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ORIGINAL ARTICLE

Predicting Factors of Surgical Mortality in Children and Adolescents Undergoing Correction of Tetralogy of Fallot

Patrícia de Souza Pinheiro,[1] Vitor Manuel Pereira Azevedo,[2] Gabrielle Rocha[3]

Instituto Nacional de Cardiologia,[1] Laranjeiras, Rio de Janeiro, RJ – Brazil
Instituto Nacional de Cardiologia,[2] Botafogo, Rio de Janeiro, RJ – Brazil
Universidade Federal Fluminense,[3] Niterói, Rio de Janeiro, RJ – Brazil

Abstract

Background: In tetralogy of Fallot, correction surgery is a priority choice, seeing that it is desirable to minimize pulmonary hypoflow and severe hypoxemia, which result in hypoxemic crises, with sudden worsening of cyanosis, tachypnea, and, in some cases, loss of consciousness, seizures, and even death.

Objective: To evaluate the predicting factors of surgical mortality in children and adolescents undergoing correction of tetralogy of Fallot.

Methods: Retrospective cross-sectional study carried out by consulting all surgical records of the Child and Adolescent Cardiology Service of the Brazilian National Institute of Cardiology, during the period from 2007 to 2010. Results with p values < 0.05 were considered significant.

Results: The study evaluated 93 medical records. In relation to the characteristics of the population at the time of surgery, the median age was 3.69 (2.13 to 5.79) years, and 58.06% (n = 54) were male. White was the most common skin color, accounting for 55.43% (n = 51) of cases. In relation to assessment of nutritional status, median weight was 13.25 (10.10 to 17.60) kg, and body mass index was 14.49 (13.44 to 16.28) kg/m2. Down syndrome was present in 11.83% (n = 11) of the patients. All patients underwent correction surgery (n = 93, 100%). Prior cyanotic crisis was found in 53.85% (n = 49) with p = 0.013; surgical procedure duration was 218.83 ± 60.63 minutes, with p = 0.003, and lactate was 1.88 ± 1.33 mg/dL during the immediate postoperative period, with p = 0.009. Regarding the outcome of surgical death, it was found in 15.05% (n = 14) of patients. Mean follow-up lasted 5.68 ± 3.76 years.

Conclusions: According to the factors analyzed, the duration of the surgical procedure, prior cyanotic crisis, and blood lactate level may be relevant to surgical mortality.

Keywords: Tetralogy of Fallot; Mortality; Surgery.

Introduction

Tetralogy of Fallot is the most common cyanotic congenital disease, with an incidence of 3 cases for every 10,000 live births, and it is responsible for approximately 5% to 7% of all congenital heart diseases.[1] For every 3,600 births, 1 child has the disease, corresponding to 3.5% of cases of congenital heart disease.[2]

Congenital heart diseases can be classified as acyanotic or cyanotic. In cyanotic heart diseases, blood from the systemic venous return passes directly from the right to the left heart without passing through the pulmonary circulation, resulting in unsaturation of systemic arterial blood and central cyanosis.[3]

Studies have demonstrated a strong association between morbidity in the intensive care unit and intraoperative factors, such as cardiopulmonary bypass time and anoxia time, in addition to the surgical techniques employed.[4]

Among the signs and symptoms that can be identified in children with congenital heart disease who are hospitalized

Mailing Address: Patrícia de Souza Pinheiro
Instituto Nacional de Cardiologia, Serviço de Nutrição. Rua das Laranjeiras, 374, Rio de Janeiro, RJ. Postal code: 22240-006 – Brazil
E-mail: pspinheiro30@yahoo.com.br

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in clinical and surgical units, the following stand out: nutritional inadequacy, risk of infections, ineffective airway clearance, impaired hematosis, hyperthermia, acute pain, growth and development deficit, altered sleep pattern, risk of intestinal constipation, and altered skin integrity.[5]

The severity of cardiac injuries and malnutrition place this group of patients at a greater risk of operative mortality. Thus, adequate nutritional treatment should be considered, because there is the possibility of nutritional recovery after the surgical procedure.[6]

Therefore, it is necessary to identify the predicting factors of surgical mortality in children and adolescents undergoing surgery to correct tetralogy of Fallot, which are an important tool in predicting the immediate results and providing subsequent assistance with the medical and nutritional interventions available. Studies have shown that there is a strong association between morbidity/mortality in the intensive care unit and intraoperative factors. Accordingly, the objective of this article was to evaluate the predicting factors of surgical mortality in children and adolescents undergoing correction of tetralogy of Fallot, at the Brazilian National Institute of Cardiology (Instituto Nacional de Cardiologia, INC).

