ISSN 2782-9510

PEDIATRIC INFECTIOUS DISEASE SOCIETY OF THE PHILIPPINES

PIDSP JOURNAL

Vol. 24 No. 2
July – December 2023

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 2 July-December 2023
Dy-Co, AS. Aye, AI Captain!
https://doi.org/10.56964/pidspj20232402001

EDITORIAL

Arlene S. Dy-Co, MD, FPPS, FPIDSP
Editor-in-Chief, PIDSP Journal

Correspondence:
Dr. Arlene S. Dy-Co
Email: pidsp2009@yahoo.com

Aye, **AI** Captain!

Before we say aye to an innovation whether in healthcare or other fields it is prudent to evaluate its merits and perils. The rapid rise of artificial intelligence (AI) has created both a growing excitement and cautious wary amongst many in the medical and research fields. And this includes the field of medical publishing. AI is being used today across different fields, also known as machine intelligence, it focuses on building and managing technology that can learn to autonomously make decisions and carry out actions on behalf of a person. As the technology has become more embedded in everyday applications, the interest focused on seeking to emulate the human brain through the design of programs and algorithms with real-time processing.

Much debate about AI's potential to revolutionize is countered with significant concerns. There are many areas in which AI is valuable in publishing from simple formatting, checking of grammar to the more complex like-text auto-tagging to improve discoverability of research and much more. It can improve and speed up many processes in the editorial workflow. Some of its potential drawbacks include concerns about privacy and data security, loss of personal touch and limited emotional connection, bias and possibly discriminatory algorithms and ethical concerns. Such that the International Committee of Medical Journal Editors (ICMJE) updated the criteria for authorship in the advent of AI. It states that chatbots should not be listed as authors because they cannot be responsible for the accuracy, integrity, and originality of a work, and these responsibilities are required for authorship. Authors should not list AI and AI-assisted technologies as an author or co-author, nor cite AI as an author. Further, ICMJE issued that journals should require authors to disclose whether they used artificial intelligence (AI)-assisted technologies in the production of submitted work. Authors who use such technology should describe, in both the cover letter and the submitted work, how they used it.

While AI surely has an enormous potential for great advances much of their power comes from their ability to outperform human abilities in terms of speed and accuracy. I believe that AI's existence in the medical publishing field is unavoidable. It is poised to have a dramatic influence in the way we share new discoveries, health advances and findings in research and innovations. At its best, it can spread landmark breakthroughs instantaneously but this makes accountability very critical to its application. I hope that measures will be employed to ensure that humans run the system—not the other way around.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

ORIGINAL ARTICLE

THE CORONAVIRUS DISEASE 2019 (COVID-19) IMMUNOGLOBULIN (IgG) LEVELS USING CHEMILUMINESCENCE IMMUNOASSAY (CLIA) ANTI-S-RBD TEST IN TERM NEONATES BORN TO COVID-19 FULLY VACCINATED MOTHERS

Ray C. Mendoza II, MD, Belle M. Ranile, MD, FPPS, FPIDSP, Nathalie Anne R. Hernaez, MD, FPPS, FPSNbM
Cebu Doctors' University Hospital
1ST PLACE 2023 PIDSP RESEARCH CONTEST

ABSTRACT

Background: Though protective levels of neonatal SARS-CoV2 IgG still warrant further studies, maternal antibodies from COVID-19 vaccination may be the key to neonatal protection against COVID-19 related complications. This study aimed to correlate SARS-CoV2 IgG titers of term newborns delivered to fully vaccinated/boosted mothers with the time of dose completion to delivery and the type of COVID-19 vaccine received by the mothers.

Methodology: A single center prospective cohort study that utilized CLIA Anti-S-RBD IgG determination in cord blood was done. Kruskal-Wallis and Mann-Whitney U Test were used to determine significant differences between IgG titers from vaccine types and groups as to trimester when COVID-19 dose was completed. Spearman's rank was used to determine the correlation between IgG levels and interval of dose completion to delivery.

Results: All 177 newborns enrolled in the study had reactive results ($\geq$ 1 AU/ml) regardless of vaccine type received and trimester of maternal vaccination completion. The highest titers recorded per group was 19,340 AU/ml from the booster group and 5,960 AU/ml from the primary series group. The mRNA vaccinated group exhibited higher titers compared to other vaccine types regardless of the trimester completion for both groups.

Conclusions: A significant difference between IgG levels showed that higher titers were noted in the booster group compared to the primary series group across all trimesters. There was also a significant correlation between titer levels and time of dose completion to delivery with higher titers associated with more recent dose completion for both groups.

KEYWORDS: *SARS-CoV2 IgG S-RBD, COVID-19 Vaccine, Neonates*

Correspondence:
Dr. Ray C. Mendoza II
Email: raimen3173@gmail.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

INTRODUCTION

The COVID-19 pandemic has already affected 452 million people globally with 3.6 million cases from the Philippines and counting as recorded by the World Health Organization (WHO). The increasing cases accounts for over 6 million COVID-19 related deaths with pregnant women having the greatest risk for severe to critical symptoms.[1,2,3,4] A systematic review and meta-analysis involving 67, 271 pregnant women concluded that their odds of intensive care unit admissions were significantly increased compared to the general reproductive age population. The risk increases in the presence of maternal co-morbidities with pre-eclampsia as the most common cause of morbidity and mortality.[5] Locally, a cross-sectional study conducted in Philippine General Hospital showed that maternal mortality rates due to severe COVID-19 was at 1.91 per 100 cases.[6]

Due to the increasing number of severe-critical COVID-19 among pregnant women, an emergency use authorization of COVID-19 vaccines was issued by WHO and was supported by international and local obstetrical societies. This emphasized the importance of vaccination during pregnancy amidst the pandemic.[7,8,9] These were the vaccine types approved for pregnant and lactating mothers: Messenger RNA (mRNA), inactivated, and viral vector vaccines.[10] Aside from maternal protection, this strong recommendation was due to several reports of symptomatic newborns of COVID-19 unvaccinated and infected mothers. It was noted that transplacental transmission of SARS-CoV2 was due to high levels of maternal viremia.[11] Multisystem Inflammatory Syndrome in Neonates (MIS-N), another entity of concern, is a post-infectious complication of COVID-19 infection. The passive transmission of maternal post-infectious antibodies was theorized to be one of the pathogenesis of MIS-N.[12] Thus studies on the vertical transfer of SARS-CoV2 IgG after maternal vaccination and the measurement of its levels using cord blood samples were conducted to emphasize the importance of maternal vaccination. Transplacental ratio studies between the newborns and mothers' antibodies showed direct correlation when tested.[13]

This study aimed to determine the correlation between the SARS-CoV2 Anti-S-RBD IgG levels in cord blood of term neonates and the time of maternal COVID-19 vaccine primary series or booster completion from delivery of mothers without history of COVID-19. This study also determined the neonate's demographic profile, mother's vaccination profile, and the difference between the median serum IgG levels across each trimester and vaccine type received.

MATERIALS AND METHODS

Study Design and Subjects

This was a single center prospective cohort study on term neonates born to COVID-19 fully vaccinated or boosted mothers who delivered in a tertiary hospital from July to September 2022. To avoid discrepancies in the IgG titers, mothers with documented or prior history of COVID-19 infection during the course of pregnancy were excluded. To avoid delays in the neonatal resuscitation, newborns with poor APGAR and those who experienced adverse events during the perinatal period such as, asphyxiation or Intrauterine Fetal Demise (IUFD) were also excluded. Hemolyzed specimens that could not be read by the machine, or untoward events during the intrapartum course (e.g., umbilical cord detached from placenta, placenta adhered to the uterus) causing insufficient cord blood samples were considered dropouts.

Definition of Terms

- **Fully Vaccinated Mother:** A mother who has completed the recommended course of COVID-19 vaccination at least 2 weeks from delivery.
- **COVID-19 Booster:** an additional COVID-19 vaccine dose after completing the initial full course vaccination.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

- **Term Newborn**: A newborn who is ≥ 37 weeks age of gestation by Ballard scoring.
- **Reactive CLIA Anti-S-RBD IgG:** Defined as a value of ≥ 1 arbitrary unit per ml (AU/ml)
- **Documented COVID-19 Maternal Infection:** A SARS-CoV2 RT-PCR and/or Rapid Antigen Test (RAT) positive (+) result during pregnancy either for screening purposes or if the mother developed symptoms.

Sample Size and Sampling

A 95% confidence interval was estimated to quantify the difference in mean serum COVID-19 IgG levels. The researcher set the margin of error to be no more than 50 AU/ml. The sample size computation was based on the standard deviation (SD) of a previous study by Kashani-Ligumsky, L., et. al. which had a mean serum IgG level of 224.7 U/ml and a standard deviation of 64.3.[14]

In this study, a power of 80% was considered with α=0.05 (1-0.95), then z=1.96. Given these, the study needed at least 27 IgG titer readings per group for the following sets of comparisons: mothers who completed their vaccination and had booster in the 1st, 2nd, and 3rd trimester to have an actual power of 80.06%. The comparisons were done per trimester to minimize the effect of time as a confounding variable. Therefore, a total of 162 neonates were needed in this study obtained by quota sampling for the study to be 95% confident with an estimate within 50 units of the true mean serum SARS-CoV2 IgG levels in AU/ml, and considering a power of 80% for every comparison to be done.

Data Collection

Once an expectant mother arrived at the labor room or emergency room, the researcher or his co-investigator interviewed and reviewed the mother's prenatal and vaccination history for screening and were documented in the data collection sheet. The mother's vaccine/booster card was requested to validate the vaccination status. If a mother met the criteria, the research protocol and objectives of the study were then explained, and consent was obtained. The attending obstetrician and pediatrician were informed of the study and the mother's decision. In the event the mother was unable to consent for certain reasons (i.e. in active labor/labor pains, exhaustion), the husband/partner or legal guardian of the newborn signed the consent in her stead.

The SARS-CoV2 S-RBD CLIA IgG levels were taken immediately after delivery of the placenta through cord blood samples (4-5 ml) from the umbilical vein obtained aseptically by the researcher and placed in a red-top tube. The samples were sent to the medical technologist on duty for centrifugation while another trained medical technologist ran the test. For optimal results, the specimens should be free of fibrin, red blood cells, or other contaminants that may cause inconsistent results. The samples were tested using MAGLUMI® CLIA SARS-CoV2 S-RBD IgG test which has a clinical specificity of 99.6% and clinical sensitivity of 100%. This assay uses an indirect CLIA for the quantitative determination of IgG antibodies to SARS-CoV2 and utilizes the MAGLUMI® fully-auto CLIA analyzer. An IgG titer of ≥ 1 AU/ml was considered reactive for this study. The sample is mixed along with the buffer and magnetic microbeads coated with S-RBD recombinant antigen, then incubated to form immune-complexes. After precipitation, the sample undergoes several wash cycles and treatment until a chemiluminescent reaction occurs. This reaction was measured using a photomultiplier wherein the light signal was measured as relative light units (RLUs) which was proportional to the concentration of S-RBD IgG in the sample. Manual errors in handling and labeling the samples were avoided by using the provided barcode labels attached on the test tubes. Quality control (negative and positive) was done to achieve satisfactory levels based on acceptable analyte values within the laboratory's control range. If ever these values do not fall within the range, a repeat measurement was done and if ever the repeat still did not fall within the acceptable range the results were not reported and the following troubleshoot methods were done: the

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

material's expiry date was checked, maintenance was performed, if test kit instructions were followed, rerun the assay with fresh quality specimens, or to contact technical support or distributor for assistance.

After processing, the samples were discarded to the infectious waste disposal as per institutional protocol. The mother's type of vaccine, months from dose completion to delivery, newborns' demographics, SARS-CoV2 IgG S-RBD result, and interpretation were then entered in the data collection sheet.

Data Analysis and Statistical Considerations

Descriptive statistics such as mean, standard deviation (SD), median, interquartile range (Q1-Q3), minimum and maximum values were reported to describe the numerical variables under clinical profile of neonates. The same were used to present the following: serum SARS-CoV2 IgG levels of term neonates in AU/ml; and time in months from vaccination completion or booster to delivery. Frequency distribution and percentage were used to present the categorical variables with frequency counts under vaccination profile of mothers. Mann-Whitney U Test was used to determine if there was a significant difference between two groups in terms of serum SARS-CoV2 IgG levels of term neonates in AU/ml. Kruskal-Wallis Test was used to determine if there was a significant difference among 3 groups in terms of serum SARS-CoV2 IgG levels of term neonates in AU/ml. Spearman's Rank Correlation was used to determine if there was a significant correlation between SARS-CoV2 IgG levels in AU/ml and time in months from vaccination completion to delivery; and also between IgG levels and time in months from booster to delivery.

For all tests, a confidence interval was set at 95%, comparison and association significance at <0.05, all hypotheses were tested at 0.05 level of significance.

Ethical Issues

This study was approved by the research technical committee and the institutional ethics review committee of Cebu Doctors' University Hospital, Protocol Code: 2-2022-011. Written informed consents were obtained from the mothers prior to enrollment of the newborns. Letter of request for sponsorship was sent to Mr. Artis L. Pinote, the CEO of LabSolutions Technologies, Inc. and Distributor of SNIBE MAGLUMI® SARS-CoV2 CLIA S-RBD IgG who provided the test kits used in this study. The researcher shouldered the cost of processing and storage of the samples. The author and co-authors declared that they had no conflicts of interests in the conduct of this study.

Data Privacy

All personal information of the subjects were held confidential in accordance to the Data Privacy Act of 2012. Only the researcher and the co-authors knew the identity of the subjects and were able to review the data. All documents containing the subjects' data were safely secured in a locker accessible only to the researcher and all the data collected were used for the sole purpose of this study. After the study was finalized and all data were encoded, tallied, and treated, all documents with the subjects' personal data were discarded and shredded.

RESULTS

During the study period, a total of 260 newborns (3 sets of twins) were born to 257 mothers. From the 260 newborns, 57 were excluded from the study due to the following exclusion criteria: Prematurity (21), mothers with documented history of COVID-19 infection (16), mother's RT-PCR was positive on admission (13), Intrauterine fetal demise (3), incomplete maternal COVID-19 vaccination (2), and unvaccinated mothers (2). From the remaining 203 eligible newborns, 26 were considered drop-outs due to the following reasons: eligible but group's sample size quota was reached (19), hemolyzed specimens (3), umbilical cord detached from placenta (2), and

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

placenta adhered to the uterus (2); ending with 177 subjects in the study. All eligible mothers enrolled in this study signed the informed consent and were informed of their respective results. The 177 newborns delivered had a mean Ballard score of 38.07 weeks AOG, 102 (42.37%) were male and 75 (57.63%) were female.

Table 1. Vaccination profile of mothers

Mothers' Vaccination Profile	N=176		
	Mean (SD)	Median (Q1-Q3)	Min-Max
Time *in months* from primary series completion to delivery	9.47 (4.12)	10.00 (7-12)	1-17
Time *in months* from booster to delivery	5.23 (2.99)	5.00 (3-8)	0.60-13
	No.	**%**	
Completed COVID-19 Vaccination			
Without booster	92	52.27	
With booster	84	47.73	
COVID-19 Primary Series Completion			
Prior to pregnancy	22	12.5	
At 1st trimester	29	16.48	
At 2nd trimester	14	7.95	
At 3rd trimester	27	15.34	
COVID-19 Booster Completion			
At 1st trimester	27	15.34	
At 2nd trimester	30	17.05	
At 3rd trimester	27	15.34	
Type of COVID-19 vaccine			
Inactivated	64	36.36	
mRNA	68	38.64	
Viral Vector	44	25	

Table 1 presents the mothers' vaccination profile, trimester of dose completion, and distribution of vaccine types. On average, the time in months from primary series completion to delivery was at 9.47 months (SD 4.12) and 5.23 months (SD 2.99) for the booster group. There were 176 eligible mothers (1 mother of term twins), 92 (52.27%) had only completed the primary series while 84 (47.73%) mothers received booster shots. Only the primary series completed during 2nd trimester sub-group was not able to fulfill the minimum required quota of 27 samples (14, 7.95%). Because of this, a non-parametric test was used utilizing the median of the IgG titers. The distribution of vaccine type amongst the mothers shows that 38.64% received the mRNA vaccine, 36.36% with inactivated vaccine, and 25% received the viral vector vaccine.

Table 2. Serum SARS-CoV2 IgG levels of neonates using CLIA Anti-S-RBD test in AU/ml

Neonates' COVID-19 IgG Titer	N=177		
	Mean (SD)	Median (Q1-Q3)	Min-Max
Serum IgG *in AU/ml* for ALL neonates	763 (1705)	467 (195-794)	5.4 – 19,340
Serum IgG *in AU/ml* for neonates of primary series group	398.7 (639.9)	277.9 (89-503.5)	5.4 – 5,690
Serum IgG *in AU/ml* for neonates of booster group	1,167 (2323)	731 (450-971)	100 – 19,340
Qualitative Assessment	**N**	**%**	
Reactive CLIA Anti S-RBD IgG	177	100.00	
Non-Reactive CLIA Anti S-RBD IgG	00	00.00	

Table 2 presents the neonates' IgG levels for both primary series and booster group. All 177 cord blood samples showed reactive results with the highest recorded from the primary series group at 5,690 AU/ml and 19,340 AU/ml from the booster group.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

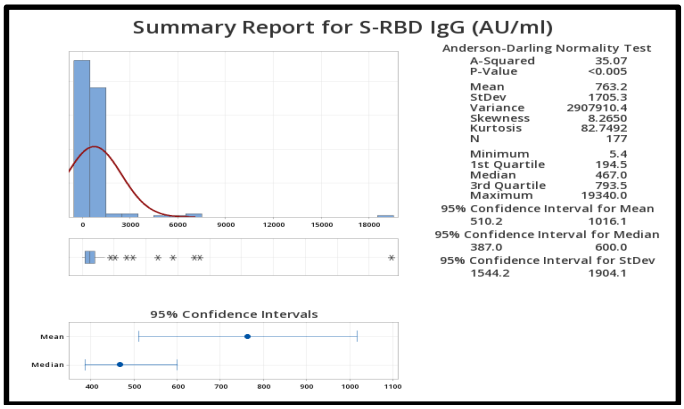

Figure 1. Serum SARS-CoV2 IgG levels of neonates in AU/ml

Figure 1 shows the population distribution in terms of IgG levels which shows an asymmetrical distribution and a mean IgG of 763.2 AU/ml. A standard deviation of 1,705 AU/ml, tells us how spread out the data are from the mean and that the values vary widely. About 25% of the neonate's titer readings were less than or equal to 194.5 (Q1). Median titer is 467 AU/ml which means that half of the sampled neonates had IgG titers below this value. About 25% of the IgG titers in the sampled population are greater than 793.5 AU/ml, i.e., 75% of the neonates considered in this study had titers of 793.5 and below (Q3).

