applied RA.

Table 1. Demographic characteristics and general distributions of patients (n,%)

|               | Group I (n=49, %) | Group II (n=53, %) | Total (n=102, %) | p     |
|---------------|------------------|-------------------|-----------------|-------|
| Sex           |                  |                   |                 |       |
| Female        | 18 (36.7)        | 16 (30.2)         | 34 (33.3)       | 0.624 |
| Male          | 31 (63.3)        | 37 (69.8)         | 68 (66.7)       |       |
| Body weight (grams) | 12220.1±10816.3 | 16107.5±15532.1   | 14239.98±13550.47 | 0.149 |
| Age groups    |                  |                   |                 |       |
| 0-28 days     | 11 (22.4)        | 3 (5.7)           | 14 (13.7)       | <0.001|
| 29 day-1 year | 13 (26.5)        | 23 (43.4)         | 36 (35.3)       |       |
| 1.1-3 year    | 4 (8.2)          | 5 (9.4)           | 9 (8.8)         | 0.117 |
| 3.1-6 year    | 7 (14.3)         | 8 (15.1)          | 15(14.7)        |       |
| 6.1-16 year   | 14 (28.6)        | 14 (26.4)         | 28(27.5)        |       |
| ASA risk class|                  |                   |                 |       |
| ASA 1         | 1 (2)            | 42 (79.2)         | 43(42.2)        |       |
| ASA 2         | 23 (46.9)        | 9 (17)            | 32(31.4)        | <0.001|
| ASA 3         | 15 (30.6)        | 23 (43.4)         | 38(36.7)        |       |
| ASA 4         | 10 (20.4)        | 0 (0)             | 10 (9.8)        |       |
| Surgical procedures performed |                  |                   |                 |       |
| Thorax+gastrointestinal | 21 (42.9) | 12 (22.6) | 33(32.4) | 0.006 |
| Urogenital    | 18 (36.7)        | 33 (62.3)         | 51 (50)         |       |
| Head and neck | 2 (4.1)          | 6 (11.3)          | 8 (7.8)         |       |
| Other*        | 8 (16.3)         | 2 (3.8)           | 10 (9.8)        | <0.001|
| Previous surgery |              |                   |                 |       |
| Yes           | 20 (40.8)        | 4 (7.5)           | 24(23.5)        |       |
| No            | 29 (59.2)        | 49 (92.5)         | 78(76.5)        |       |
| Surgical procedure |           |                   |                 | 0.845 |
| Urgent        | 10 (20.4)        | 10 (18.9)         | 20(19.6)        |       |
| Elective      | 39 (79.6)        | 43 (81.1)         | 82(80.4)        |       |

* minor surgeries such as burn debridement, foreign body ingestion, tongue ligament

Group I: Patients with congenital anomaly, Group II: Patients without congenital anomaly

ASA: American Society of Anesthesiologists
It was observed that of the patients with a congenital anomaly, 8 were diagnosed with Down Syndrome, 5 with VACTREL syndrome, 6 with other syndromes (Griscelli, Frajil-X, Jarcho-Levin, Wolf- Hirschhrom, Sotos and Prader-Willi Syndrome). The remaining 30 had only single anomalies or comorbid pathologies and had no genetic diagnoses. There was a significant difference in terms of anesthesia-surgery-recovery durations. They were longer in patients with congenital anomalies (p<0.001).

All patients were monitored and heating blankets were used to prevent hypothermia. The iv method was frequently used when transferring patients from the service to the theatre after checking their vascular access safety and re-opened in problematic cases. There was no significant difference in terms of anesthesia method or iv analgesic agents (p>0.005), or in terms of anesthesia induction method or the agents (p>0.005) (Table 2). Sevoflurane was used to maintain general anesthesia in 98.1% in Group II (p=0.001) (Table 2). A total of 16 patients in Group I had easy mask ventilation, this rate was 77.6% in Group I and 50.9% in Group II (p=0.002). While 77.6% in Group I had easy mask ventilation, this rate was 98.1% in Group II (p=0.001) (Table 2). A total of 16 patients in Group I experienced difficulty in the airway [mask ventilation]. Two patients urgently operated in Group I, they were used in 34% in Group II (p=0.001) (Table 2). In Group I, 61.2% of the patients received endotracheal intubation, while LMA was used in 50.9% in Group II (p=0.002). While 77.6% in Group I had easy mask ventilation, this rate was 98.1% in Group II (p=0.001) (Table 2). A total of 16 patients in Group I experienced difficulty in the airway [mask ventilation].