Methods

This study was approved by the Research Ethics Committee of the INC under CAAE number 53654816.8.000.5272 (Appendix 1), and it was submitted to the Brazilian registry platform for clinical trials under submission number 013900/2016. Upon submission, waiver of the free and informed consent form was requested, given that this was a retrospective study.

This was a retrospective cross-sectional study carried out by consulting all surgical records of patients from the Child and Adolescent Cardiology Service of the INC who underwent surgery for tetralogy of Fallot from 2007 to 2010. The patient flowchart is in Figure 1.

Inclusion criteria

All patients from 0 to 18 years of age who underwent surgery for tetralogy of Fallot were eligible for inclusion.

Exclusion criteria

Patients who underwent surgery for tetralogy of Fallot associated with other concomitant surgeries were excluded.

Data collection

The following data related to the preoperative period were collected from medical records: age at the time of surgery; gestational age; sex; weight; height; presence of hypoxemic crisis; blood exchange (with the objective of reducing hematocrit values); history of associated genetic disease, including Down syndrome; general laboratory data (blood count, glucose, lactate); gasometry; echocardiogram; cardiac catheterization; and sociodemographic data.

The following data related to the postoperative period were collected: mechanical ventilation time; need for extracorporeal membrane oxygenation; anoxia time (aortic clamping); echocardiogram; fasting time (from the preoperative to the postoperative period); gasometry; general laboratory data (blood count, glucose, lactate); water restriction; degree of water restriction; and nutritional access route.

Assessment of nutritional status

Regarding nutritional assessment, the following parameters were used for nutritional diagnosis: weight and height by age and body mass index (BMI), before surgery. All parameters were collected from medical records, normalized by percentile and z score using the values standardized by the World Health Organization in 2006 and 2007 as a reference.

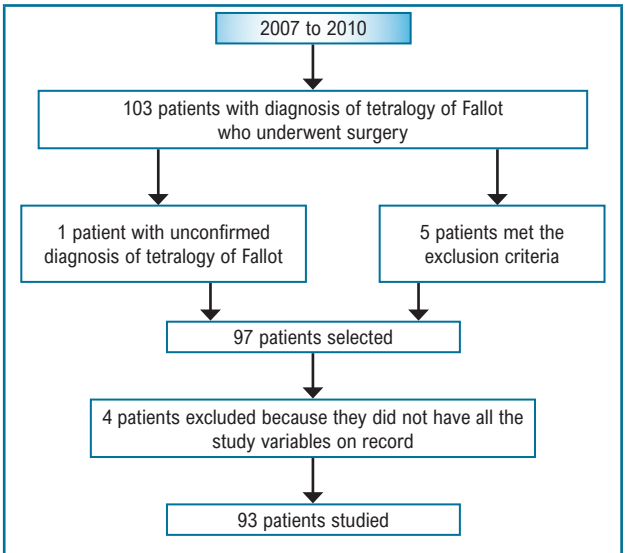

Figure 1 – Patient data flowchart.

Data were collected from the medical records of the Child and Adolescent Cardiology Service of the INC for patients who underwent surgery for tetralogy of Fallot from 2007 to 2010.

Statistical analysis

In descriptive analysis, continuous variables were presented through median and interquartile range and analyzed for the outcome of death using the Mann-Whitney U test. Categorical variables were expressed as numbers and percentages and analyzed using the chi-square test or Fisher's exact test when appropriate. To verify the normality of the data, the Shapiro-Wilk test was used. Univariate and multivariate logistic regression were performed for the outcome. In multivariate logistic regression, the backward method was used, which sequentially removed the variables with the highest p-value until only variables with a statistically significant coefficient remained. The best cutoff point for predicting the outcome for the continuous variables was evaluated by the receiver operating characteristic (ROC) curve. Estimated survival was analyzed using the Kaplan-Meier curve. Results with p values < 0.05 were considered significant. The statistical program STATA MP version 14.2 by Stata Corp was used for data analysis.