Table 3. Comparison of serum SARS-CoV2 IgG levels of neonates depending on maternal vaccination

	Computed Values			
Trimester Completed	**Primary Series**	**w/ Booster**	**p***	**Interpretation**
First trimester	N=29, η_1=147.7	N=24, η_2=540.0	<0.001	**Significant difference**
Second trimester	N=14, η_1=302	N=30, η_2=726	<0.001	**Significant difference**
Third trimester	N=27, η_1=690	N=27, η_2=991	0.004	**Significant difference**
All Titers, regardless of time	N=93, η_1=277.9	N=84, η_2=730.5	<0.001	**Significant difference**

Vaccine Type	**Inactivated**	**mRNA**	**viral vector**	**P****	**Interpretation**
Vaccine Type, regardless of booster and time	N=65, η_1=332	N=68, η_2=653	N=44, η_3=357	0. 001	**Significant difference**

**Median values compared; Comparison done with Mann-Whitney U Test; significant at <0.05*
***Median values compared; Comparison done with Kruskal-Wallis Test; significant at <0.05*

Table 3 presents the results comparing the average serum IgG levels between mothers who completed the primary series and those who got booster shots. All p-values were <0.05, the differences between the median IgG levels of each population were all statistically significant. The average IgG levels of neonates from the booster group were significantly higher regardless of trimester (730.5 vs 277.9 AU/ml; p-value <0.001). This table also presents the comparison between the average serum IgG levels across the three vaccine types. With p-value of 0.001, the differences between the median IgG levels of each population across all groups was statistically significant. The average IgG level is significantly higher among mothers who received mRNA vaccines.

Table 4. Relationship between serum COVID-19 IgG levels (AU/ml) and time (months) from vaccination to delivery

IgG levels (AU/ml) and time (months)	**Correlation coefficient ρ (95% CI for ρ)**	**p-Value ***	**Interpretation**
Completed Vaccination w/o Booster	-0.627 (-0.745, -0.470)	<0.001	**Significant relationship**
Completed Vaccination w/ Booster	-0.487 (-0.642, -0.292)	<0.001	**Significant relationship**

**Relationship tested using Spearman's Rank Correlation; significant at <0.05*

Table 4 presents the relationship between SARS-CoV2 IgG levels (AU/ml) and time in months from primary series or booster completion to delivery. Since the p-values for both groups were <0.001, the association between time from dose completion to delivery and the IgG levels were statistically significant. Which means a higher IgG titer was associated with more recent vaccinations with respect to the date of delivery. It must be noted however that this correlation does not imply a causal relationship (cause and effect) between the two measurements. This relationship is presented in the

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 3-12 July-December 2023
Mendoza RC, Ranile BM, Hernaez NAR. The Coronavirus Disease 2019 (COVID-19) Immunoglobulin (IgG) Levels Using Chemiluminescence Immunoassay (CLIA) Anti-S-RBD Test in Term Neonates Born to COVID-19 Fully Vaccinated Mothers.
https://doi.org/10.56964/pidspj20232402002

following matrix plots for the primary series group (Figure 2) and booster group (Figure 3).

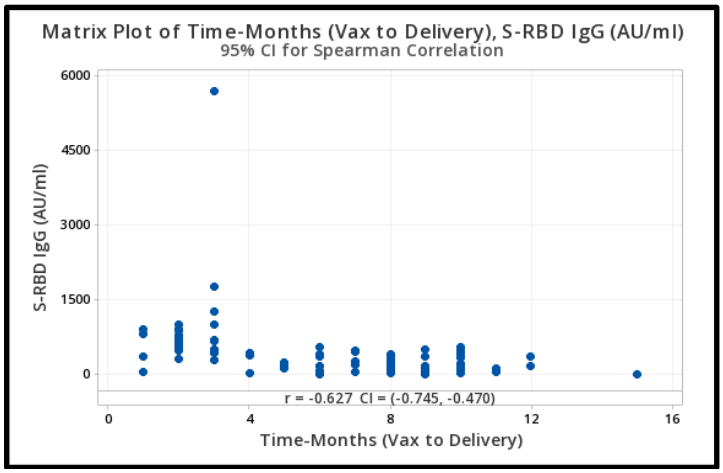

Figure 2. SARS-CoV2 IgG levels (AU/ml) and time (months) from primary series completion to delivery

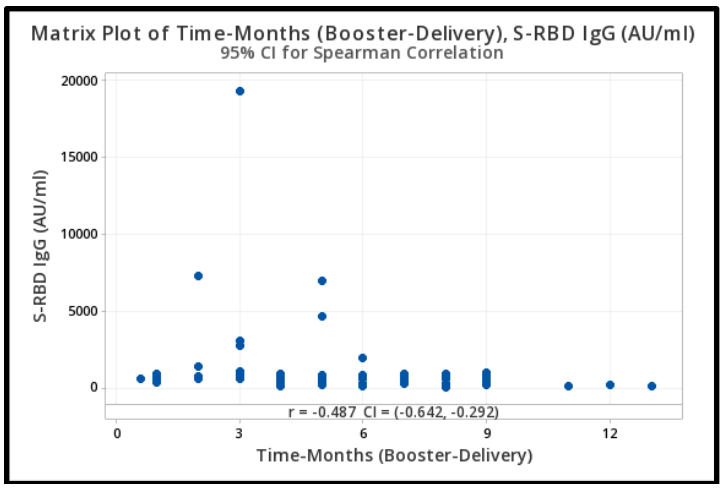

Figure 3. SARS-CoV2 IgG levels (AU/ml) and time (months) from booster completion to delivery

DISCUSSION

Until now, the COVID-19 pandemic still has its infectious and post-infectious effects on the pediatric population even after the roll out of vaccinations for age 5 years old and above implying that the younger and more vulnerable population remain at risk.[15] Cardiovascular manifestations such as arrhythmias, AV blocks to severe complications such as cardiac dysfunction leading to shock and death were documented in a case series conducted on newborns of unvaccinated mothers with maternal COVID-19 infection during the course of pregnancy.[12] Vaccination has proven to have been the solution to lessen both maternal and neonatal morbidity and mortality. As of March 2023, 79 million Filipinos had already completed the primary series while only 24 million received booster shots.[16]

The protective antibody level of SARS-CoV2 has not yet been fully established; However, benefits of maternal COVID-19 vaccination on newborns delivered to vaccinated mothers showed that it decreases the risk of hospital admissions. This was evident in the case-control test negative study by Halasa et. al., which assessed the effectiveness of maternal COVID-19 mRNA vaccination against hospitalization and their infants younger than 6 months old. The study showed that overall effectiveness of maternal COVID-19 vaccination was at 52% as it reduced the risk for hospitalization (COVID or non-COVID related admissions) by 80%. There was also a decrease in pediatric ICU admissions by 70% for COVID-19 related cases and 47% in non-COVID-19 cases. Overall, the study concluded that complete maternal COVID-19 vaccination was associated with a reduced risk of hospitalization for COVID-19 and critical illness among infants younger than 6 months.[17]

This single center prospective cohort study has demonstrated that maternal antibodies to SARS-CoV2 were passed on to the fetus as exhibited by a reactive result on all cord blood samples, regardless of which trimester vaccination was completed or which type of vaccine or booster was given. The results of this study were in congruent with the prospective cohort study by Sourouni et. al. that showed a 100% reactivity to SARS-CoV2 IgG antibodies from cord blood samples of mRNA vaccinated mothers.[18] In another similar prospective cohort study by Kugelman et. al., which involved newborns of both vaccinated and boosted mothers during the third trimester, the results were similar to this study as the booster group had significantly higher antibody titers. The mothers in their study were presumed to have no prior infection to COVID-

19 based on their clinical history alone, like our study. One limitation to their study was the test kits used also detects antibodies to nucleocapsid indicating that natural infection despite being asymptomatic may have altered some of their results.[19]

In a large observational study by Lo Sasso et. al, they determined the SARS-CoV2 IgG Anti-S-RBD titers of the following groups: mRNA vaccinated without prior COVID-19 infection, COVID-19 recovered, and COVID-19 recovered with vaccination. Those in the COVID-19 recovered group had the lowest antibody titer, with the highest belonging to the vaccinated without prior infection group indicating the S-RBD's specificity to antibodies after vaccination.[20] The significance of anti-spike protein S1 and S2 (anti-S) after vaccination, particularly the receptor binding domain (RBD) was identified to be responsible in eliciting robust immune response while on the other hand elevated levels of anti-nucleocapsid (Anti-N) were prominent in patients with prior infection.[21,22] In this study, the test kits used were the MAGLUMI® CLIA SARS-CoV2 S-RBD IgG which has a clinical specificity of 99.6% and sensitivity of 100%.

Compared to other types, mRNA vaccines exhibited higher antibody levels in this study. This finding was supported by Lau et. al.'s comparative study on the kinetics of neutralizing and SARS-CoV2 antibodies after inactivated and mRNA vaccination. Lau's study concluded that mRNA vaccines showed significant antibody response compared to the inactivated vaccines.[23] This study also showed the significant relationship between the time of vaccine completion or booster from the time of delivery. A shorter time in months (1-2 months) from vaccine or booster completion to the time of delivery wherein peak SARS-CoV2 IgG titers achieved was observed. This is congruent with the longitudinal study by Naaber et. al., in which peak SARS-CoV2 IgG Anti-S-RBD titers were noted at 1 week and 6 weeks after the 2nd dose of mRNA vaccination. It was good to note that antibodies in their study, declined and reached a nadir of 2-25% (median of 7%) from peak levels by 6 months post-vaccination, indicating the importance of booster vaccination to augment antibody titers.[24] This was supported by Shook et. al who concluded that majority of the newborns delivered to vaccinated mothers had higher and more persistent anti-S antibodies even at six months old. [25]

This study emphasizes the significant effect of booster vaccines on the antibody titers. Furthermore, all mothers enrolled in this study had no documented or known history of COVID-19. This study has several limitations. It was conducted as a single center study only in a private institution and may not represent the local population. Screening of mothers for previous history of COVID-19 was based on history and retrieval of previous positive RT-PCR or RAT results. Therefore, some mothers with mild symptoms or those who previously had asymptomatic course may have been enrolled to the study possibly affecting some of the results. This study utilized a quota sampling method and during the study period, one group's quota was not reached (primary series 2nd trimester) thus utilizing the median of each IgG in non-parametric testing was used instead of the parametric tests.

CONCLUSION

The study showed that all samples were reactive to SARS-CoV2 IgG. The average IgG levels of neonates of the booster group were significantly higher compared to the primary series group regardless of which trimester it was completed. In addition, significantly higher titers of SARS-CoV2 IgG were noted among neonates whose mothers were vaccinated with mRNA vaccines. Lastly, there was a significant correlation between IgG titer levels of neonates and time from vaccination/booster completion to delivery with higher titers associated in more recent dose completion with respect to time of delivery.

RECOMMENDATIONS

Future research with a multi-center design involving government hospitals to get a better representation of the local population is recommended. Another recommendation is to compare the IgG titers of

newborns born to mothers who are fully vaccinated without prior history of COVID-19 infection, COVID-19 recovered mothers or with prior history, and COVID-19 recovered with vaccination to assess which group has the best antibody response. A longitudinal study on the newborns in this study to reassess their IgG titers and status or condition at 3 and 6 months old would have been ideal.

ACKNOWLEDGEMENTS

To Mr. Alex Phil Ponce, for his contribution in the statistical analysis of this study.

To Dr. Samuel Yu, Chairman – Department of Pediatrics, for allowing the researcher to conduct this study.

To Dr. Ma. Asuncion Tremedal, Chairman – Department of OB-GYN and the Obstetrics and Gynecology Consultants, for allowing the researcher to interview and extract cord blood samples on their patients.

To Dr. Alma Malilong, Chairman – Department of Pathology and Mrs. Marmi Sesaldo, Chief Medical Technologist - CDUH, for allowing the researcher to utilize their equipment, for the processing and storage of the specimens.

To Mr. Artis Pinote, CEO of LabSolutions Technologies, Inc. and Distributor of SNIBE MAGLUMI® SARS-COV2 S-RBD IgG, for graciously providing the test kits used in this study.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. WHO Coronavirus (COVID-19) Dashboard [Internet]. Covid19.who.int. 2022 [cited 13 March 2022]. Available from: https://covid19.who.int/
2. COVID-19 Tracker | Department of Health website [Internet]. Doh.gov.ph. 2022 [cited 13 March 2022]. Available from: https://doh.gov.ph/covid19tracker
3. Keni R, Alexander A, Nayak PG, Mudgal J, Nandakumar K. Covid-19: Emergence, spread, possible treatments, and global burden. Frontiers in Public Health. 2020;8.
4. Evidence used to update the list of underlying medical conditions that increase a person's risk of severe illness from COVID-19 [Internet]. Stacks.cdc.gov. 2022 [cited 19 March 2022]. Available from: https://stacks.cdc.gov/view/cdc/89840
5. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ. 2020;:m3320.
6. Clemente M, Amosco M, Octavio M, Bravo S, Villanueva-Uy E. Maternal and Neonatal Outcomes of Pregnant Women with Clinically Confirmed COVID-19 Admitted at the Philippine General Hospital. Acta Medica Philippina. 2021;55(2).
7. Adhikari, E. and Spong, C., 2021. COVID-19 Vaccination in Pregnant and Lactating Women. *JAMA*, 325(11), p.1039.
8. COVID-19 Vaccination Considerations for Obstetric–Gynecologic Care [Internet]. Acog.org. 2021 [cited 18 August 2021]. Available from: https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2020/12/covid-19-vaccination-considerations-for-obstetric-gynecologic-care
9. COVID-19 vaccines, pregnancy and breastfeeding [Internet]. Royal College of Obstetricians & Gynaecologists. 2022 [cited 18 January 2022]. Available from: https://www.rcog.org.uk/en/guidelines-research-services/coronavirus-covid-19-pregnancy-and-womens-health/covid-19-vaccines-and-pregnancy/covid-19-vaccines-pregnancy-and-breastfeeding/
10. List of FDA issued Emergency Use Authorization - Food and Drug Administration [Internet]. Food and Drug Administration -. 2021 [cited 18 August 2021]. Available from: https://www.fda.gov.ph/list-of-fda-issued-emergency-use-authorization/
11. Vivanti A, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J et al. Transplacental transmission of SARS-CoV-2 infection. Nature Communications. 2020;11(1).
12. Pawar R, Gavade V, Patil N, Mali V, Girwalkar A, Tarkasband V et al. Neonatal Multisystem Inflammatory Syndrome (MIS-N) Associated with Prenatal Maternal SARS-CoV-2: A Case Series. Children. 2021;8(7):572.
13. Flannery D, Gouma S, Dhudasia M, Mukhopadhyay S, Pfeifer M, Woodford E et al. Assessment of Maternal and Neonatal Cord Blood SARS-CoV-2 Antibodies and Placental Transfer Ratios. JAMA Pediatrics. 2021;175(6):594.
14. Kashani-Ligumsky L, Lopian M, Cohen R, Senderovich H, Czeiger S, Halperin A et al. Titers of SARS CoV-2 antibodies in cord blood of neonates whose mothers contracted SARS CoV-2 (COVID-19) during pregnancy and in those whose mothers were vaccinated with mRNA to SARS CoV-2 during pregnancy. Journal of Perinatology. 2021;41(11):2621-2624.
15. Pediatric Vaccination for the prevention of COVID-19 for 5 to 11 age group | HTA [Internet]. Hta.doh.gov.ph. 2022

[cited 26 April 2022]. Available from: https://hta.doh.gov.ph/2022/02/11/pediatric-vaccination-for-the-prevention-of-covid-19-for-5-to-11-age-group/

16. National COVID-19 vaccination dashboard [Internet]. Gov.ph. [cited 2023 Mar 21]. Available from: https://doh.gov.ph/covid19-vaccination-dashboard
17. Halasa NB, Olson SM, Staat MA, Newhams MM, Price AM, Pannaraj PS, et al. Maternal vaccination and risk of hospitalization for Covid-19 among infants. N Engl J Med [Internet]. 2022;387(2):109–19. Available from: http://dx.doi.org/10.1056/NEJMoa2204399
18. Sourouni M, Braun J, Oelmeier K, Möllers M, Willy D, Hennies MT, et al. Assessment of neonatal cord blood SARS-COV-2 antibodies after COVID-19 vaccination in pregnancy: A prospective cohort study. Geburtshilfe und Frauenheilkunde. 2022;82(05):510–6.
19. Kugelman N, Nahshon C, Shaked-Mishan P, Cohen N, Lahav Sher M, Barsha H, et al. Third trimester messenger RNA COVID-19 booster vaccination upsurge maternal and neonatal SARS-CoV-2 immunoglobulin G antibody levels at birth. Eur J Obstet Gynecol Reprod Biol [Internet]. 2022;274:148–54. Available from: http://dx.doi.org/10.1016/j.ejogrb.2022.05.029
20. Lo Sasso B, Giglio RV, Vidali M, Scazzone C, Bivona G, Gambino CM, et al. Evaluation of anti-SARS-CoV-2 S-RBD IgG antibodies after COVID-19 mRNA BNT162b2 vaccine. Diagnostics (Basel) [Internet]. 2021;11(7):1135. Available from: http://dx.doi.org/10.3390/diagnostics11071135
21. Barin B, Kasap U, Selçuk F, Volkan E, Uluçkan Ö. Comparison of SARS-CoV-2 anti-spike receptor binding domain IgG antibody responses after CoronaVac, BNT162b2, ChAdOx1 COVID-19 vaccines, and a single booster dose: a prospective, longitudinal population-based study. The Lancet Microbe. 2022;.
22. Van Elslande J, Oyaert M, Ailliet S, Van Ranst M, Lorent N, Vande Weygaerde Y et al. Longitudinal follow-up of IgG anti-nucleocapsid antibodies in SARS-CoV-2 infected patients up to eight months after infection. Journal of Clinical Virology. 2021;136:104765.
23. Lau CS, Oh MLH, Phua SK, Liang YL, Li Y, Huo J, et al. Kinetics of the neutralizing and spike SARS-CoV-2 antibodies following the Sinovac inactivated virus vaccine compared to the Pfizer mRNA vaccine in Singapore. Antibodies (Basel) [Internet]. 2022;11(2):38. Available from: http://dx.doi.org/10.3390/antib11020038
24. Naaber P, Tserel L, Kangro K, Sepp E, Jürjenson V, Adamson A, et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. Lancet Reg Health Eur [Internet]. 2021;10(100208):100208. Available from: http://dx.doi.org/10.1016/ j.lanepe.2021.100208
25. Shook L, Atyeo C, Yonker L, Fasano A, Gray K, Alter G et al. Durability of Anti-Spike Antibodies in Infants After Maternal COVID-19 Vaccination or Natural Infection. JAMA. 2022;327(11):1087

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

ORIGINAL ARTICLE

COMPARISON OF THE EFFECTIVENESS, SAFETY, COMPLIANCE, AND COST OF THE 6-MONTH ISONIAZID VS 3-MONTH ISONIAZID-RIFAMPICIN REGIMEN FOR LATENT TUBERCULOSIS IN CHILDREN

Hannah Pamela D. Tan, MD, Arlene S. Dy-Co, MD, FPPS, FPIDSP
National Children's Hospital, Quezon City, Philippines
2ND PLACE 2023 PIDSP RESEARCH CONTEST

ABSTRACT

Background: Tuberculosis remains to be a major cause of morbidity in children and treatment of latent tuberculosis is important to prevent children from developing active tuberculosis. This study aimed to compare the effectiveness, safety, compliance, and cost of the currently available Latent Tuberculosis Infection treatment regimens, 6 months isoniazid (6H) and 3 months isoniazid plus rifampicin (3HR), based on the 2020 Department of Health National Tuberculosis Control Program Tuberculosis Preventive Treatment guidelines for children.