**Table 2. Comparisons between groups**

|                     | Group I (n=49, %) | Group II (n=53, %) | Total (n=102, %) | P     |
|---------------------|-------------------|-------------------|------------------|-------|
| Anesthesia induction methods |                   |                   |                  |       |
| IV                  | 38 (77.6)         | 41 (77.4)         | 79 (77.5)        | 0.619 |
| Inhilation          | 0 (0)             | 1 (1.9)           | 1 (1)            |       |
| IV + Inhilation     | 11 (22.4)         | 11 (20.8)         | 22 (21.6)        |       |
| N’ agents used in induction |             |                   |                  |       |
| Propofol           | 27 (55.1)         | 27 (52.9)         | 54 (54)          | 0.522 |
| Ketamine           | 7 (14.3)          | 4 (7.5)           | 11 (11)          |       |
| Fentanyl           | 0 (0)             | 1 (2)             | 1 (1)            |       |
| Propofol+fentanyl  | 15 (30.6)         | 18 (37.3)         | 33 (34)          |       |
| Use of neuromuscular blocker |             |                   |                  |       |
| Yes                | 30 (61.2)         | 18 (34)           | 48 (47.1)        | 0.011 |
| No                 | 19 (38.8)         | 35 (66)           | 54 (52.9)        |       |
| Anesthesia technique |                  |                   |                  |       |
| LMA                | 11 (22.4)         | 27 (50.9)         | 38 (37.3)        | 0.022 |
| ETE                 | 30 (61.2)         | 18 (34)           | 48 (47.1)        |       |
| Mask ventilation    | 4 (8.2)           | 4 (7.5)           | 8 (7.8)          |       |
| Sedation           | 4 (8.2)           | 4 (7.5)           | 8 (7.8)          |       |
| Mask ventilation difficulty |             |                   |                  |       |
| Easy               | 38 (77.6)         | 52 (98.1)         | 90 (88.2)        | 0.001 |
| Difficult          | 11 (22.4)         | 1 (1.9)           | 12 (11.8)        |       |
| Intraoperative complications associated with anesthesia |             |                   |                  |       |
| Difficult airway   | 16 (32.7)         | 1 (1.9)           | 17 (16.7)        | <0.001|
| Cardiac depression  | 6 (12.2)          | 1 (1.9)           | 7 (6.9)          |       |
| Laryngospasm / bronchospsam | 6 (12.2) | 2 (3.8) | 8 (7.8) | <0.001 |
| Aspiration         | 0 (0)             | 2 (3.8)           | 2 (2)            |       |
| Other*             | 21 (42.9)         | 47 (88.7)         | 68 (66.7)        |       |

Group I: Patients with congenital anomaly. Group II: Patients without congenital anomaly. iv: intravenous; LMA: laryngeal mask airway, ETE: Endotracheal intubation; * Difficult vascular access, hypothermia, allergy, pain etc. minor complications

Patients with adequate spontaneous breathing and SpO₂ ≥ 96% were extubated, others were transferred to ICU. In addition to the patients diagnosed with TEF + esophageal atresia (n:10) and ileus (n:3; patients with Down syndrome with dysmorphic facial features and not diagnosed genetically and without anomalies), and one patient with Griscelli syndrome was intubated and transferred to ICU due to laryngo/bronchospasm. In addition to intubated patients (n:14), three patients [TEF + esophageal atresia (n:2), colostomy closure (n:1, Down syndrome)] were also intubated and transferred to ICU. All 10 patients who lost their lives due to cardiac arrest on around the 14th day in the ICU were patients with anomalies. The 30-day mortality rate in ICU was 59%.