Results

All 93 medical records were evaluated. Regarding the characteristics of the population at the time of surgery, the median age observed was 3.69 (2.13 to 5.79) years. Males represented 58.06% (n = 54) of patients. White was the most common skin color, accounting for 55.43% (n = 51) of patients. The most common city of origin was Rio de Janeiro, with 38.71% (n = 36) of patients. In relation to comorbidities presented, Down syndrome was present in 11.83% (n = 11) of the patients. Regarding mother's and father's level of education, the most common were incomplete elementary school, with 30.11% (n = 28), and complete high school, with 25.81% (n = 24). The families had running water and sewage treatment in 98.57% (n = 69) and 81.43% (n = 57) of cases, respectively. The vaccination schedule was complete for the patient's age in 93.75% (n = 75). These data are displayed in Table 1. The patients' homes had an average of 4.59 rooms; an average of 4.34 inhabitants lived in the same house, and average family income corresponded to 1.89 times monthly minimum wage.

In relation to assessment of nutritional status, median weight was 13.25 (10.10 to 17.60) kg, and BMI was 14.4 (13.44 to 16.28) kg/m^2. Fasting time (from the preoperative to the postoperative period) was 60.95 (57.00 to 84.00) hours, and diet was administered orally in 95.24% (n = 80), with water restriction of 40 ml/kg in 95.18% (n = 79). These data are displayed in Table 1.

In univariate analysis of surgical mortality, Fisher's exact test was used to verify, among the analyzed factors, which would have prognostic significance for the analyzed outcome. This result is displayed in Table 2.

In multivariate analysis of independent predicting factors of surgical mortality, in step 1, using multivariate

Table 1 – Anthropometric and nutritional characteristics of the patients studied

Variables	
	Median (p25 – p75)
Weight, kg	
At birth	2.74 (2.40 – 3.10)
Surgery	13.25 (10.10 – 17.60)
Discharge	13.35 (10.82 – 18.4)
Last consultation	38.00 (26.10 – 49.50)
Delta surgery weight and discharge	−0.15 (−0.615 – 0.22)
Delta surgery weight and last consultation	24.50 (14.54 – 34.70)
Height, m	
Surgery	1.09 (0.93 – 1.25)
Discharge	1.075 (0.93 – 1.18)
Surgery BMI kg/m^2	14.49 (13.44 – 16.28)
Fasting time in hours	60.95 (57.00 – 84.00)
	Number (percentage)
Nutritional access route %(N)	
Enteral	4 (4.76%)
Oral	80 (95.24%)
Postoperative WR %(N)	83 (98.81%)
WR 40 ml/kg %(N)	79 (95.18%)

Values expressed as median; p25: 25th percentile; p75: 75th percentile; BMI: body mass index; kg: kilograms; m: meters; N: number; WR: water restriction. Source: Research data (2020).

Table 2 – Univariate analysis of surgical mortality

Risk factor	Surgical mortality				p
	Yes		No		
	N	Percentage	N	Percentage	
Sex					
Male	5	9.26	49	90.74	0.737
Female	5	12.82	34	87.18	
Down syndrome					
Yes	1	9.09	10	90.91	1.000
No	9	10.98	73	89.02	
Cyanotic crisis					
Yes	9	18.37	40	81.63	0.018
No	1	2.38	41	97.62	
Vaccine schedule up to date					
Yes	8	10.67	67	89.33	1.000
No	0	0.00	5	100.00	
Prior BT					
Yes	2	9.09	20	90.91	1.000
No	8	12.12	58	87.88	
Prior echocardiogram					
Yes	4	10.00	36	90.00	1.000
No	6	11.32	47	88.68	
Severe PVS					
Yes	1	25.00	3	75.00	0.174
No	3	3.61	80	96.39	
Valve type					
Biological	1	50.00	1	50.00	0.176
Monocusp	3	6.67	42	93.33	
Plasty	6	13.04	40	86.96	
Skin color					
White	7	13.73	44	86.27	0.682
Mixed	3	9.09	30	90.91	
Black	0	0.00	8	100.00	
Running water					
Yes	9	13.04	0	86.96	1.000
No	0	0.00	1	100.00	