Methodology: In this open label randomized controlled trial pilot study, 30 participants were assigned to receive either 6H or 3HR. Medications were administered daily by either participants (with direct supervision of treatment supporters) or treatment supporters (for younger participants). Data on outcome measures in terms of effectiveness, safety, and compliance were obtained. Direct cost of treatment was computed per patient's weight category. Independent Z-test for proportion (for effectiveness, safety, and compliance) and mean (for cost) at 5% level of significance was used to compare the outcomes for each treatment group.

Results: Twelve subjects (67%) in the 6H group completed per-protocol therapy, compared to 10 subjects (87%) in the 3HR group. The proportion of adverse events was higher in the 6H group (22%) compared to the 3HR group (8%), but statistical tests showed no significant difference for both compliance and frequency of adverse events. No participant developed active TB disease in both groups. The cost of the 6H treatment regimen was 2,180.18 Php while the cost of the 3HR treatment regimen was 1,526.41 Php, with a p-value of 0.0470 which was statistically significant.

Conclusions: Both 6H and 3HR are effective treatments for latent TB infection in patients 0-18 years old. Both treatments were comparable in terms of safety and ease of compliance, but overall cost was higher in the 6H treatment regimen.

KEYWORDS: *latent tuberculosis, 6H, 3HR*

Correspondence:
Dr. Hannah Pamela D. Tan
Email: hahnpam@gmail.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

INTRODUCTION

Tuberculosis (TB) is the leading cause of death from infectious diseases for children of all ages globally, with the Philippines as one of the 30 countries in the WHO high TB burden list[1]. It affected approximately 10.6 million people worldwide in 2021[1]. Around a fourth of the global population have Latent Tuberculosis Infection (LTBI), a state of persistent immune response to stimulation by *M. tuberculosis* antigens with no evidence of active TB. [1]

TB is both curable and preventable. The National Tuberculosis Control Program (NTCP) of the Philippine Department of Health (DOH) includes Tuberculosis Preventive Treatment (TPT) as one of its core program indicators[2]. Currently available regimens for children include daily isoniazid for 6 months (6H) and daily isoniazid and rifampicin for 3 months (3HR)[2].

Studies comparing the effectiveness, safety, compliance, and cost between the two regimens have been conducted internationally but to our knowledge, there are no studies yet comparing the 6H and 3HR regimen in Filipino children, hence, this study looked into the effectiveness, safety, compliance, and cost of the currently available LTBI treatment regimens under the DOH NTCP TPT for children.

This study aims to compare the currently available LTBI treatment regimens, 6H and 3HR, under the DOH NTCP TPT for children in terms of effectiveness, safety, compliance, and cost. The results of this study can guide pediatricians and policy makers as to which of the two currently available regimens, 6H or 3HR, under DOH NTCP TPT will yield better outcomes.

MATERIALS AND METHODS

Study Design

The study was an open label randomized controlled trial pilot study conducted in a 250-bed pediatric tertiary government institution in Quezon City, Philippines.

Population and Sample Size

Children 0-18 years old who were eligible for TPT according to the NTCP guidelines were enrolled in the hospital's TB Directly-Observed Therapy, Short-Course (DOTS) Center. A sample size of 30 was enrolled following the recommendations by Hertzog[3] on group comparisons to demonstrate intervention efficacy. Randomization was done using a research randomizer software at www.randomizer.org (Version 4.0, 2013) and yielded the following independent groups: 18 subjects for group 1 and 12 subjects for group 2.

Sampling was done as follows: One subject per index patient referred to the TB DOTS center was included in the study. Once evaluated and considered eligible for TPT, and after ruling out active TB disease, the subjects were randomly assigned to one of the treatment regimens. Other contacts of the index case were also evaluated for TB and those who were eligible for TB treatment but not included in the study were still enrolled at the hospital's TB DOTS center.

Subjects included children 0-18 years old who were eligible for TPT after exclusion of active TB disease following the guidelines in the NTCP Manual of Procedures (MOP)[4]. The following were the inclusion criteria: (1) Children less than 5 years old who are household contacts of bacteriologically confirmed pulmonary TB; (2) Children 5-18 years old who are household contacts of bacteriologically confirmed pulmonary TB with no other risk factors for TB and with a positive TST; (3) Children less than 5 years old who are household contacts of clinically diagnosed pulmonary TB with positive TST; and (4) Children 0-18 years old who are close contacts of bacteriologically confirmed pulmonary TB with positive TST.

The following cases were excluded: (1) People living with HIV and other risk groups such as those with other immune-suppressive medical conditions[4]; (2) Children who have been previously treated for LTBI or active TB disease; (3) Children exposed to drug-resistant TB cases; (4) Children with pre-existing liver disease; and (5) Children with any sign of hepatic abnormalities such as jaundice, ascites, bleeding, etcetera, at the time of initial examination.

The following enrolled study participants were considered dropouts: (1) Those who failed to present at the TB DOTS Center within 3 days from the scheduled follow-up and could not be contacted after at least 3 phone calls within the 3-day allowance for

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

follow-up; (2) Those who presented with symptoms suggestive of hepatitis as assessed by the TB DOTS physician; and (3) Those who had treatment interruption for two months or more.

Data Collection

The conduct of the study was discussed thoroughly with the parents and/or legally authorized representative as well as with the subjects in a manner that was comprehensible to all. Informed consent was signed by at least 1 parent or guardian who was identified as the subject's treatment supporter. Assent forms were signed by subjects more than 7 years old.

Children enrolled in the study were randomly assigned to either of 2 treatment groups: Group 1 – six (6) months daily isoniazid; Group 2 – three (3) months daily isoniazid + rifampicin. Dosing according to the latest guidelines set by NTCP MOP was followed. Supplemental pyridoxine of 5-10 mg/day was also given to infants who participated in the study. All medications were provided by the TB DOTS clinic free of charge. Allocation concealment was done by a designated resident. The treatment group assignment from the random number generator software was placed in a sealed envelope and given to the investigators.

Participants were registered as regular patients at the TB DOTS Clinic with printed and electronic records. All information were available only to the researchers and de-identified and coded during data analysis. The forms contained the following details: demographic and baseline clinical characteristics such as age, sex, civil status, nationality, educational status, family size, history of BCG vaccination, presence of co-morbidities, and indication for TPT. The following information were noted on each follow up visit: weight, presence of TB signs and symptoms, adverse reactions and issues on compliance. Laboratory (such as complete blood count and liver enzymes) and imaging tests during treatment and until 1 month after completion of treatment were also requested whenever indicated.

Treatment supporters were oriented at the start of TPT regarding possible adverse reactions and given a list of things to monitor during treatment. Adverse drug reactions were graded and managed according to guidelines. Patients and treatment supporters were advised to contact the TB DOTS center or the researcher to report any adverse reactions. Baseline liver function tests (LFTs) were done for subjects 15-18 years old. For other participants, LFTs were only done any time during treatment when symptoms suggestive of hepatitis as assessed by the TB DOTS physician developed.

Subjects with treatment interruptions and those who satisfy the withdrawal criteria were considered dropouts. For treatment interruptions of less than two months, treatment was continued and prolonged to compensate for missed doses. If more than two months of TPT were missed, patients were advised to restart the same regimen from the beginning after ruling out active TB.

This study was conducted according to the ICH-GCP Rules and Regulations and the Data Privacy Act of 2012, and commenced upon IRB approval. Subjects were given the option to withdraw at any point in the study. There was no financial incentive for participants. The authors declare no conflicts of interest regarding the conduct of this study. Partial research funding was granted by the Pediatric Infectious Disease Society of the Philippines.

Outcome Measures

The currently available LTBI treatment regimens, 6H and 3HR, under the DOH NTCP TPT were compared in terms of:

1. Effectiveness described as the proportion of compliant patients who did not develop active TB disease (non-development of chest radiograph changes and/or symptoms suggestive of active TB disease) during the course of treatment until 1 month after completion;
2. Safety described to be inversely related to the proportion of adverse events encountered during the course of treatment until 1 month after completion, classified according to severity and by organ system following the NTCP TPT guidelines

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

3. Compliance described as the proportion of patients who were able to successfully complete the prescribed duration of treatment; and
4. Cost described as the average total cost inclusive of TB medications and diagnostics for the whole duration of the assigned treatment, and clustered by weight range.

Statistical Analysis

The statistical treatment of the study outcomes was as follows:

1. Effectiveness was measured as the proportion of compliant patients who did not develop active TB disease for each treatment regimen, and was compared using the independent Z-test for proportion at 5% level of significance.
2. Safety was measured as inversely related to the proportion of adverse events encountered during treatment for each regimen and was compared using the independent Z-test for proportion at 5% level of significance;
3. Compliance was measured as the proportion of patients considered to have completed treatment for each regimen and compared using the independent Z-test for proportion at 5% level of significance;
4. Cost was measured as the mean total cost for each regimen and was compared using the independent Z-test for mean at 5% level of significance. Parametric statistical tests such as Z-test rely on the normality assumption to make accurate inferences about the population. Hence, to ensure that assumptions for parametric analysis were met, the Normality test was applied to the cost data including weight.

RESULTS

Trial Participants

From September 2021 to July 2022, 30 subjects who met the inclusion criteria and agreed to participate in the pilot study were enrolled and randomly assigned to the treatment regimens. The demographic and clinical characteristics of the trial participants were similar between the two treatment groups except for the indication for TPT (Table 1) where most of the 6H group were exposed to household contacts, while the participants in the 3HR group were exposed to close contacts.

Table 1. Baseline demographic and clinical characteristics

Characteristic	6H (N=18)	3HR (N=12)	P-value
Age			
<5 years old	12 (67%)	4 (33%)	0.125
5 to <15 years old	5 (28%)	8 (67%)	
15-18 years old	1 (5%)	0	
Sex			
Male	5 (28%)	6 (50%)	0.266
Female	13 (72%)	6 (50%)	
Educational status			
No formal schooling	14 (78%)	6 (50%)	0.332
Elementary	3 (17%)	5 (42%)	
High School	1 (5%)	1 (8%)	
Family size			
Less than 4 members	2 (11%)	2 (17%)	1.000
4-6 members	11 (61%)	7 (58%)	
More than 6	5 (28%)	3 (25%)	
Place of residence			
Metro Manila	16 (89%)	10 (84%)	1.000
Cavite	1 (5.5%)	0	
Rizal	1 (5.5%)	1 (8%)	
Others	0	1 (8%)	
Indication for treatment			
Less than 5 years old household contacts of BC-PTB[1]	11 (61%)	3 (25%)	0.017
5-18 years old household contacts of BC-PTB[1] with no other risk factors for TB and with (+) TST	5 (28%)	3 (25%)	
Less than 5 years old household contacts of CD-PTB[2] with (+) TST	2 (11%)	1 (8%)	
0-18 years old close contacts of BC-PTB[1] with (+) TST	0	5 (42%)	
Body Mass Index			
Obese	2 (11%)	1 (8%)	0.060
Overweight	0	0	
Risk for overweight	2 (11%)	0	
Normal	6 (33%)	10 (84%)	
Wasted	3 (17%)	1 (8%)	
Severely wasted	5 (28%)	0	

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

Table 1. Baseline demographic and clinical characteristics (continued)

Characteristic	6H (N=18)	3HR (N=12)	P-value
BCG			
Given	18 (100%)	12 (100%)	--
Not given	0	0	
Unknown	0	0	
Presence of Comorbidity			
Yes	3 (17%)	0	0.255
No	15 (83%)	12 (100%)	

[1]BC-PTB: Bacteriologically confirmed pulmonary tuberculosis
[2]CD-PTB: Clinically diagnosed pulmonary tuberculosis

Compliance

The proportion of those who completed treatment was higher in the combination 3HR group (Table 2). However, independent z-test at 5% level of significance showed that the proportion of overall treatment completion was not significantly different between the two groups.

Safety

The proportion of adverse events reported was higher in the 6H group (Table 2). However, independent z-test at 5% level of significance showed that the proportion of adverse events was not significantly different between the two treatment groups. In those who reported adverse reactions, 80% were from the 6H group. Most reports of adverse events for both treatment groups involved nausea (Grade 1, mild). There was one report of jaundice with elevated liver enzymes (Grade 3, severe) from the 6H group, which resolved after one week. The same regimen was resumed after resolution of symptoms and no further complications were noted.

Effectiveness

Among the participants in both groups who completed treatment, no case of active tuberculosis was diagnosed during treatment and up to 1 month post-treatment (Table 2). Since both groups had an equal proportion of treatment effectiveness, independent z-test was no longer done. Moreover, since no trial participant developed active TB disease while on TPT and 1 month thereafter, per protocol analysis and intention-to-treat analysis of the effectiveness between the two treatment regimens were also not done.

Cost

The mean cost for each treatment group, inclusive of cost for medicine and diagnostics, was calculated in those who completed treatment. Computed costs were based on the doses of the treatment regimens and, therefore, were dependent on the weights of the participants in the two treatment groups. Independent t-test at 5% level of significance of the weights of the trial participants who completed treatment under each group showed that there was no significant difference between the weights of both groups. Independent z-test at 5% level of significance showed that the mean cost is significantly higher in the 6H group compared to the 3HR group. However, both p-values computed for the mean weights (P = 0.051) and costs (P = 0.047) were too close to P = 0.05 to be considered not significant and significant, respectively. They can be statistically inconclusive and that additional trial participants can potentially make the results significant and not significant, respectively.

Table 2. Independent Z-test results for effectiveness, safety, compliance, and cost between 6H and 3HR.

Outcome	Group 1 (6H) n= 18 Count (%), Mean (SD)	Group 2 (3HR) n= 12 Count (%), Mean (SD)	P-value	Sig[1]
Non-development of TB Disease	12 (100%) (of 12 completed)	10 (100%) (of 10 completed)	--	ns[4]
Adverse Events	4 (22%)	1 (8%)	0.31	ns[4]
Treatment Completion	12 (67%)	10 (83%)	0.33	ns[4]
Weight (in kg)[2]	17.125 (1.45)	24.4 (3.42)	0.0501	ns[4]
Cost[3]	2180.18 (774.79)	1526.41 (649.64)	0.0470	s[5]

[1]5% Level of Significance; ns-not significant; s-significant
[2]Independent t-test for mean
[2,3]Only those who completed the treatment
[4]NS: not significant
[5]S: significant

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 13-19 July-December 2023
Tan HPD, Dy-Co, AS. Comparison of the effectiveness, safety, compliance, and cost of the 6-month isoniazid vs 3-month isoniazid-rifampicin regimen for latent tuberculosis in children.
https://doi.org/10.56964/pidspj20232402003

DISCUSSION

Our study showed that the overall treatment completion rate was higher in the 3-month combination therapy than the 6-month monotherapy. Although there was no noted statistically significant difference between the two treatment groups, a larger sample size is needed to produce more conclusive results. Similarly, an 11-year RCT by Spyridis *et al*.[5] showed that compliance is better with the short-course combination therapy. Moreover, systematic reviews and RCTs on LTBI treatment in children and adolescents by Assefa *et al*.[6] and Cruz *et al*.[7] showed that overall completion rate was either equivalent or higher in the short-course combination therapy.

It was found that in those who did not complete treatment (8 from each of the treatment groups), 50% or 4 in 8 were not able to visit the TB DOTS Center due to loss of accompanying treatment supporters secondary to either migration *i.e*. moved to provinces away from Metro Manila, or to limitations in public transportation during the COVID-19 surges early in 2022. In the 6H group, 2 subjects had treatment supporters who opted to discontinue treatment because participants persistently complained of nausea after intake of isoniazid. The last 2 subjects were declared lost to follow-up after failure to visit the TB DOTS Center within 3 days from the scheduled regular follow-up and 3 failed phone calls within the 3-day allowance for follow-up. These factors which led to non-compliance and discontinuation of therapy are important aspects of the program that should be investigated and dealt with to ensure success.