**Table 3. Comparisons between groups**

|                     | Group I (n=49, %) | Group II (n=53, %) | Total (n=102, %) | P     |
|---------------------|-------------------|-------------------|------------------|-------|
| Intraoperative invasive monitoring requirement |             |                   |                  |       |
| Yes                 | 26 (53.1)         | 3 (5.7)           | 29 (28.4)        | <0.001|
| No                  | 23 (46.9)         | 50 (94.3)         | 73 (71.6)        |       |
| Intraoperative steroid use |             |                   |                  |       |
| Yes                 | 20 (40.8)         | 5 (9.4)           | 25 (24.5)        | 0.001 |
| No                  | 29 (59.2)         | 48 (90.6)         | 77 (75.5)        |       |
| Intraoperative antiemetic use |             |                   |                  |       |
| Yes                 | 17 (34.7)         | 14 (26.4)         | 31 (30.4)        | 0.488 |
| No                  | 32 (65.3)         | 39 (73.6)         | 71 (69.6)        |       |
| Intraoperative atropine use |             |                   |                  |       |
| Yes                 | 9 (18.4)          | 2 (3.8)           | 11 (10.8)        | 0.018 |
| No                  | 40 (81.6)         | 51 (96.2)         | 91 (89.2)        |       |
| Intraoperative neostigmine use |             |                   |                  |       |
| Yes                 | 12 (24.5)         | 4 (7.5)           | 16 (15.7)        | 0.038 |
| No                  | 37 (75.5)         | 49 (92.5)         | 86 (84.3)        |       |
| Presence of postoperative nausea/vomiting |             |                   |                  |       |
| Yes                 | 4 (8.2)           | 1 (1.9)           | 5 (4.9)          | 0.142 |
| No                  | 45 (91.8)         | 52 (98.1)         | 97 (95.1)        |       |
| Postoperative analgesia methods |             |                   |                  |       |
| Tramadol            | 17 (34.7)         | 16 (30.2)         | 33 (32.4)        | 0.774 |
| Paral               | 11 (22.4)         | 15 (28.3)         | 26 (25.5)        |       |
| Local infiltration anaesthesia |             |                   |                  |       |
| Yes                 | 21 (42.9)         | 22 (41.5)         | 43 (42)          |       |

**Postoperative exit conditions**

|                     | Group I (n=49, %) | Group II (n=53, %) | Total (n=102, %) | P     |
|---------------------|-------------------|-------------------|------------------|-------|
| Intubated           | 13 (26.5)         | 1 (1.9)           | 14 (13.7)        | 0.001 |
| Exubated            | 36 (73.5)         | 52 (98.1)         | 88 (86.3)        |       |
| Postoperative follow-up place |             |                   |                  |       |
| ICU                 | 16 (32.7)         | 1 (1.9)           | 17 (16.7)        | <0.001|
| Service             | 33 (67.3)         | 52 (98.1)         | 85 (83.3)        |       |

Group I: Patients with congenital anomaly. Group II: Patients without congenital anomaly. ICU: Intensive care unit.
Discussion

It was determined that since the surgical interventions in patients with congenital anomalies are complicated, they were in higher ASA risk categories and had longer anesthesia-surgery-recovery periods. It was identified that these patients were frequently intubated, that they had more frequent complications related to difficult airway, and higher ICU transfers.

Preoperative evaluation is vital for safe anesthesia and a successful perioperative process. It should include the patient’s clinical history, complementary examinations and informing the patient and relatives [1, 14, 15]. In the study, during the physical examination of the infants, anomalies, facial structure, nasal and oral aperture, and the structures of tongue, teeth, and neck that could affect airway management were carefully examined, and in cases of asymmetric face, mandibular hypoplasia, micrognathia, and tongue anomalies, additional consultations and examinations were requested.