Sewage treatment					
Yes	9	15.79	48	84.21	0.193
No	0	0.00	13	13	
Nutritional access route					
Enteral	0	0.00	4	100.00	1.000
Oral	2	2.50	78	97.50	
Water restriction					
Yes	2	2.41	81	97.59	1.000
No	0	0.00	1	100.00	
Water restriction volume					
20 ml/kg	0	0.00	1	100.00	
25 ml/kg	0	0.00	1	100.00	
40 ml/kg	1	1.27	78	98.73	0.095
50 ml/kg	0	0.00	1	100.00	
100 ml/kg	1	100.00	0	0.00	

Fisher's exact test. BT: Blalock-Taussig surgery; kg; kilogram; ml: milliliter; PVS: pulmonary valve stenosis.

logistic regression, applying the backward method, with the removal of the variable with the highest p value at each step, the z score of weight at surgery was the parameter in comparison with the other factors. In step 1, there were 10 factors. During the process, factors with p values without statistical significance were eliminated. These results are displayed in Table 3.

After performing multivariate analysis of independent predicting factors of surgical mortality in step 1, there was evidence that the predicting factors that had significance in the analyzed outcome were: duration of the surgical procedure, previous cyanotic crisis, and postoperative blood lactate, as demonstrated in the multivariate analysis of surgical mortality in the final stage. These results are displayed in Table 4.

Most of the deaths (n = 14, 15%) occurred within the first postoperative month, and the remaining occurred during the follow-up period. Estimated survival analysis was performed using the Kaplan-Meier curve, as displayed in Figure 2.

The best cutoff point for predicting the outcome in relation to serum lactate was found through analysis of the ROC curve of lactate; starting with the value of 2.5 mg/dL, the risk of death increased, as shown in Figure 3.

The best cutoff for predicting the outcome in relation to duration of the surgical procedure was found through analysis of the ROC curve of duration of the surgical procedure; after 220 minutes of procedure, the risk of surgical mortality increased, as shown in Figure 4.

Discussion

The main objective of this study was to discover, among several possible factors, which could be predictors of surgical mortality in children and adolescents undergoing surgery for tetralogy of Fallot, within the context of the institution. In the survey, we found 14 surgical deaths (30 days after surgery) and 1 death (5 months after surgery) during the follow-up period. Among the factors that were relevant for death, the following stand out: duration of the surgical procedure, prior cyanotic crisis, and postoperative blood lactate value.

In relation to the characteristics of the population at the time of surgery, patients in the sample were predominantly White males; the results found were similar to those observed in the literature, which may suggest that tetralogy of Fallot affects the male sex more. In the retrospective review by McCrary et al from 2013, with 133 patients with tetralogy of Fallot, 84 (61%) male patients were found and 76 (57%) were White.[7] In the study by Egbe et al from 2014, which followed 97 patients with tetralogy of Fallot, from January 2001 to

Table 3 – Multivariate analysis of independent predictors of surgical mortality considering the z score of weight at surgery, step 1

Surgical mortality	Odds ratio	Standard error	Z score	95% CI		p value
Z score	2.10	2.09	0.75	0.30	4.80	0.46
Surgical age, years	0.12	0.20	−1.28	0.00	3.07	0.20
Duration of surgery, minutes	1.04	0.03	1.30	0.98	1.10	0.19
Surgeon's exp, 20 surgeries	0.79	2.19	−0.09	0.00	82.35	0.93
Prior cyanotic crisis	7.58	8.78	1.57	0.01	5.41	0.12
Hemoglobin, mg/dL	0.21	0.26	−1.24	0.02	2.45	0.21
Potassium, mg/dL	43.70	140.57	1.17	0.08	239.57	0.24
Lactate, mg/dL	46.41	111.48	1.60	0.42	514.02	0.11
Creatinine, mg/dL	12.92	1.36	1.34	0.00	1.14	0.18
CPB time, minutes	1.01	0.03	0.38	0.95	1.07	0.70

CI: confidence interval; CPB: cardiopulmonary bypass; dL: deciliters; exp: experience; mg: milligrams.