The proportion of adverse effects was higher by three times as much in the 6H group compared to the 3HR group. The difference was not statistically significant, but a larger sample size is needed to provide more conclusive results. Our findings are consistent with the systematic review by Assefa *et al*.[6] and the RCT by Spyridis *et al*.[5] where pediatric patients on isoniazid monotherapy were reported to be more likely to have transient elevation of liver enzymes than those under the combination therapy of isoniazid + rifampicin. In contrast, a systematic review by Cruz *et al*.[7] showed that children given isoniazid therapy had similar or lower frequency of adverse events including hepatoxicity compared with those given isoniazid + rifampicin therapy. Also, in this review, Cruz *et al*. cited studies showing that isoniazid is actually well tolerated by pediatric patients.[7]

No participant developed active TB disease among those who completed treatment. This suggests that both treatment regimens are effective, although a larger sample size is needed to derive more conclusive results. It is interesting to note that in the study of Assefa *et al*.[6] and in the RCT by Spyridis *et al*.[5], pediatric patients given isoniazid monotherapy had twice the risk of developing active TB disease than those given isoniazid + rifampicin combination therapy. In both studies, TB disease was reported along with findings on chest radiographs and suggested that combination therapy has a faster effect in decreasing the microbial load, which might have impeded the development of radiologic changes in participants on combination therapy. In contrast, Cruz *et al*.[7] showed in a systematic review that the estimated efficacy of LTBI treatment in children was equivalent in both treatment groups similar to our findings.

This pilot study showed that the cost of treatment with 6H was statistically higher than that of 3HR inclusive only of medications and laboratory procedures during treatment until 1 month after completion of treatment. Cost of treatment is not limited to direct costs as what was included in this study but should also include indirect costs incurred such as transportation cost, and loss of work wages.

CONCLUSION AND RECOMMENDATIONS

This pilot study showed that both 6H and 3HR can be effective treatments for latent TB infection in children and adolescents 0-18 years of age with no significant difference in terms of compliance and adverse reactions between treatment groups. The cost of the 6H treatment regimen is significantly higher than that of the 3HR treatment regimen. Although medications are given for free at the TB DOTS Center where this study was conducted, policy-makers can

make use of this financial advantage in future amendments of the TB DOTS guidelines.

ACKNOWLEDGEMENTS

NCH TB DOTS Staff for the technical assistance

CONFLICT OF INTEREST

None declared.

REFERENCES

1. World Health Organization. Global tuberculosis report 2022 [Internet]. Geneva: World Health Organization; 2022 October 27. Available from https://www.who.int/publications/i/item/9789240061729
2. Department of Health. Decline in the reported TB cases an effect of the pandemic [Press Release]. Manila: Department of Health; 2020 July 21. Available from: https://doh.gov.ph/press-release/DECLINE-IN-REPORTED-TB-CASES-AN-EFFECT-OF-THE-PANDEMIC-DOH#:~:text=In%202019%2C%20the%20Philippines%20had,of%20death%20in%20the%20country
3. Hertzog MA. Considerations in determining sample size for pilot studies. *Research in nursing & health* [Internet]. 2008;31(2):180–191. Available from: https://doi.org/10.1002/nur.20247
4. Department of Health. National tuberculosis control program manual of procedures, 6th ed. Manila: Department of Health; 2020. Available from https://doh.gov.ph/sites/default/files/publications/NTP_MOP_6th_Edition.pdf
5. Spyridis NP, Spyridis PG, Gelesme A, Sypsa V, Valianatou M, Metsou F, *et al*. The effectiveness of a 9-month regimen of isoniazid alone versus 3- and 4-month regimens of isoniazid plus rifampicin for treatment of latent tuberculosis infection in children: Results of an 11-year randomized study. *Clinical Infectious Disease* [Internet]. 2007;45:715-722. Available from: https://doi.org/10.1086/520983
6. Assefa Y, Assefa Y, Woldeyohannes S, Hamada Y, Getahun H. 3-month daily rifampicin and isoniazid compared to 6- or 9-month isoniazid for treating latent tuberculosis infection in children and adolescents less than 15 years of age: an updated systematic review. *Eur Respi J* [Internet]. 2018;52:1800395. Available from https://doi.org/10.1183/13993003.00395-2018
7. Cruz AT, Ahmed A, Mandalakas AM, Starke, J. R. Treatment of latent tuberculosis infection in children. *Journal of the Pediatric Infectious Diseases Society* [Internet]. 2018;2(3):248-258. Available from: https://doi.org/10.1093/jpids/pit030

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

ORIGINAL ARTICLE

CLINICAL AND MICROBIOLOGICAL PROFILE AND FACTORS AFFECTING OUTCOME AMONG PEDIATRIC FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES

Josh Nathan L. Ngai, MD, Kristian Dorell T. Masacupan, MD, Allan Robert R. Racho, MD,
Maria Luz U. Del Rosario, MD, Loralyn Mae O. Lagaya-Arañas, MD, Imelda A. Luna, MD
Institute of Pediatrics and Child Health, St. Luke's Medical Center - Quezon City
3RD PLACE 2023 PIDSP RESEARCH CONTEST

ABSTRACT

Objective: To evaluate the clinical and microbiological profile and factors affecting outcome among pediatric febrile neutropenic (FN) patients with hematologic malignancies (HM)

Methodology: This was a cross-sectional study which looked into medical records of Filipino children 0-18years old diagnosed with FN and HM and admitted from June 2016 up to June 2022 at the St. Luke's Medical Center, Quezon City (SLMC-QC). Data on age, sex, underlying malignancy, stage of treatment, site of infection, presence of central line, initial antibiotic therapy, culture positivity and isolates were retrospectively evaluated. Incomplete records were excluded. The relationship between clinical & microbiologic profile and outcomes were analyzed using T-test and Chi-square test. Significance was set at $p<0.05$.

Results: This study included 267 episodes of FN. Patients had a mean age of 8.3 years with male preponderance (59%). The most frequent underlying malignancy was acute lymphoblastic leukemia (61%). Episodes occurred primarily during the induction (40%) and consolidation phases (28%) of chemotherapy. Most (65%) had an absolute neutrophil count (ANC) of $<100/mm^3$. Central line catheter was present in 59% of episodes and 52% had an implanted port. There was no identifiable focus of infection in 52% of cases. Gram-negative bacteria, specifically *Klebsiella pneumoniae* (13%) and *Escherichia coli* (11%) were the most common isolates. Most patients (88%) recovered. Age >10years, male sex, diagnosis of acute myelogenous leukemia, relapse disease, ANC $<100/mm^3$, presence of a central line, and central line associated bloodstream infection were significantly associated with duration of hospital stay. Presence of central venous line was the most significant factor associated with mortality.

Conclusions: Several clinical and microbiological factors, specifically age >10years, male sex, diagnosis of acute myelogenous leukemia, relapse disease, ANC $<100/mm^3$, presence of a central line, and central line associated bloodstream infection, were documented to significantly affect outcome in Filipino pediatric FN patients with HM.

KEYWORDS: *Pediatric, Febrile Neutropenia, Hematologic Malignancies, Leukemia, Philippines*

Correspondence:
Dr. Josh Nathan L. Ngai
Email: jnlngai@gmail.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

INTRODUCTION

Febrile neutropenia (FN) is a medical emergency posing a high mortality rate if not treated promptly and aggressively.[1] The most effective empirical antimicrobial regimen must be rapidly administered to patients as delay in treatment may result in septicemic shock and increase mortality.[2] FN has been observed to be much more common in hematologic malignancies.[3] Mortality has been associated with several factors such as duration and degree of neutropenia, bacteremia, isolation of resistant organisms, identifiable focus, performance status, comorbidities, and type of underlying malignancies.[4]

Treatment practices for FN vary between centers and depend on institutional policies, hospital isolates, and physician preferences. Governing bodies recommend the use of empiric agents that specifically cover for invasive gram-negative bacteria, especially *Pseudomonas aeruginosa,* but defer to the physician's best judgment to suit the situation and needs of the patient.[5] A high prevalence of infections in the community and antibiotic resistance due to the unrestricted and rampant use of broad-spectrum antibiotics further complicates disease management.[6]

It is worthwhile to evaluate the clinical profile, pathogenic organisms, pattern of antibiotic sensitivity, and outcome of treatment among high risk hematologic malignancies with febrile neutropenia. While there are innumerable studies from Western countries on febrile neutropenia and its etiology, choice of empiric antibiotic and outcome, literature on febrile neutropenia patients in developing countries is gradually evolving.[6] In the Philippines, local data remain sparse and there is absence of local standard protocols established for the management of febrile neutropenia in pediatric patients with hematologic malignancies thus, this study looked into the clinical and microbiological profile and factors affecting outcome among pediatric febrile neutropenic patients with hematologic malignancies.[7-9]

MATERIALS AND METHODS

Study Design

A cross-sectional analytic study design was utilized which included records of Filipino patients 0 to 18 years old diagnosed with hematologic malignancies who were admitted for and/or developed febrile neutropenia during their admission from June 2016 to June 2022 at St. Luke's Medical Center, Quezon City (SLMC-QC). For the purpose of this study, hematologic malignancies refer to the three main types, specifically: leukemia, lymphoma, and multiple myeloma.

Inclusion Criteria

Filipino children aged 0 to 18 years and 354 days admitted at SLMC-QC between June 2016 to June 2022 and diagnosed with hematologic malignancy and admitted for or developed febrile neutropenia during the period of admission were included.

Exclusion Criteria

Patients with the following conditions were excluded: (1) fever attributed to malignancy, blood transfusion, or medications; (2) concurrent COVID-19 infection; and (3) those with incomplete data.

Sample Size and Sampling

To estimate the sample size, Cochran's formula was used where the margin of error (e) was set at ±5% (95% confidence). In a study by Anirban and Amit, prevalence was estimated at 12.8%.[10] Based on this, the computed sample size for this study was 171. Patients were screened for eligibility until a total of 267 unique episodes of FN in 96 distinct patients were obtained.

Data Collection

Convenience sampling of charts at the medical records of SLMC-QC was performed and documents were screened for eligibility using the set criteria. No randomization was done since all patients were assigned under one group and no blinding procedures on patients, investigators, and study staff was implemented.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

Clinical and microbiological profile of pediatric patients with febrile neutropenia and hematologic malignancies admitted from June 2016 to June 2022 was obtained. The following data were collected: age, sex, underlying malignancy, stage of treatment, absolute neutrophil count (ANC), site of infection, presence of central venous catheter, initial antibiotic therapy, culture positivity and culture isolate. Charts with incomplete data were excluded.

Outcomes noted were mean duration of hospital stay, recovery from infection, and status upon discharge whether recovered or expired.

Data Analysis

Data encoding utilized Microsoft Excel 2018. EpiInfo 3.5.3 software was used in processing and analyzing the data gathered. Descriptive statistics were generated for all variables using mean and standard deviation. Frequencies and percentages were computed for nominal data. T-test for numerical data and Chi-square test for nominal data were used to determine whether there were significant differences between factors. Clinical and microbiologic profiles of pediatric febrile neutropenia were analyzed using frequency and percentage for categorical profiles and mean and standard deviation for continuous profiles. Determination of the relationship between duration of hospital stay with the clinical and microbiologic profile was analyzed using T-test and Chi-square test. Level of significance was set at $p < 0.05$.

Ethical Issues

The study abided by the Principles of the Declaration of Helsinki and was conducted following the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP). The Clinical Protocol and all relevant documents were reviewed and approved by the SLMC-QC Institutional Ethics Review Committee. Only the investigator was allowed to view the patient's files within the vicinity of the medical records section. Patient confidentiality was respected by ensuring anonymity of records. Each patient document was coded and did not contain any identifying information to ensure confidentiality.

All study data were recorded and investigators were responsible for the integrity of the data (i.e. accuracy, completeness, legibility, originality, timeliness and consistency).

RESULTS

Clinical Profile

There were 267 episodes of FN documented in this study. Table 1 shows the clinical profile of subjects. Average age was 8.3 years old with 64% belonging to the <10 years age group. Male-female distribution showed a male preponderance at 59%.

Acute lymphoblastic leukemia (ALL, 61%) was most frequent among the hematologic malignancies, followed by acute myelogenous leukemia (AML, 36%). Majority of febrile neutropenia episodes developed during the induction phase (40%) and consolidation phase (28%) of chemotherapy. Most (65%) had an ANC <100/mm^3 upon diagnosis of febrile neutropenia.

Central line catheter was present in 157 episodes (59%) with 139 (52%) having a central venous access device (CVAD). Most did not have an identifiable focus of infection (52%), while in those with a focus, the most common were pulmonary (17%), gastrointestinal (14%), and catheter-associated (10%).

Table 1. Clinical profile of pediatric febrile neutropenia patients with hematologic malignancies

Parameter	N= 267
Age (years)	8.25±4.986
Less than 10	171 (64%)
10 or more	96 (36%)
Sex	
Male	158 (59%)
Female	109 (41%)
Malignancy	
ALL	164 (61%)
AML	97 (36%)
Lymphoma	3 (1%)
Chronic myelogenous leukemia	3 (1%)

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

Table 1 continued. Clinical profile of pediatric febrile neutropenia patients with hematologic malignancies

Parameter	N= 267
Treatment Phase	
Induction	107 (40%)
Consolidation	75 (28%)
Relapse	52 (19%)
Maintenance	33 (12%)
ANC (/mm^3)	
Less than 100	174 (65%)
>100-500	58 (22%)
>500-1000	35 (13%)
Presence of Central Line	
Central Access Venous Device	
None	139 (52%)
Peripherally Inserted Central	110 (41%)
Catheter	16 (6%)
Internal Jugular	2 (1%)
Identified Site of Infection	
None	138 (52%)
Pulmonary	46 (17%)
Gastrointestinal	38 (14%)
Catheter-associated	27 (10%)
Skin and Soft tissue	14 (5%)
Genitourinary	4 (1%)

Microbiological Profile

Most episodes of febrile neutropenia had negative cultures with only 29% culture positivity. Overall, 93 culture isolates were identified. Majority were gram-negative bacteria (63.4%), followed by gram-positive (25.8%), and fungal isolates (10.8%). The most common gram-negative isolate was *Klebsiella pneumoniae*. The most common gram-positive isolate was *Staphylococcus epidermidis*.

Table 2. Microbiological profile of pediatric febrile neutropenia patients with hematologic malignancies

Culture Profile	Values
Culture Positivity	N=267
No growth	190 (71%)
Growth	77 (29%)

Table 2 continued. Microbiological profile of pediatric febrile neutropenia patients with hematologic malignancies

Culture Isolate	N=93
Gram-negative	**59 (63.4%)**
Klebsiella pneumoniae	13 (4.9%)
Escherichia coli	11 (4.1%)
Escherichia coli ESBL+	8 (2.6%)
Pseudomonas putida	5 (1.9%)
Klebsiella pneumoniae ESBL+	3 (1.1%)
Acinetobacter baumannii	3 (1.1%)
Aeromonas hydrophila	2 (0.7%)
Salmonella spp.	2 (0.7%)
Bacillus cereus	1 (0.4%)
Bacillus megaterium	1 (0.4%)
Enterobacter cloacae	1 (0.4%)
Enterococcus faecalis	1 (0.4%)
Enterococcus faecium	1 (0.4%)
Enterococcus spp.	1 (0.4%)
Kingella kingae	1 (0.4%)
Neisseria flava	1 (0.4%)
Proteus penneri	1 (0.4%)
Pseudomonas aeruginosa	1 (0.4%)
Sphingomonas paucimobilis	1 (0.4%)
Stenotrophomonas maltophilia	1 (0.4%)
Gram-positive	**24 (25.8%)**
Staphylococcus epidermidis	11 (4.1%)
Streptococcus oralis	3 (1.1%)
Staphylococcus aureus	2 (0.7%)
Staphylococcus capitis	2 (0.7%)
Micrococcus luteus	1 (0.4%)
Staphylococcus cohnii	1 (0.4%)
Staphylococcus haemolyticus	1 (0.4%)
Staphylococcus hominis	1 (0.4%)
Streptococcus mitis	1 (0.4%)
Streptococcus viridans	1 (0.4%)
Fungal	**10 (10.8%)**
Candida parapsilopsis	3 (1.1%)
Candida tropicalis	3 (1.1%)
Candida albicans	2 (0.7%)
Candida norvegensis	1 (0.4%)
Candida spp.	1 (0.4%)
Rhodotorula mucilaginosa	1 (0.4%)

Table 3 shows that the most common antibiotics administered were cefepime (66%) and meropenem (9%). Fifteen (6%) were given metronidazole and thirteen (5%) given fluconazole.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

Table 3. Antibiotics used during for febrile neutropenia

Antibiotics	Frequency of Use
Cefepime	175 (66%)
Meropenem	24 (9%)
Metronidazole	15 (6%)
Fluconazole	13 (5%)
Piperacillin Tazobactam	11 (5%)
Vancomycin	9 (3%)
Cefuroxime	7 (3%)
Clindamycin	7 (3%)
Ceftazidime	6 (2%)
Amikacin	5 (2%)
Amoxyclav	5 (2%)
Cefixime	3 (1%)
Acyclovir	2 (1%)
Amphotericin B	2 (1%)
Levofloxacin	2 (1%)
Azithromycin	1 (0%)
Ceftriaxone	1 (0%)
Ganciclovir	1 (0%)
Micafungin	1 (0%)

Of 93 isolates identified, 17 were documented to be resistant to 3rd and 4th generation cephalosporins (i.e. ceftriaxone, ceftazidime, and cefepime) and of these, 11 were ESBL-positive, comprising a mixture of *E. coli* and *K. pneumoniae*.

Outcome

The average duration of hospital stay was 30.1 days. Of 267 episodes, 235 (88%) were discharged alive and improved while 32 (12%) expired (Table 4).