Premedication with sedatives is usually not recommended for newborns and pediatric patients due to possible side effects [1, 14, 15]. In our clinical practices, premedication is not administered to pediatric patients, and parents are allowed to accompany the child until the induction stage.

ASA reports physical condition, age, urgent surgery, and underlying disorders as risk factors for post-op critical events in pediatrics [5-13]. It is common for pediatric anesthesiologists to add 1 to ASA risk for newborns and infants due to the increased perioperative critical event risk [7, 13]. ASA 1 and 2 patients constitute the majority of pediatric cases for us. In this study, most of the patients with anomalies were in an ASA-2 or a higher risk category. We believe that newborn and neonatal patients in a pediatric surgery clinic are at a higher risk because these surgeries are frequently performed due to emergencies. Monitoring of infants and children is the same as for adults, except for a few small modifications. Additional invasive monitoring might be required depending the disease and surgical procedure [1, 5, 15], this is also the case in our clinic. In the study, 26 patients with anomaly and 3 patients without anomaly were placed urinary catheter. The reasons were the duration-type of the surgery (frequently thorax + gastrointestinal and urogenital) and the need for monitoring in the ICU. The patients’ body temperatures were monitored non-invasively, blankets were used to eliminate intraoperative hypothermia, and iv fluids were administered post-heating.

The pediatric group’s respiratory-tract anatomy and physiological properties differ from those of adults [1, 15]. With the improvements in the last decade, current pediatric airway equipment is now competing with those for the adult population and expands the pediatric anesthesiologist’s toolkit for airway management [1, 15-17]. In our clinical practice, anesthesia is planned considering the features of the upcoming surgery, and the use of supraglottic airway devices for pediatric patients is common. In the study, endotracheal intubation (61.2%) and LMA (22.4%) were used depending on the type and duration of the surgery.

Acquired or congenital-pathology-related airway problems increase morbidity and mortality in pediatric patients [6-8, 10, 12, 13]. Murat et al. [7] investigated anesthesia applications and morbidity in 24,165 pediatric patients over a 30-month period and determined difficult intubation rate as 0.42% and difficult ventilation rate 0.15%. In this study, difficult mask ventilation was 11.8% (n:12) and difficult intubation was 4.9% (n:5) for all patients. While there was no difficult intubation in the patients without congenital anomaly, there was only one difficult mask ventilation.

Airway management in pediatric anesthesia is particularly important and requires knowledge and experience. To avoid problems, it is necessary to be prepared. Intubation must be performed by anesthesiologists experienced in difficult intubation, bronchoscopy, and tracheostomy [4]. Airway management in newborns and children with congenital anomalies is rather difficult. In fact, in such cases, lethal respiratory problems and intubation difficulty are quite common [18, 19]. In our clinic, other clinics’ pediatric operations are carried out by implementing standard applications. Intubation and monitoring are performed by senior anesthesiology assistants and anesthesiology specialists experienced in pediatric anesthesia using necessary equipment (including video laryngoscopy). In the study, difficult intubation resulting from comorbid craniofacial anomalies happened only in 5 patients (with Fragile-X, Wolf-Hirschorn, Sotos, Down syndrome and dysmorphic facial structure). They were intubated in repeated trials using video laryngoscope.

The literature suggests that the same induction sequence and agents used in adult patients can be employed and that depolarizing agents as neuromuscular should be used with care due to possible arrhythmia, hyperkalemia and malignant hyperthermia [1, 5, 15]. We determined that, while anesthesia induction was frequently done with propofol and propofol + fentanyl combination, as there was already an open vascular access, induction in patients with vascular-access problems was performed by iv + inhalation, after opening vascular access by using inhalation agent, and that rocuronium was preferred as neuromuscular agent. As the operations in the study were usually thorax+gastrointestinal system surgeries that last long and require postoperative monitoring, the use of endotracheal intubation was frequent with more neuromuscular blocker use. It is possible that steroid, atropine and neostigmine were used in larger amounts because endotracheal intubation was used more in these patients and there were more complications like laryngo-bronchospasm.