Table 4 – Multivariate analysis of surgical mortality, final step

Surgical mortality	Odds ratio	Standard error	Z score	95% CI		p value
Duration of surgery, minutes	1.02	0.01	2.96	1.01	1.04	0.003
Prior cyanotic crisis	189.91	402.71	2.47	2.98	121.68	0.013
Lactate, mg/dL	3.22	1.45	2.60	1.33	7.78	0.009

CI: confidence interval; dL: deciliters; mg: milligrams.

December 2012, the majority of patients were also male, namely 51 (53%).[8] The median age at the time of surgery was 3.69 (2.13 to 5.79) years; this median can be justified due to the previous Blalock-Taussig surgery that (22) 25.00% of the patients underwent before total correction.

Down syndrome was present in some patients studied. Down syndrome is the most associated with congenital heart disease, and cardiac malformations are present in 40% to 50% of children with trisomy 21. This result was also observed by Egbe et al., in the 2014 study that followed 97 patients with tetralogy of Fallot, during the period from January 2001 to December 2012, with 11 (11%) patients with Down syndrome in their sample. Heredity may play an important role in this heart defect, as children with genetic disorders such as Down syndrome are at increased risk of tetralogy of Fallot.[8]

In a 2003 study by Van Dogen et al.,[4] with 78 patients with tetralogy of Fallot, 6 had Down syndrome. In the 1995 study by Granzotti et al., which included 86 patients with Down syndrome from 1987 to 1991, in the study population, 53% were male; 85% were White, and 3 (7%) had tetralogy of Fallot.[9]

All the patients studied underwent correction surgery at the INC, a result that demonstrates the improvement of the surgical technique currently used in the INC. In the 2008 study carried out by Vohra et al.,[10] analyzing 8 clinical studies with a total of

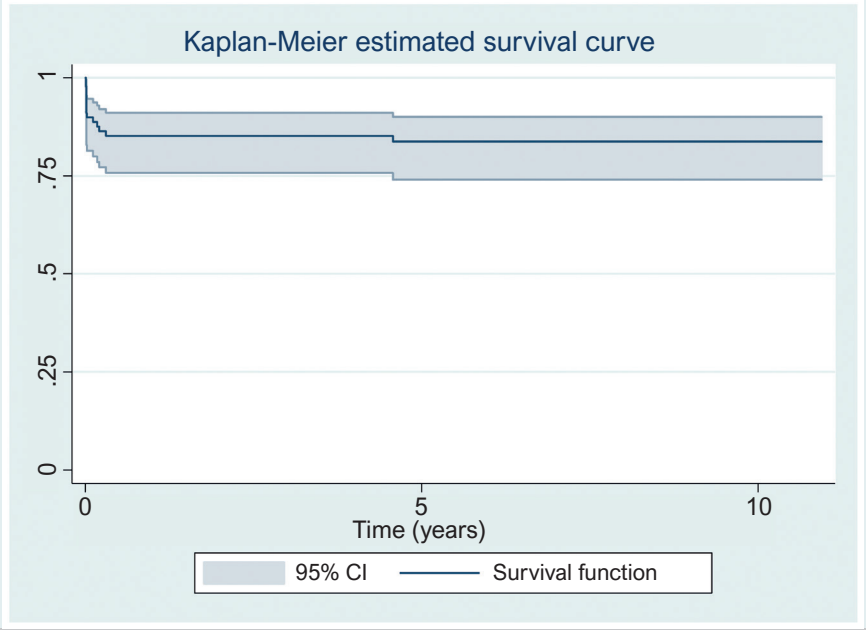

Figure 2 – Estimated survival curve.