Table 4. Duration of hospital stay and outcome

Outcome	
Duration of hospital stay (days)	30.10±34.04
Status upon discharge	N=267
Alive	235 (88%)
Expired	32 (12%)

Factors Affecting Outcome

Age >10 years old (31.3 vs 28, p=0.01) and male sex (35.63 vs 22.08, p=0.01) were identified to have significantly longer duration of hospitalization.

The type of malignancy was identified to affect duration of hospital stay, with AML having a significantly longer duration of hospital stay (39.9 days, p=0.01) compared with other hematologic malignancies. Those who were on relapse had longer hospitalization (40.56 days, p=0.01) compared with those who were on standard phases of chemotherapy. Having an ANC of <100/mm^3 had significantly longer duration of hospital stay at 34.5 days (p=0.01).

Presence of CVAD and PICC had significantly longer hospital stay at 38.5 days and 35.9 days, respectively (p<0.01 for both). Among all the factors studied, having catheter-associated infection was associated with the longest duration of hospital stay at 67.2 days (p<0.01). The presence or absence of growth in culture was not associated with significant differences in duration of hospital stay.

Table 5. Correlation of clinical and microbiologic factors with duration of hospital stay

Factor	N= 267	Duration of hospital stay (days)	p-value
Age (years)			
10 or more	**171**	**31.27**	**0.01***
Less than 10	96	28.00	
Sex			
Male	**158**	**35.63**	**0.01***
Female	109	22.08	
Malignancy			
AML	**97**	**39.87**	
CML	164	26.00	**<0.01***
ALL	3	24.67	
Lymphoma	3	15.00	
Treatment Phase			
Relapse	**52**	**40.56**	
Induction	107	31.04	**0.01***
Consolidation	75	28.55	
Maintenance	33	14.09	
ANC (/mm^3)			
Less than 100	**174**	**34.51**	**0.01***
>100-500	58	24.78	
>500-1000	35	16.97	
Presence of Central Line			
CVAD	**139**	**38.48**	
PICC	**16**	**35.88**	**<0.01***
None	110	18.90	
IJ	2	17.00	
Site of Infection			**<0.01***
Catheter-associated	**27**	**67.15**	
Pulmonary	46	35.28	
Gastrointestinal	38	25.84	
Skin and Soft tissue	14	25.14	
None	138	23.08	
Genitourinary	4	20.25	
Culture	77	36.30	0.051
Growth	190	27.58	
No growth			

*Significant at p-value <0.05

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 20-30 July-December 2023
Ngai JNL, Masacupan KDT, Racho ARR, Del Rosario MLU, Lagaya-Arañas LMO, Luna IA. Clinical and Microbiological Profile and Factors Affecting Outcome among Pediatric Febrile Neutropenic Patients with Hematologic Malignancies.
https://doi.org/10.56964/pidspj20232402004

As for status upon discharge (Table 6), the only variable correlated with outcome was the presence of central venous catheters. Specifically, having a CVAD was identified to have a 6 times higher (OR=6.277, 95% CI 1.890-20.851, p=0.003) likelihood of death while for PICC, there was a 9 times higher likelihood of death (OR=9.106, 95% CI 1.652-50.181, p=0.011).

Table 6. Correlation of clinical and microbiologic factors with status upon discharge

Factor	Odds Ratio	95% Confidence Interval	p-value
Age	2.815	0.964-8.225	0.058
Sex	1.336	0.521-3.43	0.546
Malignancy	0.109	0.004-3.031	0.191
ALL			
AML			
Lymphoma			
CML			
Treatment Phase	3.093	0.289-33.072	0.350
Relapse			
Induction			
Consolidation			
Maintenance			
ANC (/mm^3)	0.189	0.007-5.139	0.323
Less than 100			
>100-500			
>500-1000			
Central Line			
PICC	**9.106**	**1.652-50.181**	**0.011***
CVAD	**6.277**	**1.89-20.851**	**0.003***
Identified site of Infection	0.748	0.214-2.614	0.649
Gastrointestinal			
Catheter-associated			
Pulmonary			
None			
Skin and Soft tissue			
Genitourinary			
Culture Growth	1.622	0.153-17.21	0.688
Growth			
No Growth			

*Significant at p-value <0.05

DISCUSSION

This study identified that among the different hematologic malignancies, ALL was the most common underlying malignancy followed by AML. A similar incidence was also seen in other studies.[11] Febrile neutropenia was more common in diseases treated with dose-intensive therapies and bone marrow involvement such as ALL and AML. On the other hand, the higher incidence of ALL compared to AML could be attributed to the fact that ALL is more common in children with a lower incidence of childhood AML compared to adults.

In our study, the frequency of febrile neutropenia was highest in patients on the induction and consolidation phases of chemotherapy, consistent with findings across literature.[12-14] Induction and consolidation are considered as intensive chemotherapy while CNS consolidation and maintenance are considered as non-intensive phases.[12] Chemotherapy regimens administered during these intensive phases are of higher dose and considered to be myeloablative resulting in significant bone marrow suppression and consequently may cause longer neutropenia.[13,14]

Similar to other studies, the risk of developing febrile neutropenia was greater in patients with ANC <500/mm^3 and increases dramatically in those with ANC <100/mm^3.[1] In a study by Oderoi et al., ANC <100/mm^3 was identified as an independent predictor for febrile neutropenia.[12]

Most episodes of febrile neutropenia did not have an identifiable focus of infection. However, the absence of clinical signs or symptoms of infection in a great proportion of febrile neutropenic episodes does not exclude its presence.[15] Granulocytopenia markedly alters the host's inflammatory response making the classic signs and symptoms of infection undetectable. In addition, children with febrile neutropenia are less likely to have a clinically apparent site of infection compared to adults.[11]

We observed that the culture positivity rate in our study was only 29%. In general, the causative agent was not demonstrable in 60 to 70% of cases of febrile neutropenia episodes even with the best laboratory conditions.[16] This finding is consistent with various studies from both developed and developing countries reporting similar rates from 13 to 34%.[17-19] These results were also comparable with the study of Zahid et al. who reported microbiologically documented infection in only 31% of febrile neutropenic episodes.[20]

This study also demonstrated that gram-negative organisms are still the most predominant pathogens in febrile neutropenia as is observed among other developing countries.[16] In a study by Malabagi et al. in India of febrile neutropenia in hematologic malignancies, the most common organisms isolated were gram-negative bacilli.[3] A surveillance study at the pediatric oncology unit of the University Hospital in Kuala Lumpur showed that gram-negative bacteria comprise majority of isolates and identified these as the etiology of bacteremia in this group of patients.[21] Although there is a rising incidence of gram-positive bacteremia in febrile neutropenic patients this is observed more commonly among developed countries and have attributed this to the increasing use of indwelling catheters.[15]

On review of our institutions own and most recent hospital antibiogram (based mostly on cultures from adult patients), *K. pneumoniae* remains to be one of the top 3 bacterial isolates as a cause of infection in different organ systems cultured, consistent with what was shown in our study. The spectrum of bacterial isolates in our study was similar to what has been reported internationally.[22] This finding is congruent with the study of Harrifin et al. in Thailand, where *K. pneumoniae* was consistently the most common bacterial isolate in cancer patients, accounting for up to 20% of blood-culture isolates yearly.[23] In a study in Taiwan where they examined bacteremia in hematological and oncological children with febrile neutropenia, the most common isolates were Gram-negative bacteria, including *K. pneumoniae*.[24] In addition, it has been reported that there is an alarming increase in the isolation of extended-spectrum β-lactamase (ESBL)–producing bacteria. A recent study identified ESBL-producing isolates in 51.6% of *K. pneumoniae* bacteremia in children with hematologic/oncologic disease.[23-25] The rate of ESBL-producing isolates in this study was 11.82%, similar to findings which peg the incidence at 12 to 44% globally.[24,26] Factors responsible for the acquisition of ESBL-producing bacterial infections are prolonged hospital stay and prior use of broad-spectrum cephalosporins. Some consider the use of antimicrobial prophylaxis, specifically fluroquinolones to have some association with the development of ESBL production in *E. coli* and *K. pneumoniae,* although the mechanisms behind this association has yet to be elucidated on.[25] No patients in this study was given fluroquinolones as antibacterial prophylaxis for FN.

The first line antibiotic therapy used in this study was cefepime. It provides good activity against most Gram-negative bacteria and has been extensively studied for febrile neutropenia with good control of the disease.[27,28] Due to its good gram-negative, as well as gram-positive coverage, including coverage for methicillin-sensitive *S. aureus* and penicillin-non-susceptible alpha-streptococci, cefepime is a good candidate for use as empiric treatment for neutropenic fever.[27] In addition, this agent offers the advantage of Gram-positive coverage similar to that of cefotaxime and ceftriaxone, as well as good activity against *Pseudomonas aeruginosa* and many enteric bacilli that are resistant to third generation cephalosporins, including clinical isolates of *Enterobacter spp*. and *Citrobacter freundii*.[29] According to the IDSA guidelines, among the cephalosporins, cefepime can be used as a single agent for treating febrile neutropenic patients.[30]

Among the different identified clinical and microbiological parameters, it was found that age >10 years, male sex, diagnosis of AML, relapse state, having ANC <100/mm^3, presence of central line, and having catheter-associated infections had a significantly longer hospital stay.

With regard to age, younger children have a better prognosis and outcome for both ALL and AML. Holmes et al. rationalized that in malignancies like ALL, tumor cell type significantly influenced survival, with older children 10-19 years at diagnosis, being more likely to be diagnosed with T-cell ALL as opposed to B-cell ALL. They also showed that children in the 10-19 years age group were two times as likely to die compared to younger children.[31] In general, younger children respond better to treatment which may be related to the previously described good risk features. In a study by Hann et al.

comparing outcomes from febrile neutropenic episodes in children, they observed that the younger group also had a less defined site of infection.[11]

To our knowledge, there are no studies available directly correlating sex and outcome, specifically male sex and duration of hospital stay in febrile neutropenic patients. In a study done by Sulivan et al., in patients with hematologic malignancies, male sex was a risk factor for the development of perianal infections.[32] The pathogenesis of perianal infection in the neutropenic patient is similar to that in the immunocompetent patient. Male sex was a statistically significant predictor for early mortality among patients with chemotherapy-induced febrile neutropenia.[33] Although not well understood, the above findings may contribute to the longer duration of hospitalization and poorer outcomes in males given the difficulty of management and high rate of recurrence of anorectal complications.[34]

Cytogenomic subtypes may play a role in affecting the prognosis of hematologic malignancies. In ALL, the T-cell immunophenotype is reportedly twice as common in males and associated with poorer outcomes when compared to the B-cell immunophenotype which may contribute to the observation of increased likelihood of mortality in males.[31,35]

The type of hematologic malignancy affected outcomes, with this study identifying AML as having a longer duration of hospital stay. In a study by Ylmaz et al., they identified that AML was generally associated with significantly longer duration of neutropenia, fever resolution, and antibiotic administration.[21] In another study, they attributed this to the high dose Ara-C (cytarabine) regimen of AML which is also associated with high infectious mortality.[36]

In terms of relapse, it is known that the risk of febrile neutropenia is higher in children with cancer who have relapsed and who have advanced disease regardless of treatment. Additionally, as a consequence of receiving higher doses of chemotherapy for treatment of advanced disease, the risk for febrile neutropenia increases.[37] In a similar study, they identified that a greater percentage of acute leukemia patients in relapse tended to have longer febrile episodes and would require antibiotic changes than leukemia patients in remission, resulting in significantly longer hospital stay.[38]

The severity of febrile neutropenia, as reflected in the ANC, has consistently been correlated with morbidity, mortality and treatment delays in malignancies.[1] Children hospitalized for fever and neutropenia who have persistent fever and an ANC of <100/mm^3 are at high risk for morbidity and more likely to require antibiotic changes and antifungal therapy.[39] This is because the risk of clinically important infection rises as the neutrophil count falls.[40] On the other hand, children with ANC of 100/mm^3 who are classified as low risk would be candidates for early hospital discharge. This includes those with a diagnosis of ALL, non-progressive/relapse disease, not in the intensive phase of chemotherapy for ALL, and clinical stability that would otherwise not require inpatient care.[13]

In our study, the presence of central line and catheter-associated infection, among all the factors identified, was the one shown to be associated with the longest duration of hospital stay. Furthermore, the presence of PICC or CVAD was the only factor identified to be associated with a higher likelihood of mortality at 9 and 6 times higher, respectively.

In general, studies have identified central line associated blood stream infection (CLABSI) as a serious event. Although there is a large body of published evidence regarding CLABSI attributed to morbidity and mortality in adult populations with malignancies, information is still lacking in the pediatric population.[41]

Studies have shown that CLABSI results in prolonged hospital stay, increased mortality, and substantial costs to the healthcare system.[42] In a study by Roger et al. on CLABSI in children with AML, there is a significant increase in morbidity and admission to the pediatric ICU and a trend toward increased mortality.[43] Important consequences of CLABSI aside from extended hospital stay also include

interruption of chemotherapy or other treatment, catheter removal, intravascular thrombosis, endocarditis, and sepsis.[41] Prevention of CLABSI during episodes of febrile neutropenia is essential to further improve the long-term outcomes in pediatric patients with hematologic malignancies.

The major limitation of this study was its retrospective nature and single center experience. Although we only included pediatric febrile neutropenic patients with hematologic malignancies, complexities of the underlying malignancies makes the patient population heterogenous which may cause bias. This however may be remedied in future studies by increasing the sample size. Other clinical and microbiologic factors that have been shown to affect outcome (i.e. race, risk stratification, serial monitoring of ANC, duration of neutropenia, type of chemotherapy used, duration from last chemotherapy, cause of death) were also not included in this study. These may be addressed in a more systematic and comprehensive manner in future studies.

This study serves as a snippet of the microbiologic epidemiology of febrile neutropenic patients with hematologic malignancies, from a single medical center in a resource limited country. It is hoped that findings in this study may help improve practices to prevent infection-related outcomes in pediatric cancer patients.

CONCLUSION

This study showed that several clinical and microbiological factors significantly affect outcomes in Filipino pediatric febrile neutropenic patients with hematologic malignancies. Age >10 years old, male sex, AML diagnosis, relapse state, ANC <100/mm^3, presence of central line, and catheter-associated infection had significantly longer duration of hospital stay. Specifically, those with catheter-associated infection was identified to have the longest duration of hospital stay. Presence of PICC or CVAD were the only factors associated with a higher chance for mortality.

ACKNOWLEDGEMENTS

We are thankful to our colleagues and mentors from the Institute of Pediatrics and Child Health at St. Luke's Medical Center – Quezon City for their insightful comments, endless encouragement, and expert guidance. We are equally thankful to our patients, without whom, this study would not have been possible.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Krishnamani K, Gandhi LV, Sadashivudu G, Raghunadharao D. Epedimiologic, clinical profile and factors affecting the outcome in febrile neutropenia. South Asian J Cancer. 2017 Jan-Mar;6(1):25-27.
2. Perron T, Emara M, Ahmed S. Time to antibiotics and outcomes in cancer patients with febrile neutropenia. BMC Health Serv Res. 2014 Apr 10;14:162.
3. Lakshmaiah KC, Malabagi AS, Govindbabu, Shetty R, Sinha M, Jayashree RS. Febrile Neutropenia in Hematological Malignancies: Clinical and Microbiological Profile and Outcome in High Risk Patients. J Lab Physicians. 2015 Jul-Dec;7(2):116-120.
4. Osmani AH, Jabbar AA, Gangwani MK, Hassan B. Outcomes of High Risk Patients with Febrile Neutropenia at a Tertiary Care Center. Asian Pac J Cancer Prev. 2017 Oct 26;18(10):2741-2745.
5. Jin J, Lee YM, Ding Y, Koh LP, Lim SE, Lim R, et al. Prospective audit of febrile neutropenia management at a tertiary university hospital in Singapore. Ann Acad Med Singap. 2010 Jun;39(6):453-459.
6. Mishra K, Kumar S, Ninawe S, Bahl R, Meshram A, Singh K, et al. The clinical profile, management, and outcome of febrile neutropenia in acute myeloid leukemia from resource constraint settings. Ther Adv Infect Dis [Internet]. 2021 Aug 4 [cited 2022 July 12];8:20499361211036592. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8358573/ DOI: 10.1177/20499361211036592.
7. Isais-Agdeppa A, Bravo L. A Five-Year Retrospective Study on the Common Microbial Isolates and Sensitivity Pattern on Blood Culture of Pediatric Cancer Patients Admitted at the Philippine General Hospital for Febrile Neutropenia. Pediatr Infect Dis Soc J Philippines. 2005 Jan-Jun; 9(2):19-24.
8. Celiz-Pasual C, Garcia R. Infections in Febrile Neutropenia Cancer Patients Who Were Undergoing Chemotherapy at the Makati Medical Center. Pediatr Infect Dis Soc J Philippines. 2011 Jan-Jun; 12(1):10-16.