There is a growing interest in pain management in pediatric patients, especially with the use of RA techniques [20-23]. However, safe and effective regional examinations in pediatric patients with anomalies require experience because of restrictions in positioning and difficulty in cooperation [24]. In pediatric patients, parenteral ketorolac, morphine, fentanyl, meperidine can be used as a postoperative analgesic, and in the age group of 1-12 years, oral diclofenac and oral, rectal or iv acetaminophen can be used [1, 5, 15, 24]. It is quite uncommon for our clinic to use RA application in pediatric patients; IV acetaminophen is used frequently. Tramadol is preferred for children under 12 years of age. However, tramadol was also used in patients under 12 years of age when their pain was not relieved despite acetaminophen use and local infiltration.

The anesthesia complications in pediatrics are difficult
The 30-day mortality rate in the ICU was quite high (59%). The ICU lost their lives. They were in the smaller age group, surgery. In our study, 10 of the 17 patients with anomalies in anesthetics; It was 1.6 per 10,000 anesthetics for non-cardiac rate associated with cardiac surgery was 115.5 per 10,000 the US as 6.8:10,000 anesthetics. In the study, the mortality reported the overall perioperative mortality rate for children in or cardiac surgery. The main causes of mortality are airway complications were at higher rates compared to the patients with anomalies, minor complications such as difficult vascular access, hypothermia, allergy, pain, etc. were observed at a high rate of 88.7%. The reason why these complications were at higher rates compared to the patients with anomalies could be that, since it is harder to open vascular access in patients with anomalies, this issue was better taken care of in the service and that, as these children were younger, more care was taken during their transfer to the surgery room in order to protect them from hypothermia and to minimize the pathologies that could result from their current conditions. Patients might leave the theatre intubated or with oxygen support. If patients with syndromes have no severe postoperative airway obstruction or are clinically stable, they can be extubated. Ensuring postoperative hemodynamic stability in major surgeries is the most important issue and due to the drain, urine outlet, oxygenation and the need for monitoring, they can be accepted to ICU [24]. In our study, 86.3% of the patients were extubated; 13 patients with anomalies (26.5%) and 1 patient without (1.9%) were transferred to the ICU as intubated, and 3 patients as extubated. Pediatric perioperative mortality rates have increased over the past decade. These rates are higher in Brazil (9.8 per 10,000) and other developing countries (10.7-15.9 per 10,000), compared to developed countries (0.41-6.8 per 10,000), with the exception of Australia (13.4 per 10,000). The major risk factors are as follows: newborn or less than 1 year old, ASA-3 or higher, and undergoing emergency surgery, general anesthesia, or cardiac surgery. The main causes of mortality are airway problems and cardio-circulatory events [25]. Flick et al. [11] reported the overall perioperative mortality rate for children in the US as 6.8:10,000 anesthetics. In the study, the mortality rate associated with cardiac surgery was 115.5 per 10,000 anesthetics; It was 1.6 per 10,000 anesthetics for non-cardiac surgery. In our study, 10 of the 17 patients with anomalies in the ICU lost their lives. They were in the smaller age group, operated under general anesthesia, and had an ASA-2 status. The 30-day mortality rate in the ICU was quite high (59%). This study has some limitations. The first limitation is that this is a retrospective, single-center study and the results cannot be generalized. Secondly, all operations were performed by one surgeon, the number of patients with anomalies was relatively small, it was not possible to determine mortality factors. Thirdly, premedication applications in pediatric patients were not carried out in our center, and RA applications, including neuaxial anesthesia, were rare. Finally, surgical procedures included operations ranging from simple to complicated.

In conclusion, in pediatric patients with more challenging airway management, additional anatomic problems make it even more difficult. Relatively more complicated surgical interventions in pediatric patients with congenital anomalies require longer intubation of these patients and their follow-up in ICU. The researchers believe that since it would affect morbidity and mortality in pediatric patients with congenital anomalies, with ideal airway management, in addition to effective preoperative preparations, keeping difficult airway equipment available and efficient perioperative clinic management are important.

Scientific Responsibility Statement
The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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