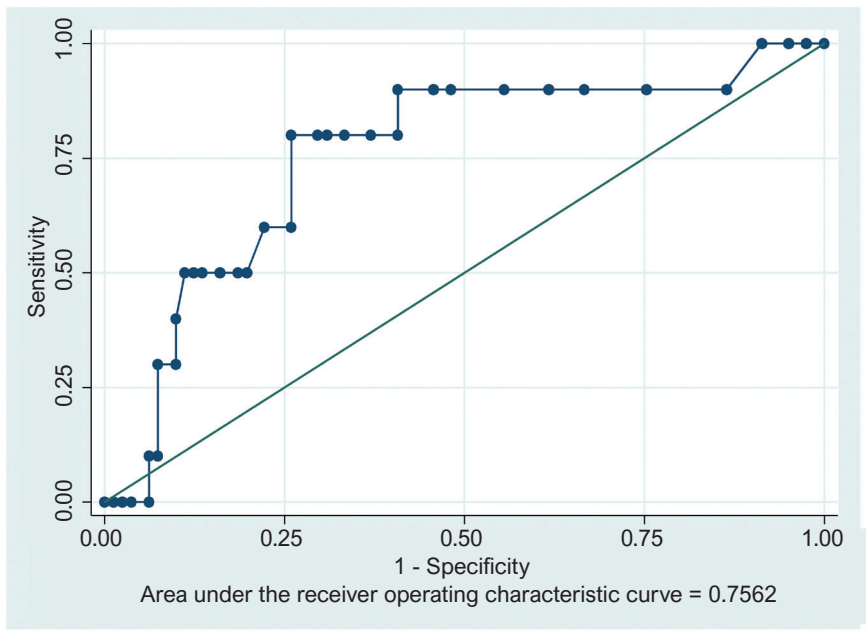

Figure 3 – Receiver operating characteristic curve for lactate.

1,720 patients, total correction surgery was present in all the referred patients. Correction surgery is a priority choice, seeing that it is desirable to minimize pulmonary hypoflow and severe hypoxemia, which result in hypoxemic crises, with sudden worsening of cyanosis, tachypnea, and, in some cases, loss of consciousness, seizures, and even death. Additionally, it prevents the secondary consequences of the palliative procedure, such as progressive obstruction of pulmonary systemic anastomoses, pulmonary artery retraction, left

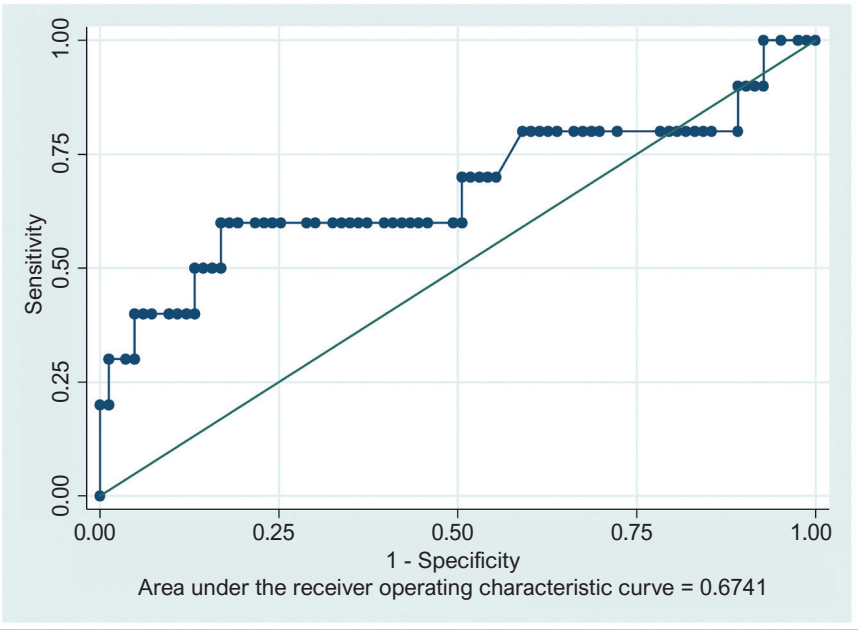

Figure 4 – Receiver operating characteristic curve for duration of surgical procedure.

ventricular volume overload, and even the possibility of developing pulmonary vascular disease, according to Star et al., in 2010.[11]

Cardiopulmonary bypass and aortic clamping were used in total correction surgery for tetralogy of Fallot, with the following results: 109.35 ± 32.54 and 86.78 ± 26.24 minutes, respectively. These variables are directly influenced by the duration of the surgical procedure; the longer the surgical procedure, the longer the cardiopulmonary bypass and aortic clamping times. However, the duration of the surgical procedure is closely related to the anatomy and the difficulty presented by each patient. The result found was different from that of the 2009 study by Silva et al.,[5] who, between April 1986 and December 2007, studied 734 patients with tetralogy of Fallot who underwent total correction surgery, with cardiopulmonary bypass and aortic clamping for 150.2 minutes and 68 minutes, respectively.