9. Panes A, Villar C, Madrid M. Microbiologic Profile and Predictors of Severe Outcome of Pediatric Cancer with Febrile Neutropenia Admitted at a Tertiary Medical Center. Pediatr Infect Dis Soc J Philippines. 2018 Jul-Dec; 19(2):37-50.
10. Anirban M, Amitabh S. Changing Microbiological Pattern of Pediatric Febrile Neutropenia. J Antimicrob Agents. 2016 Oct;2(126):2472-1212.
11. Hann I, Viscoli C, Paesmans M, Gaya H, Glauser M. A comparison of outcome from febrile neutropenic episodes in children compared with adults: results from four EORTC studies. International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC). Br J Haematol. 1997 Dec;99(3):580-588.
12. Oberoi S, Das A, Trehan A, Ray P, Bansal D. Can complications in febrile neutropenia be predicted? Report from a developing country. Support Care Cancer. 2017 Nov;25(11):3523-3528.
13. Davis K, Wilson S. Febrile neutropenia in paediatric oncology. Paediatr Child Health (Oxford). 2020 Mar;30(3):93-97.
14. Zhang Y, Zheng Y, Dong F, Ma H, Zhu L, Shi D, et al. Epidemiology of Febrile Neutropenia Episodes with Gram-Negative Bacteria Infection in Patients Who Have Undergone Chemotherapy for Hematologic Malignancies: A Retrospective Study of 10 Years' Data from a Single Center. Infect Drug Resist. 2020 Mar 26;13:903-910.
15. Meir HM, Balawi IA, Meer HM, Nayel H, Al-Mobarak MF. Fever and granulocytopenia in children with acute lymphoblastic leukemia under induction therapy. Saudi Med J. 2001 May;22(5):423-427.
16. Rosenblum J, Lin J, Kim M, Levy AS. Repeating blood cultures in neutropenic children with persistent fevers when the initial blood culture is negative. Pediatr Blood Cancer. 2013 Jun;60(6):923-927.
17. Jacob LA, Lakshmaiah KC, Govindbabu K, Suresh TM, Lokanatha D, Sinha M, Vijaykumar BR, Sumathi BG, Jayashree RS. Clinical and microbiological profile of febrile neutropenia in solid tumors and hematological malignancies at a tertiary cancer care center in South India. Indian J Cancer. 2014 Oct-Dec;51(4):464-468.
18. Goruk M, Dal M, Dal T, Karakuş A, Tekin R, Özcan N et al. Evaluation of febrile neutropenic patients hospitalized in a hematology clinic. Asian Pacific Journal of Tropical Biomedicine. 2015 Dec;5(12):1051-1054.
19. Al-Tawfiq JA, Hinedi K, Khairallah H, Saadeh B, Abbasi S, Noureen M, Raza S, Alkhatti A. Epidemiology and source of infection in patients with febrile neutropenia: A ten-year longitudinal study. J Infect Public Health. 2019 May-Jun;12(3):364-366.
20. Zahid KF, Hafeez H, Afzal A. Bacterial spectrum and susceptibility patterns of pathogens in adult febrile neutropenic patients: a comparison between two time periods. J Ayub Med Coll Abbottabad. 2009 Oct-Dec;21(4):146-149.
21. Yilmaz S, Oren H, Demircioğlu F, Irken G. Assessment of febrile neutropenia episodes in children with acute leukemia treated with BFM protocols. Pediatr Hematol Oncol. 2008 Apr-May;25(3):195-204.
22. Taj M, Farzana T, Shah T, Maqsood S, Ahmed SS, Shamsi TS. Clinical and Microbiological Profile of Pathogens in Febrile Neutropenia in Hematological Malignancies: A Single Center Prospective Analysis. J Oncol [Internet]. 2015 Jul 28 [cited 2022 July 12];2015:596504. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4531203/ DOI: 10.1155/2015/596504.
23. Ariffin H, Navaratnam P, Mohamed M, Arasu A, Abdullah WA, Lee CL, et al. Ceftazidime-resistant Klebsiella pneumoniae bloodstream infection in children with febrile neutropenia. Int J Infect Dis. 2000;4(1):21-25.
24. Han SB, Jung SW, Bae EY, Lee JW, Lee DG, Chung NG, et al. Extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae bacteremia in febrile neutropenic children. Microb Drug Resist. 2015 Apr;21(2):244-251.
25. Kim SH, Kwon JC, Choi SM, Lee DG, Park SH, Choi JH, et al. Escherichia coli and Klebsiella pneumoniae bacteremia in patients with neutropenic fever: factors associated with extended-spectrum ß-lactamase production and its impact on outcome. Ann. Hematol. 2013 May;92:533–541.
26. Zaoutis TE, Goyal M, Chu JH, Coffin SE, Bell LM, Nachamkin I, et al. Risk factors for and outcomes of bloodstream infection caused by extendedspectrum ß-lactamase-producing Escherichia coli and Klebsiella species in children. Pediatrics. 2005 Apr;115(4):942–949.
27. Cherif H, Björkholm M, Engervall P, Johansson P, Ljungman P, Hast R, Kalin M. A prospective, randomized study comparing cefepime and imipenem-cilastatin in the empirical treatment of febrile neutropenia in patients treated for haematological malignancies. Scand J Infect Dis. 2004;36(8):593-600.
28. Tamura K, Imajo K, Akiyama N, Suzuki K, Urabe A, Ohyashiki K, et al.; Japan Febrile Neutropenia Study Group. Randomized trial of cefepime monotherapy or cefepime in combination with amikacin as empirical therapy for febrile neutropenia. Clin Infect Dis. 2004 Jul 15;39 Suppl 1:S15-24.
29. Kessler RE. Cefepime microbiologic profile and update. Pediatr Infect Dis J. 2001 Mar;20(3):331-336.
30. Goulenok T, Fantin B. Antimicrobial treatment of febrile neutropenia: pharmacokinetic-pharmacodynamic considerations. Clin Pharmacokinet. 2013 Oct;52(10):869-883.

31. Holmes L Jr, Hossain J, Desvignes-Kendrick M, Opara F. Sex variability in pediatric leukemia survival: large cohort evidence. ISRN Oncol [Internet]. 2012 Apr 3 [cited 2022 July 12];2012:439070. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3324896/ DOI: 10.5402/2012/439070.
32. Sullivan PS, Moreno C, Shaib WL. Management of anorectal and intra-abdominal infections in the neutropenic cancer patient. Curr Probl Cancer. 2015 Sep-Oct;39(5):274-286.
33. Gangat N, Khan MA, Mujib M, Khurshid M. Pulmonary infiltrates during chemotherapy-induced febrile neutropenia: incidence, patterns and outcomes. J Pak Med Assoc. 2004 May;54(5):285-288.
34. Solmaz S, Korur A, Gereklioğlu Ç, Asma S, Büyükkurt N, Kasar M, et al. Anorectal Complications During Neutropenic Period in Patients with Hematologic Diseases. Mediterr J Hematol Infect Dis [Internet]. 2016 Mar [cited 2023 April 13]; 1;8(1):e2016019. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4771136/ DOI: 10.4084/MJHID.2016.019.
35. Williams LA, Spector LG. Survival Differences Between Males and Females Diagnosed With Childhood Cancer. JNCI Cancer Spectr [Internet]. 2019 Jun [cited 2022 July 12];3(2):pkz032. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6580869/ DOI: 10.1093/jncics/pkz032.
36. Orudjev E, Lange BJ. Evolving concepts of management of febrile neutropenia in children with cancer. Med Pediatr Oncol. 2002 Aug;39(2):77-85.
37. Kebudi R, Ayan İ, Görgün Ö, Gürler N. Studies in pediatric febrile neutropenia: 14 years experience. Pediatr Blood Cancer 2004;43:368.
38. Lucas KG, Brown AE, Armstrong D, Chapman D, Heller G. The identification of febrile, neutropenic children with neoplastic disease at low risk for bacteremia and complications of sepsis. Cancer. 1996 Feb 15;77(4):791-798.
39. National Collaborating Centre for Cancer (UK). Neutropenic Sepsis: Prevention and Management of Neutropenic Sepsis in Cancer Patients. London: National Institute for Health and Clinical Excellence (NICE); 2012 Sep. p.5-22.
40. Koh AY. Prolonged febrile neutropenia in the pediatric patient with cancer. Am Soc Clin Oncol Educ Book. 2012:565-569.
41. Karagiannidou S, Zaoutis T, Maniadakis N, Papaevangelou V, Kourlaba G. Attributable length of stay and cost for pediatric and neonatal central line-associated bloodstream infections in Greece. J Infect Public Health. 2019 May-Jun;12(3):372-379.
42. Wolf J, Curtis N, Worth LJ, Flynn PM. Central line-associated bloodstream infection in children: an update on treatment. Pediatr Infect Dis J. 2013 Aug;32(8):905-910.
43. Rogers AE, Eisenman KM, Dolan SA, Belderson KM, Zauche JR, Tong S, et al. Risk factors for bacteremia and central line-associated blood stream infections in children with acute myelogenous leukemia: A single-institution report. Pediatr Blood Cancer [Internet]. 2017 Mar [cited 2022 July 12];64(3). Available from: https://onlinelibrary.wiley.com/doi/10.1002/pbc.26254 DOI: 10.1002/pbc.26254.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

ORIGINAL ARTICLE

THE USE OF ABSOLUTE NEUTROPHIL COUNT AND NEUTROPHIL-LYMPHOCYTE RATIO AS PREDICTORS OF EARLY ONSET NEONATAL SEPSIS

Michelle Jane M. Manding, MD, Expedito T. Yala, MD, FPPS, FPIDSP
Central Luzon Doctors' Hospital, Tarlac City Philippines
4TH PLACE 2023 PIDSP RESEARCH CONTEST

ABSTRACT

Background: Neonatal sepsis contributes to significant morbidity and mortality. Blood culture, the gold standard in its diagnosis, has low sensitivity and is affected by multiple factors. Hence the need for markers derived from routine tests to improve diagnosis deserves further studies.

Objectives: This study aims to determine the association and optimal cut-off value and diagnostic performance of absolute neutrophil count (ANC) and neutrophil lymphocyte ratio (NLR) with early-onset neonatal sepsis in term neonates.

Methodology: This was a retrospective, analytical, single-center study of admitted patients from January 2016 to December 2021. Clinical factors were analyzed and NLR and ANC were derived from CBC and interpreted using the Manroe chart.

Results: Included were 200 neonates with a median birth AOG of 38 weeks. Microorganisms were isolated from nine of 154 neonates with blood culture, corresponding prevalence of 5.84% (95% CI 2.71–10.80). Initial CBC showed elevated mean WBC and 76.5% of neonates were considered to have elevated ANC. Optimal cut-off point of NLR for detecting culture-proven sepsis was 2.86, with a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). The ANC gave the best balance of sensitivity and specificity with an accuracy of 75.50%.

Conclusions: The NLR demonstrated good discriminative ability for predicting clinical neonatal sepsis based on ANC. However, individually or simultaneously, these markers demonstrated poor discriminative ability for culture-proven neonatal sepsis in term neonates. ANC and NLR can be used to aid in the diagnosis of clinical neonatal sepsis.

KEYWORDS: *Absolute Neutrophil Count; Neutrophil to Lymphocyte Ratio, Neonatal Sepsis*

Correspondence:
Dr. Michelle Jane M. Manding
Email: mjmandingmd@gmail.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

INTRODUCTION

Neonatal sepsis (NS) is a significant global problem that contributes to significant morbidity and mortality in newborns, especially in developing countries.[1] As many as 1.6 million neonates die each year due to infection, with 60% deaths occurring in developing countries.[2] It is a clinical syndrome resulting from the invasion of microorganisms into the bloodstream that occurs in the first month of life.[3] Currently, the criteria for diagnosis of NS are mainly based on clinical signs,[4] however, these clinical symptoms resemble other noninfectious neonatal conditions, thus making the diagnosis of NS challenging.[5] The gold standard for diagnosis of NS is blood culture;[6] however, it takes 48 hours to obtain results and has low sensitivity. Studies show that positive cultures are only found in 30-40% of cases[7] and can be affected by several factors such as maternal antimicrobial treatment, inadequate volume of blood, and contamination.[8]

Other parameters used for the diagnosis of NS include complete blood count, C-reactive protein (CRP), and procalcitonin. However, interpretations are challenging because they vary significantly with the day of life and gestational age or has low sensitivity (CRP).[9] Thus, it is of critical importance to identify new biomarkers that will enable fast and reliable hematological scoring systems for sepsis in its early stages. The use of absolute neutrophil count (ANC) has improved the sensitivity in screening for neonatal bacterial disease.[10] A publication by Manroe *et al*., established a method for determining whether a neonate's neutrophil count should be considered neutrophilic, normal, or neutropenic.[11] The physiological immune response of circulating leukocytes to stressful events is characterized by a raised neutrophil count and decreased lymphocyte count. A microbial infection causes an increase of the total leukocyte and neutrophil counts and results in an inflammatory reaction. For this reason, these counts might be used as diagnostic markers of microbial infection.[12,13] Sepsis could give rise to elevated neutrophil counts and decreased lymphocyte counts resulting from infection of pathogenic microorganisms, indicating that septic neonates might have a higher neutrophil-to-lymphocyte ratio (NLR).[14] NLR is considered comparatively to be more stable than absolute neutrophil or lymphocyte counts as both neutrophil and lymphocyte counts are included in the calculation.[15] In a meta-analysis done by Huang, the prognostic value of NLR in sepsis concluded that it may be a helpful prognostic biomarker of patients with sepsis and that higher NLR values may indicate unfavorable prognoses in these patients.[16] However, there have been no studies that compare the utility of NLR in relation to ANC. The predictive value of ANC in early onset neonatal sepsis (EONS) combined with NLR, which is a more accessible, simpler and cheaper inflammatory marker, can lead to early treatment and reduce morbidity and mortality.

The objective of this study was to determine the association of ANC and NLR with EONS in term neonates, adjusting for AOG, birthweight and its appropriateness, and mode of delivery. Furthermore, to describe the demographic, clinical, and laboratory profile of septic neonates and to determine the optimal cut-off value and diagnostic performance (sensitivity, specificity, PPV, NPV, LR+, LR-, diagnostic accuracy, AUC); to predict EONS of the following: ANC, NLR, and simultaneous testing of ANC and NLR; and lastly to enumerate the isolates in blood cultures in early-onset sepsis of term neonates.

MATERIALS AND METHODS

Study Design and Population

This was a retrospective, observational, analytical, single-center study, and the data were obtained from chart review of neonates admitted to the Neonatal Intensive Care Unit of a tertiary training hospital in Tarlac City, Philippines, from January 2016-December 2021. Included in the study were term neonates born by spontaneous singleton delivery and cesarean section with a gestational age of 37 to 42 weeks via Ballard's scoring, appropriate for gestational age (AGA) diagnosed with EONS. Exclusion criteria include patients with incomplete data and those born under 37 weeks and more than

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

42 weeks. Additionally, neonates with mothers who had preeclampsia and gestational diabetes mellitus; are small or large for gestational age, with significant congenital abnormalities, congenital heart diseases, hematologic diseases, and healthcare associated infections were likewise excluded due to their potential as confounders. Patients who were transferred from or to other institutions were also excluded.

Sample Size Computation

A minimum total of 481 term neonates with both CBC and blood cultures done to test for EONS is required for this study; this covers the sample size requirement for both NLR (n = 472) and ANC (n = 481), [17-19] based on a level of significance of 5%, precision at ±0.05, and an area under the curve of 0.586 of ANC to predict sepsis. A total of 472 term neonates with both CBC and blood cultures done, are required for this study based on a level of significance of 5%, precision at ±0.05, and an area under the curve of 0.63 of NLR to predict sepsis. The AUC-based sample size formula was based on Hajian-Tilaki *et al.,* 2014.

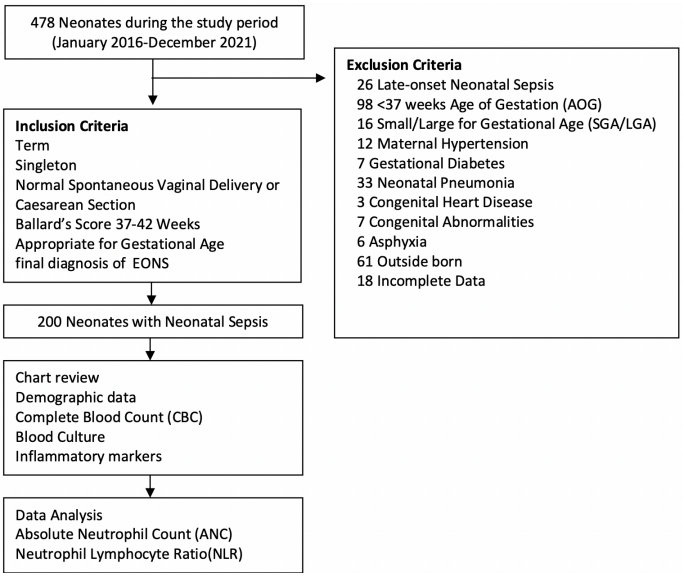

Figure 1. Study Enrollment – of the 478 neonates admitted in the study period, 200 met the study inclusion criteria. Analysis was performed to determine the relationship of EONS with NLR and ANC.

Ethical Consideration

The study protocol was approved by the University of the East Ramon Magsaysay Memorial Medical Center, Inc. Research Institute for Health Sciences Ethics Review Committee. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. This study was partially funded by the Pediatric Infectious Disease Society of the Philippines. The authors declare no conflict of interest.

Data Sampling and Collection

Digital medical records of patients who fulfilled the criteria for the study were retrieved and reviewed. Data collected were entered with a researcher-developed data collection form that included the demographic profile, prenatal and neonatal history, clinical signs, complete blood count, and other relevant laboratory results. Patient data were de-identified into an identification code. All existing data were stored electronically in a password-protected computer for five years, where access to data will only be accessible to the study proponents**.**

Statistical Analysis

Descriptive statistics were used for categorical variables- frequency and percentage. Shapiro-Wilk or test was used to determine the normality distribution. Continuous quantitative data that meet normality assumption were summarized using mean and standard deviation (SD), while those that do not were described using median and range. Continuous variables that are normally distributed were compared using an independent t-test. Otherwise, the non-parametric Mann-Whitney U test was used. For categorical variables, Chi-square test was used to compare the proportion between two groups. If the expected percentages in the cells are less than 5%, Fisher's Exact Test was used.

Receiver operating characteristic (ROC) analysis was performed, and Youden's J index was defined for all points along the ROC curve. The maximum index value (best balance of sensitivity and

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

specificity) was used to select the optimal cut-off point. ROC curves were plotted, giving areas under the curve (AUROC), which could range from 0.5 (no discriminative ability) to 1.0 (perfect discriminative ability). The optimal cut-off value's sensitivity, specificity, predictive values, likelihood ratios, and diagnostic accuracy in detecting EONS were determined. Crude and adjusted odds ratios and the corresponding 95% confidence intervals from logistic regression were computed to determine the associations of ANC and NLR (based on Manroe chart and optimal cut-off values from AUROC) with EONS.

All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 15.0 (StataCorp SE, College Station, TX, USA) was used for data analysis.