Mechanical ventilation time is an important parameter, given that the longer the mechanical ventilation time, the greater the chances of the patient developing respiratory complications such as pneumonia and acute discomfort syndrome. We found the following result: median of 18.00 (8.83 to 31.73) hours. In a study carried out between January 2013 and January 2015 with 200 patients with tetralogy of Fallot, who underwent elective correction surgery, even with shorter mechanical ventilation time (11.19 ± 4.52 minutes), with aortic clamping time of 62.76 ± 4.27 minutes and intensive care unit stay of 48.85 ± 7.26 hours, 11 (5.5%) patients died. This length of stay in the intensive care unit may have contributed as an increment to explain the postsurgical deaths, according to Ladha et al., 2016.[12]

Prior cyanotic crisis was present in most of the patients studied. This is a determining factor for decision making regarding the surgical technique to be used, because uncontrolled hypoxemic crises and deteriorated clinical conditions lead to an immediate approach, given that they can worsen and even lead to the death of the patient. Our result was higher than that found in the 2014 study by Egbe et al.,[8] in which 19 of the 97 patients with tetralogy of Fallot had a prior cyanotic crisis.

In total correction surgery, the monocusp valve was used in 45 (48.39%) of the patients. This prosthesis is widely used due to its surgical safety, with no evidence of increased mortality in patients with this type of valve prosthesis, which is corroborated by the 2010 study by Park et al.,[13] who studied 734 patients with tetralogy of Fallot during the period from April 1986 to December 2007 and concluded that the use of a monocusp patch was not associated with increased

mortality or reoperation. Our result is different from that found in another investigation by Van Dogen et al in 2003,[4] including 78 patients with tetralogy of Fallot underwent total correction surgery, where a monocusp valve was used in 13 (17%) of the patients.

In relation to prior Blalock-Taussig surgery, it was performed in 22 (25.00%) of the patients, and our result was higher than that found in the literature. This technique is still used due to the unfavorable anatomy presented by patients, such as the anatomy of the pulmonary arteries with lack of pulmonary segments, discontinuity, and hypoplasia at any age, making total correction difficult initially, according to Vohra et al., in 2008.[10] Unfavorable anatomy may justify the difference in the result found comparing the 2003 study by Van Dogen et al.,[4] where they followed 78 patients with tetralogy of Fallot, and 3 (4%) patients had undergone prior Blalock-Taussig surgery.

The duration of the surgical procedure was prolonged, seeing that it is a complex procedure that involves cardiopulmonary bypass, which could justify this prolonged time. Another study showed that major surgeries and prolonged time lead to a series of metabolic and hormonal changes in patients, leading to a 50% to 100% increase in metabolism, according to João et al., in 2003.[14]

Increased serum lactate values were observed in the postoperative period. This result has also been described in the literature as a possible increase in mortality, since lactate is a predictor of mortality. A study with 150 patients with tetralogy of Fallot, between the ages of 6 months and 12 years, who underwent total correction surgery, resulting in 139 (92.7%) patients surviving and 11 (7.3%) deaths, showed that lactate was a predictor of mortality regardless of other factors. In the 2017 study by Bhardwaj et al., including 150 patients undergoing surgery for total correction of tetralogy of Fallot, 11 patients died, and the authors concluded that high blood lactate together with oxygen partial pressure are predictors of mortality.[15]

Fasting time (between the preoperative period and the postoperative period) was prolonged; in most cases, an oral diet was initiated with water restriction of 40 ml/kg, which is regularly used in the service and has shown to be efficient in the management of these patients during the postoperative period. Feeding should be started as soon as the patient presents hydro-air sounds, usually after the first 24 hours. In stable cases, when the patient is extubated and lucid, an oral diet should be initiated, according to João et al., in 2003.[14] In other cases, an enteral diet should be started, through the placement of a post-pyloric tube, reducing the volume of liquids infused.