ANC is computed as *WBC x Neutrophil x 10*, while NLR is computed by dividing neutrophil count and lymphocyte count.

RESULTS

Our study population included 200 neonates with a median of 38 (range 37-41) weeks AOG. Subjects comprised 57% males, and 76% were delivered via cesarean section (Table 1). The birth length and weight medians were 49 (range 39-59) cm and 2975 (range 2020-3860) grams, respectively. The initial CBC was obtained at a median of the 8th hour of life. The most common clinical signs compatible with sepsis were respiratory distress (32%), poor feeding, (23%), vomiting (22%) and jaundice (11%).

Pathogenic organisms were isolated from 9 of 154 neonates with a blood culture. The corresponding prevalence was 5.84 (95% CI 2.71–10.80) per 100. Microbial growths belonged to the genera *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Pantoea*, *Shigella*, *Staphylococcus*, and *Streptococcus* from initial blood culture results.

Table 1. Neonatal characteristics of all septic neonates admitted in a Neonatal Intensive Care Unit from January 2016-December 2020

	All (n=200)	Culture-positive (n=9)
	Median (Range);	Frequency (%)
AOG, weeks	38 (37-41)	39 (37-39)
Sex		
Male	114 (57.00)	4 (44.44)
Female	86 (43.00)	5 (55.55)
Mode of delivery		
Cesarean section	152 (76.00)	8 (88.89)
Normal spontaneous	48 (24.00)	1 (11.11)
Birth length, cm	49 (39-59)	50 (45-57)
Birthweight, grams	2975 (2020-3860)	3000 (2300-3500)
2001-2500	30 (15.00)	2 (22.22)
2501-3000	88 (44.00)	3 (33.33)
3001-3500	69 (34.50)	4 (44.44)
3501-4000	13 (6.50)	0
Circumference, cm		
Head	34 (30-37)	34 (31-37)
Chest	32 (24-36)	32 (30-35)
Abdominal	30 (24-36)	29 (26-34)
CBC timing, hour of life	8 (1-144)	8 (6-48)
Clinical signs of sepsis		
Respiratory distress	64 (32.00)	4 (44.44)
Vomiting	45 (22.50)	2 (22.22)
Poor feeding	47 (23.50)	1 (11.11)
Jaundice	22 (11.00)	1 (11.11)
Cyanosis	21 (10.50)	1 (11.11)
Tachycardia	1 (0.50)	0
Apnea	0	0
Bradycardia	0	0
Lethargy	0	0
Isolated pathogen		
Pseudomonas stutzeri	-	2 (22.22)
Acinetobacter baumannii	-	1 (11.11)
Burkholderia cepacia	-	1 (11.11)
CoNS	-	1 (11.11)
Pantoea agglomerans	-	1 (11.11)
Shigella spp.	-	1 (11.11)
Staphylococcus epidermidis	-	1 (11.11)
Streptococcus agalactiae	-	1 (11.11)

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

Initial CBC showed elevated mean (±SD) WBC count of 26.25 ± 8.37 x10^9/l and ANC of 17647.88 ± 6593.41 x10^6/l (Table 2). 153 (76.5%) neonates were considered to have elevated ANC using the Manroe chart. The average neutrophil and lymphocyte differential counts were 65.61% and 21.72%, respectively, and the median NLR was 3.35 (range 0.51-11.11).

Table 2. Initial CBC and other parameters, Procalcitonin and C-Reactive Protein of all septic neonates admitted in a Neonatal Intensive Care Unit from January 2016- December 2020

Parameters	All (n=200)	Culture-positive (n=9)
	Mean ± SD; Median (Range); Frequency (%)	
Procalcitonin, ng/mL [n=6]		
<0.5	0	-
≥0.5	6 (100)	-
CRP, mg/L [n=3]		
<1.0	1 (33.33)	-
1.0-3.0	1 (33.33)	-
>3.0	1 (33.33)	-
Complete Blood Count		
Hemoglobin g/dL	17.58 ± 2.70	17.78 ± 2.99
Hematocrit %	51.92 ± 8.48	52.41 ± 8.70
Platelet x1000/µL	280.56 ± 72.95	283.44 ± 60.35
WBC x1000/µL	26.25 ± 8.37	29.89 ± 8.36
Neutrophils, %	65.61 ± 9.84	68.04 ± 6.76
Lymphocytes, %	21.72 ± 9.14	20.57 ± 7.83
NLR	3.35 (0.51-11.11)	3.68 (1.30-6.35)
ANC cells/µL	17647.88 ± 6593.41	20433.56 ± 6297.76
ANC classification		
Normal	47 (23.50)	1 (11.11)
Elevated	153 (76.50)	8 (88.89)

ANC and NLR, individually or simultaneously, demonstrated poor discriminative ability for culture-proven NS in term neonates (n=9/154 neonates), with AUCs ranging from 0.569 to 0.636 (Table 3, Figure 1).

Table 3. Predictive abilities of CBC-derived values for culture-proven EOS in term neonates (n=154)

	AUC (95% CI)
ANC	0.635 (0.430–0.840)
NLR	0.569 (0.399–0.739)
ANC and NLR	0.636 (0.422–0.850)

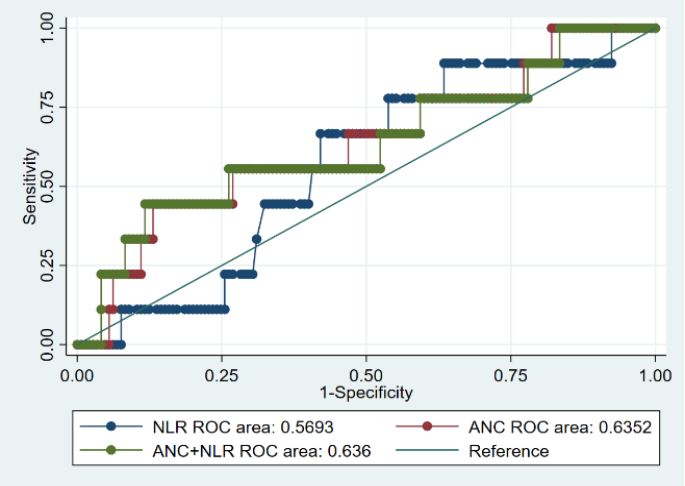

Figure 2. ROC curves of ANC and NLR, individually and simultaneously, to predict culture-proven EONS.

The optimal cut-off point of NLR for detecting culture-proven NS was 2.86, and values greater than or equal to this gave a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). Its overall accuracy was 39.61% (95% CI, 31.83–47.80%) (Table 4). For elevated ANC by Manroe chart, the sensitivity was similar at 88.89% (95% CI, 51.75–99.72%), specificity lower at 25.52% (95% CI, 18.65–33.42%), and overall accuracy lower at 29.22% (95% CI, 22.18–37.08%).

Table 4. Diagnostic performance of elevated ANC and NLR in screening for culture-proven EOS in term neonates (n=154)

	Elevated ANC	NLR ≥ 2.86
	Point Estimate (95% CI)	
Sn, %	88.89 (51.75–99.72)	88.89 (51.75–99.72)
Sp, %	25.52 (18.65–33.42)	36.55 (28.72–44.95)
PPV, %	6.90 (5.45–8.68)	8.00 (6.27–10.15)
NPV, %	97.37 (85.10–99.58)	98.15 (89.19–99.71)
Positive LR	1.19 (0.93–1.53)	1.40 (1.08–1.82)
Negative LR	0.44 (0.07–2.82)	0.30 (0.05–1.95)
Accuracy, %	29.22 (22.18–37.08)	39.61 (31.83–47.80)

Sn – Sensitivity; Sp – Specificity; LR – Likelihood Ratio

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

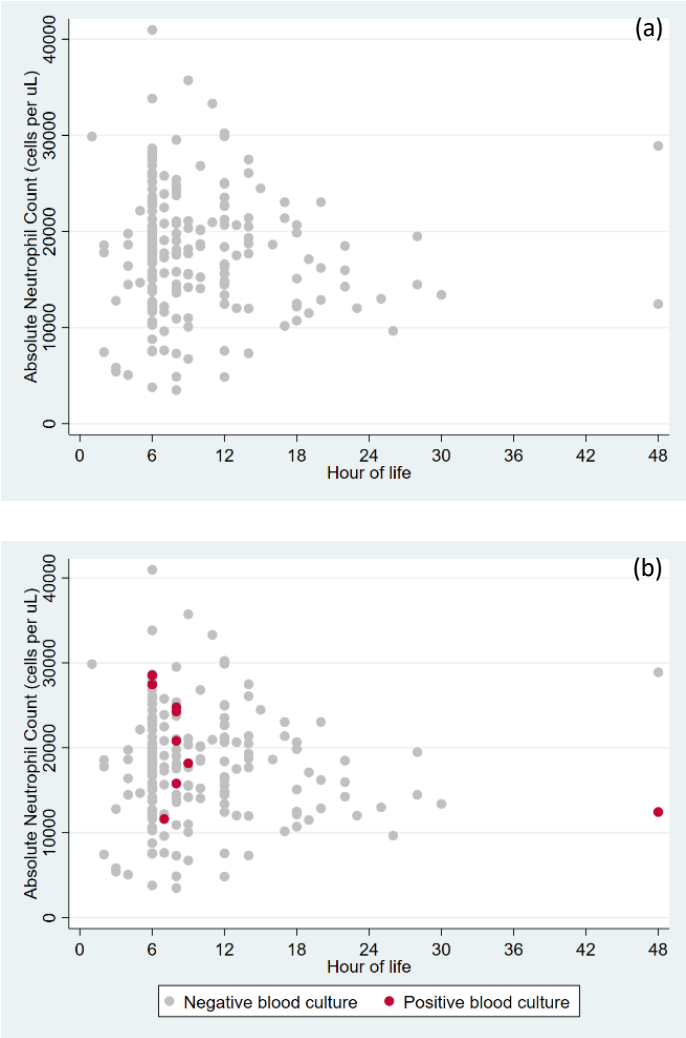

Figure 3. Absolute neutrophil counts were plotted according to hour of life when CBC was taken. (a) All neonates (n=200); (b) Neonates with blood culture (n=154). Red points indicate those that showed growth on culture.

Table 5. Association of ANC classification and NLR with culture-proven EOS in term neonates (n=154)

	Crude OR (95% CI)	*P*	Adjusted* OR (95% CI)	*P*
ANC classification				
Normal	Reference	-	Reference	-
Elevated	2.74 (0.33–22.65)	.349	1.30 (0.12–13.77)	.825
NLR				
<2.86	Reference	-	Reference	-
≥2.86	4.61 (0.56–37.87)	.155	4.09 (0.40–42.22)	.237

*Controlled for birth AOG, sex, and mode of delivery

NLR demonstrated good discriminative ability for predicting clinical NS using the ANC classification based on the Manroe chart in term neonates (AUC = 0.8580; 95% CI 0.8005 to 0.9156) (Figure 4).

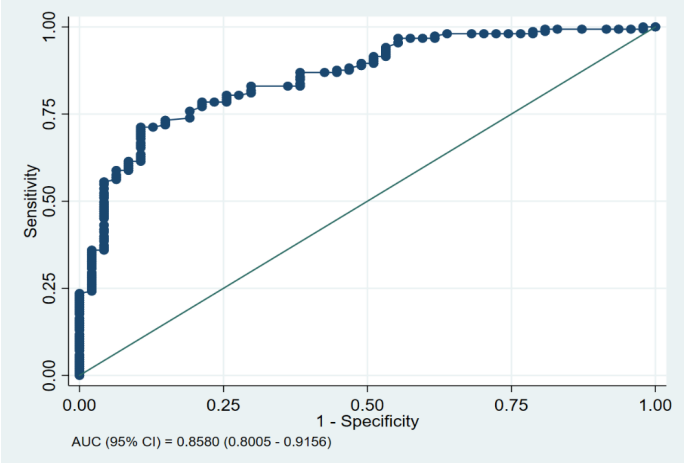

Figure 4. NLR discriminative ability for predicting clinical neonatal sepsis using the ANC classification

Youden's J index was used to determine the optimal cut-off point for NLR in predicting clinical NS using the ANC classification based on the Manroe chart, which gives the point with the best balance of sensitivity and specificity. NLR ≥ 3.06 had a fair sensitivity of 71.24%, high specificity of 89.36%, and an accuracy of 75.50% (Table 6).

Table 6. Diagnostic performance of optimal cut-off point of NLR in screening for clinical sepsis based on ANC classification from the Manroe chart (n=200)

	NLR
Disease prevalence, % (95% CI)	76.50 (70.00–82.19)
Sensitivity, % (95% CI)	71.24 (63.38–78.26)
Specificity, % (95% CI)	89.36 (76.90–96.45)
PPV, % (95% CI)	95.61 (90.44–98.05)
NPV, % (95% CI)	48.84 (42.20–55.52)
Positive likelihood ratio (95% CI)	6.70 (2.91–15.43)
Negative likelihood ratio (95% CI)	0.32 (0.25–0.42)
Accuracy, % (95% CI)	75.50 (68.94–81.29)

Term neonates with NLR ≥ 3.06 had about 20 to 24-fold increase in odds to have clinical NS based on the ANC classification from the Manroe chart, whether by crude association or after adjusting for AOG, sex, and mode of delivery.

Table 7. Association of NLR with clinical NS based on ANC classification from the Manroe chart (n=200)

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 31-40 July-December 2023
Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
https://doi.org/10.56964/pidspj20232402005

	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
NLR				
<3.06	Reference	-	Reference	-
≥3.06	**20.81 (7.72–56.06)**	**<.001**	**24.20 (8.61–68.02)**	**<.001**

Adjusted analysis accounted for the following variables: AOG, sex, mode of delivery

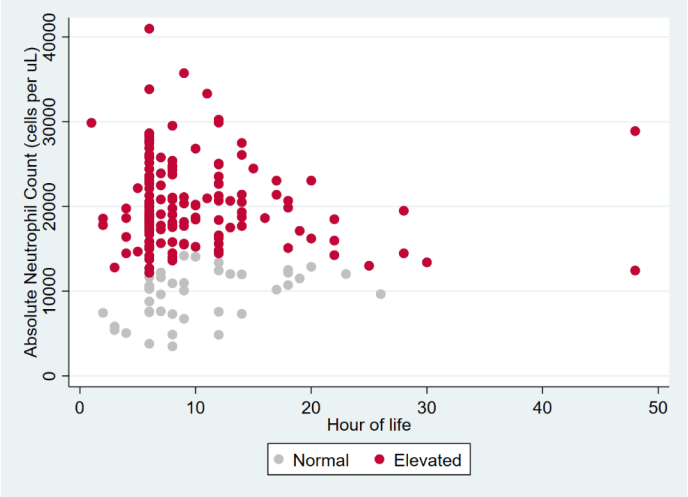

Figure 5. ANC classification based on Manroe chart

DISCUSSION

Our study showed that the most common clinical signs observed in patients with neonatal sepsis in this study included respiratory distress (32%), poor feeding (23%), and jaundice (11%). Microorganisms were isolated from nine of 154 neonates with blood culture, a corresponding prevalence of 5.84% (95% CI 2.71–10.80). Initial CBC showed elevated mean WBC and 76.5% of neonates considered to have elevated ANC. The optimal cut-off point of NLR for detecting culture-proven sepsis was 2.86, with a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). The ANC gave the best balance of sensitivity and specificity with an accuracy of 75.50%.

The clinical profile findings are in accordance with the study conducted by Nasser et al., where respiratory distress was the most frequent clinical sign of EONS at 60%, followed by tachycardia (10%), jaundice, lethargy, and apnea (6.7 %), and lastly poor feeding, bradycardia, and cyanosis by 3.3% .[20] On the other hand, Can *et al,.* found the main presenting signs were apnea and bradycardia (31%), followed by tachycardia (29%), jaundice, poor feeding, and lastly, respiratory distress (5%).[21]

Albeit the gold standard for the diagnosis of sepsis, as shown by the low prevalence of culture-proven sepsis in our study, the rate of yield of blood culture is low [22] especially in developing countries wherein clinically diagnosed sepsis is present in 49-170 per 1000 births and the culture proven sepsis was at 16 per 1000 live births.[23] Results takes up to 48 hours to obtain which may be affected by multitude of factors such as inadequate blood volume, contamination and maternal antimicrobial use.[22] In our study, we yielded no predominant microbial growth but instead included *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Pantoea*, *Staphylococcus*, and *Streptococcus* in comparison to commonly reported microorganisms in EONS which includes *S. agalactiae* and *E.coli*.[20] The blood culture were obtained from the first day of life and the results may have been influenced multiple factors.

CBC is a conventional laboratory test to aid in the diagnosis of sepsis and we derive multiple inflammatory markers which includes total leukocyte count, immature to total neutrophil count and ANC. However, WBC has been of little clinical use due to its broad variation hence Manroe chart was widely utilized[24] which shows the total neutrophil count in the first 60 hours of life.[10] In our study, initial CBC showed an elevated (±SD) WBC count of 26.25 ± 8.37 $x10^9$/l and ANC of 17647.88 ± 6593.41 $x10^6$/l. 153 (76.5%) neonates were considered to have elevated ANC as plotted in the chart.