Reoperation occurred in few patients. This result was well described by Vohra et al., in 2008, in the review where they analyzed 8 clinical studies with 1,720 patients, investigating whether surgical outcomes for the correction of tetralogy of Fallot were influenced by the age when primary repair occurred, concluding that it does not increase mortality or reoperation in children < 6 months, also observing a longer stay in intensive care units, requiring ventilatory support and inotropic agents in patients < 3 months of age undergoing total correction.[10]

Regarding the outcomes found in the study sample, 14 surgical deaths were observed. In this study, the surgical deaths may be related to the results found in relation to the level of postoperative serum lactate, duration of the surgical procedure, and prior cyanotic crisis, as these crises can deteriorate the clinical status of the patient. This result differs from the one found in another study from 2015 by Saygi et al., which included 122 patients between January 2010 and November 2013, where there were 9 (7.3%) deaths. This was similar to the findings observed by Kempny et al., 2017 and Pfitzer et al., 2017.[17,18]

Pulmonary insufficiency was present in most patients, in the severe form in a third of the patients, and pulmonary stenosis was observed in a quarter of the patients studied. The follow-up time of the study patients was sufficient to observe these dysfunctions; they are widely described in the literature and are expected consequences of the surgical procedure. Therefore, long-term follow-up of patients is important. Our result is not very different from that found in the 2015 study by Demirpençe et al.,[19] who followed 23 patients undergoing total correction surgery for 5.1 ± 3.5 years and observed that the time between palliative surgery and total correction was 4.3 ± 2.0 years and that pulmonary dysfunctions appeared approximately 6.3 ± 3.0 years after total correction. A 10-year multicenter study evaluating arrhythmia and sudden death in 795 patients who underwent complete correction of tetralogy of Fallot concluded that the lesions that predominantly influenced sudden death were ventricular tachycardia and pulmonary regurgitation. Therefore, the preservation or restoration of pulmonary

valve function reduces the risk of sudden death, as evidenced in the 2000 study by Gatzoulis et al.[20]

According to the research results, the predicting factors with statistical significance identified in this study were: duration of the surgical procedure with p = 0.003; prior cyanotic crisis with p = 0.013; and postoperative blood lactate with p = 0.009.

Every type of monitoring depends on the interpretation applied by the human brain, an ingenious machine that has never been surpassed by technology. Clinical suspicion, however subtle it may be, must be valued and investigated promptly, in order to detect alterations that can be corrected in time. A profound harmony between the pediatric cardiologist, pediatric heart surgeon, perfusionist, anesthesiologist, intensivist, hemodynamicist, echocardiographer, nurse, physiotherapist, and nutritionist is of fundamental importance. Failure to structure and, especially, maintain this team united inexorably leads to errors, or even worse, death.

This study had limitations, mainly due to its retrospective nature, which made it difficult to obtain all the variables initially proposed.

The results found in this study identified possible predicting factors of surgical mortality in children and adolescents undergoing correction surgery for tetralogy of Fallot at the INC. This result is of interest to all institutions that perform this type of surgery, as well as to research related to surgical mortality.

Conclusions

This study thus found evidence that duration of the surgical procedure greater than or equal to 220 minutes, level of blood lactate during the postoperative period greater than or equal to 2.5 mg/dL, and presence of a previous cyanotic crisis contributed to increased mortality; however, patients' nutritional status did not interfere with the final result found. Most of the deaths in the sample were observed to have occurred within the first 6 months after surgery, and starting with the fifth year after the surgical procedure, 95% of the patients developed heart failure, according to survival curve analysis.

Author contributions

Conception and design of the research: Pinheiro PS, Azevedo VMP, Rocha GS. Acquisition of data: Pinheiro PS. Analysis and interpretation of the data: Pinheiro PS, Azevedo VMP, Rocha GS. Statistical analysis: Azeredo VMP. Writing of the manuscript: Pinheiro PS. Critical revision of the manuscript for intellectual content: Pinheiro PS, Azevedo VMP, Rocha GS.

Potential Conflict of Interest

No pontential conflict of interest relevant to this article was reported.

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Study Association

This article is part of the thesis of master submitted by Gabrielle de Souza Rocha, from Instituto Nacional de Cardiologia, Universidade Federal Fluminense.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee on Animal Experiments of the Instituto Nacional de Cardiologia (INC) under the protocol number 53654836.8.0000.5272.

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