NLR is considered more stable than absolute neutrophil or lymphocyte counts since both neutrophils and lymphocytes are included in the computation.[22] Normal NLR values in healthy neonatal or pediatric populations have been reported with an average value of 0.52- 0.91.[25] In our study, the cut-off point for NLR in predicting clinical NS using ANC classification based on the Manroe chart was ≥3.06, which had fair sensitivity at 71.24% and high specificity at 89.63%. In contrast, the optimal cut-off point of NLR for detecting culture-

proven NS was lower at 2.86, and values greater than or equal to this gave a sensitivity of 88.89% and specificity of 36.55%. Various studies reported cut-off points close to our determined value: Omran *et al.,* identified that NLR, at a cut-off value of 2.7, had a sensitivity of 80% and specificity of 57.1%[26], while Wilar *et al.,* found that the mean NLR from EONS and non-EONS group was 2.82±2.29 (sensitivity of 83.3% and specificity 57.1%) and 0.82±0.32, respectively.[27] In a similar study population, higher mean NLR was found in those with positive blood cultures at 3.69 (±3.0) than those with negative blood cultures at 1.56 (±1.83).[28] These studies had similar findings which showed a higher NLR value on septic neonates versus the control group. On the other hand, Can *et al.,* found a higher predictive cut-off value of NLR for EONS at 6.76 (sensitivity 97.4%; specificity 100%) compared in our study,[21] while another study determined a lower predictive NLR cut off value in neonates with NS at 1.81 with 86.1% sensitivity, 85.1% specificity, 68.9% PPV, and 94.1% NPV.[29]

Several studies reported significantly higher NLR in septic neonates compared to healthy neonates. Comparing the determined cut-off point of NLR at 2.86 for detecting culture-proven NS in those with elevated ANC by Manroe chart, the sensitivity was similar at 88.89% specificity lower at 25.52%. Furthermore, NLR demonstrated good discriminative ability to predict clinical NS using ANC classification when compared using the classification based on the Manroe chart. In a study done by Uri *et al.,* results show that the markers ANC and NLR have similar sensitivity in identifying serious bacterial disease.[31] Term neonates with NLR >3.06 had about 20 to 24 fold increase in odds of clinical NS. However, NLR showed no significant association with culture-proven EONS outcomes. This is in contrast with the study done by Sumitro *et al.,* where neonates with an NLR ≥2.12 have almost twice the risk of giving a positive blood culture, and when combined with CRP≥2.70mg/dL, the risk of providing positive blood culture results is more than doubled.[32] On the other hand, one study showed that NLR when combined with CRP would have better accuracy in diagnosing sepsis.[28] Inclusion of NLR as a new diagnostic marker for EONS is reinforced in this study in context with previous studies and the management of sepsis are mainly based on clinical signs which are nonspecific.[4] Blood cultures lack sensitivity due to specific characteristics of the neonatal population[33] and results can be affected by multiple factors. As a result, a combination of findings may then explain the poor discriminative ability in culture proven sepsis, but a good discriminative ability in clinically diagnosed NS.

Interpretations of our findings should take into account study limitations. The study was done in a single center with relatively small sample size in a retrospective manner. Also, the study did not include healthy neonates in comparing the utility of NLR and ANC. Some subjects also did not have culture results or culture was not done which may have contributed to the low yield of positive results.

CONCLUSION AND RECOMMENDATIONS

We demonstrated the good discriminative ability of NLR in predicting clinical NS based on the ANC of Manroe. However, ANC and NLR, individually or simultaneously, demonstrated poor discriminative ability for culture-proven NS in term neonates. ANC and NLR, can be used as an additional marker of infection to aid in the diagnosis of clinical NS and can be further evaluated in a prospective analysis with larger population and in subsets of patients with culture proven sepsis. A comparison of its diagnostic performance may further be established when compared to healthy neonates and may also be correlated with other inflammatory markers such as ESR, CRP, and procalcitonin.

REFERENCES

1. Alkan Ozdemir S, Arun Ozer E, Ilhan O, et al. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal. May 2018; 32:e22338.
2. Haque KN, et al. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. May 2005; 6: S45-S49.
3. Rusell AB, Isaacs D. Rennie JM, Robertson NR. Infection in the newborn. 5th edition, London: Churchill Livingston; 2012.p.1013-1031.
4. Mukhopadhyay S, Puopolo KM. Risk assessment in neonatal early onset sepsis. Seminars in Perinatology 2012 [Cited

2021 Sep 01];36. Available from https:// doi.org/: 10.1053/j.

5. Li T, Dong G, Zhang M, et al. Association of neutrophil-lymphocyte ratio and the presence of neonatal sepsis. J of Immunol Research 2020 [Cited 2021 Sep 01];Available from https://doi.org/10.1155/2020/765071.
6. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatric Critical Care Medicine 2005 [Cited 2021 Sep 01]; 6. Available from https://doi.org/10.1097/01.PCC.0000149131.72248.E6.
7. Ferrieri P, Wallen LD, Gleason CA, et al. Avery's disease of the newborn. Philadelphia (PA) : Saunders Elsevier, 2012. p.538-550.
8. Iroh Tam PY, Bendel CM. Diagnostics for neonatal sepsis: current approaches and future directions. Pediatric Research 2017 [Cited 2021 Sep 15];82. Available from https://doi.org/10.1038/pr.2017.134.
9. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal Infectious Diseases: Evaluation of Neonatal Sepsis. North Am : Pediatr Clin, 2013, Vol. 367.
10. Manroe BL, Weinberg AG, Rosenfeld CR, et al. The neonatal blood count in health and disease.I. Reference values for neutrophilic cells. The Journal of Pediatrics 1979 [Cited 2021 Oct 03];95. Available from https://doi.org/10.1016/s0022-3476(79)80096-7.
11. Das A, Ray S, Chattopadhyay A, et al. Gestation-wise reference ranges of neutrophil counts in Indian newborns. Oman Medical Journal 2019 [Cited 2021 Mar 28]; 34. Available from https://doi.org/10.5001/omj.2019.25.
12. Elawady S, Botros SK, Sorour AE, et al. Neutrophil CD64 as a Diagnostic Marker of Sepsis in Neonates. Pediatr Infect Dis J 2014 https://doi.org/10.2310/JIM.0000000000000060
13.. Yoon NB, Son C, Um SJ. Role of the neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. Ann Lab Med 2013 Mar;33(2):105-110.
14. Shen XF, Cao K, Jiang JP, et al. Neutrophil dysregulation during sepsis: an overview and update. J of Cellular and Molecular Medicine 2017; 21: p1687-1697.
15. Dirican A, Kucukzeybek B, Alacacioglu A, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? International Journal of Clinical Oncology. 2015 [Cited 2021 Mar 28]; 20. Available from https://doi.org/doi: 10.1007/s1014.
16. Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med 2020 [Cited 2021 Mar 03]; 38. Available from https://doi.org/doi: 10.1016/j.ajem .
17. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *J Biomed Inform* 2014; 48:193 – 204. Accessed from https://www.ncbi.nlm.nih.gov/pubmed/24582925.
18. Li T, Dong G, Zhang M, Xu Z, Hu Y, Xie B, Wang Y, Xu B. Association of Neutrophil–Lymphocyte Ratio and the Presence of Neonatal Sepsis. Journal of Immunology Research. 2020 Dec 2;2020.
19. Hornik CP, Benjamin DK, Becker KC, Benjamin Jr DK, Li J, Clark RH, Cohen-Wolkowiez M, Smith PB. Use of the complete blood cell count in early-onset neonatal sepsis. The Pediatric infectious disease journal. 2012 Aug;31(8):799
20. Nasser MM, Afia AA, El-Khatib GZ, Ibrahim MI. The value of neutrophil to lymphocyte ratio and procalcitonin level for detecting early-onset neonatal sepsis. Journal of Pediatrics 2020 [Cited 2021 Jan 02]; 23. Available from https://doi.org/10.21608/azjp.2020.85895.
21. Can E, Hamilcikan Ş, Can C. et al. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol 2018 [Cited Jan 27]; 40. Available from PMID: 29219889.
22. Li T, Dong G, Zhang M, et al. Association of neutrophil lymphocyte ratio and the presence of neonatal sepsis. J of Immunology Research 2020 [Cited 2021 Mar 28]. Available from https://doi.org/10.1155/2020/7650713.
23. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. The New England Journal of Medicine 2005; 352: 1-13.
24. Stearns-kurosawa, DJ, Osuchwoski M, Valentine C, et al. The pathogenesis of sepsis. Annual Review of Pathology 2011; 6:19-48.
25. Hamiel U, Bahat H, Kozer E, et al. Diagnostic markers of acute infections in infants aged 1 week to 3 months: A retrospective cohort study. BMJ Open 2018; 8:e018092.
26. Omran A, Maaroof A, Saleh MH, Abdelwahab A. Salivary C-reactive protein, mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr 2018 [Cited 2021 Jan 27]; 94. Available from https://doi.org/10.1016/j.jped.2017.03.006. Epub 201.
27. M, Weitzman. Diagnostic utility of white blood cell counts and differential counts. Am J Dis Child 1975; 129:1183.
28. Sumitro KR, Utomo1 MT, Widodo AD. Neutrophil-to-lymphocyte ratio as an alternative marker of neonatal sepsis in developing countries. Oman Medical Journal 2021 [Cited 2021 Jan 27]; 36. Available from https://doi.org/10.5001/omj.2021.05.
29. Nugroho ID, Hidayah D, Yulidar H. Diagnostic value of neutrophil to lymphocyte ratio as an early marker in infected neonates. Universitas Sebelas Maret Surakarta / Regional General Hospital, April 2018. [Cited 2021 Nov 15]. Available from https://eprints.uns.ac.id/40470/

30. Harmansyah H, Alasiry E, Daud D. Absolute neutrophil count as predictor of early onset sepsis. Clinical Medicine Research 2015; 4. 87.10.11648/j.cmr.20150403.15.
31. Uri H., Hilla B., Eran K., Yotam H.,. Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. 2018, [Cited 2021 Nov 15]. BMJ Open.
32 Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: a meta-analysis. The American Journal of Emergency Medicine 2020; 38: [Cited 2021 Mar 15]
33. Zea-Vera A, Ochoa T J. Challenges in the diagnosis and management of neonatal sepsis. Journal of Tropical Pediatrics 2015 [Cited 2021 Feb 02]; 61. Available from https://doi.org/10.1093/tropej/fmu079.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 41-51 July-December 2023
Garcia RDJ. Multi-system Inflammatory Syndrome in Children in two private, urban, tertiary hospitals in Metro Manila, Philippines. https://doi.org/10.56964/pidspj20232402006

ORIGINAL ARTICLE

MULTI-SYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C) IN TWO PRIVATE, URBAN, TERTIARY HOSPITALS IN METRO MANILA, PHILIPPINES

Robert Dennis J. Garcia, MD, MHSA
Section Chief, Pediatric Infectious Diseases, Makati Medical Center
Active Consultant, Department of Pediatrics, Cardinal Santos Medical Center

ABSTRACT

Background: MIS-C is an infrequent, but serious complication encountered after acquiring COVID-19 illness in children. There is a lack of local data on MIS-C in the Philippines.

Objective: To identify demographic data, co-morbidities, clinical manifestations, laboratory results, 2D-echocardiography findings, acute co-illnesses and complications, treatment, and outcome of children with MIS-C, seen in two, private, urban, tertiary hospitals.

Methodology: This is a retrospective, descriptive study of all consecutive MIS-C cases, using the 2020 US CDC definition, seen between July 2020 to January 2023, by a single infectious disease physician. Demographic, epidemiologic, clinical, and physical examination findings; results of laboratory, 2-DE, and radiologic tests; co-illnesses and complications; and therapeutic and outcome data, were entered in a case report form for each patient.

Results: Thirty-six patients were seen. MIS-C cases had a median age of 6 years, presented with fever in 97%, while one-half had abdominal pain, vomiting, diarrhea and/or rash. CRP, D-dimer, ferritin, LDH and procalcitonin were generally elevated, and thrombocytopenia was seen in 39%. The most common 2-DE abnormalities were pericardial effusion (50%), coronary artery dilatation or aneurysm (39%) and mitral regurgitation (36%); the 2-DE was normal in 22%. The main complications were pneumonia (31%), myocarditis (28%) and hypotension (14%); 8% had ARDS. Treatment was with corticosteroids (89%) and IVIG (84%). Most (94%) recovered, and the hospital stay was five days, or less, in 86%. The two mortalities were a severely wasted adolescent with previously undiagnosed HIV infection; and an adolescent on chemotherapy for AML, who was also being treated for disseminated TB.

Conclusions: There is a need to create a greater awareness of MIS-C as, like Kawasaki disease, it has the potential to be an important cause of acquired heart disease among children.

KEYWORDS: *MIS-C, Filipino, Pediatric*

Correspondence:
Dr. Robert Dennis J. Garcia
Email: rdgarcia59@yahoo.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 41-51 July-December 2023
Garcia RDJ. Multi-system Inflammatory Syndrome in Children in two private, urban, tertiary hospitals in Metro Manila, Philippines. https://doi.org/10.56964/pidspj20232402006

INTRODUCTION

Among children, Multisystem Inflammatory Syndrome in Children (MIS-C) is an infrequent, but serious complication encountered after acquiring COVID-19 infection, as it can lead to myocarditis, cardiac dysfunction, coronary artery aneurysms, and in severe cases, multi-organ dysfunction and death.[1-2] The cause of MIS-C is still incompletely understood, but it is thought to result from an abnormal immune response to COVID-19 infection. Endothelial dysfunction and cytokine storm have been mechanisms proposed, that result in end-organ injury.[3]

There is scarce local data on MIS-C. There were 16 MIS-C cases in an interim report of the SALVACION registry, five in one case series, and one newborn (MIS-N) in one case report.[4-6]

There is a need to describe the epidemiology, clinical and diagnostic findings, treatment and outcome of local cases for this important and serious post-COVID-19 complication seen in children.

The purpose of this study is to identify demographic data, co-morbidities, clinical manifestations, laboratory results, 2D-echocardiography (2-DE) findings, acute co-illnesses and complications, treatment and outcome of children, 18 years and younger, diagnosed to have MIS-C, seen in two, private, urban, tertiary hospitals, who were referred to a single pediatric infectious disease physician.

MATERIALS AND METHODS

This is a retrospective, descriptive study of MIS-C cases that were seen between July 2020 to January 2023. Each consecutive inpatient admission or referral of a patient 18 year old and below, with a discharge diagnosis of MIS-C was included in this study. Demographic, epidemiologic, clinical, and physical examination findings; results of laboratory, 2-DE, and radiologic tests; co-illnesses and complications; and therapeutic and outcome data relevant to the MIS-C diagnosis, were entered in a case report form for each patient by the author. The inclusion criteria for MIS-C utilized the 2020 U.S. C.D.C. definition, as follows:[7]

1. An individual aged <21 years presenting with fever, laboratory evidence of inflammation, and evidence of clinically severe illness requiring hospitalization, with multi-system (=/>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
2. No alternative plausible diagnoses; AND
3. Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the four weeks prior to onset of symptoms.

Excluded were patients diagnosed with Kawasaki disease with no laboratory or epidemiologic exposure to anyone with COVID-19 in the four weeks prior to illness onset.

This study was approved by each hospital's Institutional Review Board. As all the cases were obtained from the author's personal files in a password-protected, personal computer, no medical records were accessed from the hospitals' medical records department.

RESULTS

Table 1. Demographics & co-morbidities of patients with MIS-C from July 2020 to January 2023 (n=36)

Age	**Years**
Mean	6.4
Median	6
Range	NB[a] to 15
Sex	**n (%)**
Male	23 (64%)
Female	13 (36%)
Comorbidity	**n (%)**
Obese/overweight	3 (8%)
Tuberculosis[b]	2 (6%)
AML[c] on chemotherapy	1 (3%)
HIV infection with severe wasting	1 (3%)
Hemophagocytic lymphohistiocytosis	1 (3%)
Hypothyroidism	1 (3%)

[a]Newborn; [b]Disseminated and pulmonary; [c]Acute myelogenous leukemia

The mean age was 6.4 years; 64% were males. There were three patients who were <4 months of age. The top co-morbidity before the hospital admission was obesity (8%).

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 41-51 July-December 2023
Garcia RDJ. Multi-system Inflammatory Syndrome in Children in two private, urban, tertiary hospitals in Metro Manila, Philippines. https://doi.org/10.56964/pidspj20232402006

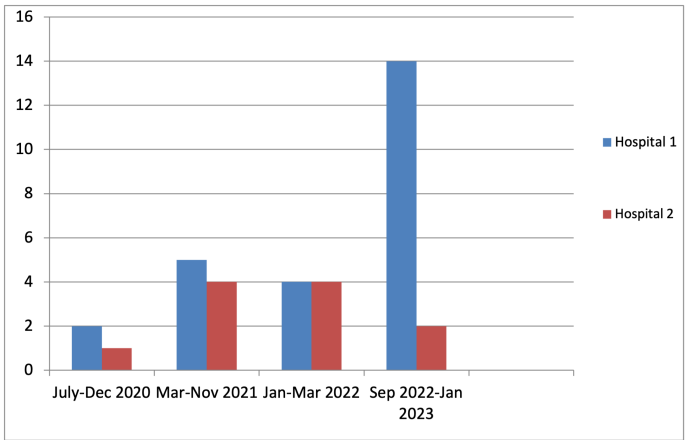

Figure 1: MIS-C cases during different time periods, July 2020 to January 2023 (n=36)

There were four surges in cases between July 2020 to January 2023, with the highest one between September 2022 to January 2023.

Table 2: Signs and symptoms of MIS-C cases, July 2020 to January 2023 (n=36)

Clinical finding	n (%)
Fever	35 (97%)
Vomiting	19 (53%)
Cough	17 (47%)
Abdominal pain	17 (47%)
Rash	15 (42%)
Diarrhea	12 (33%)
Conjunctivitis	12 (33%)
Extremity redness	6 (17%)
Mouth changes	5 (14%)
Difficulty breathing	4 (11%)
Cervical lymphadenitis	2 (6%)

All but one had fever (the only afebrile case was a newborn), while about half of cases had vomiting, cough, abdominal pain, and a rash.

Table 3: Complete blood count and inflammatory parameters of MIS-C cases, July 2020 to January 2023 (n=36)

Laboratory parameter	Result
WBC, lowest value, mean	6,700/mm^3
Hematocrit, lowest value, mean	0.33
Proportion with platelet count <150,000/mm^3	39%
CRP, highest value, mean	71 mg/l
D-dimer, highest value, mean	2,172 ng/ml
Ferritin, highest value, mean	2,246 ug/l
LDH, highest value, mean	532 IU/l
Procalcitonin, highest value, mean	1.6 ng/ml
SGPT, highest value, mean (n=17)	85 U/l

The mean lowest WBC count was within the normal range, while thrombocytopenia was seen in 39% of patients. The inflammatory markers (CRP, D-dimer and ferritin) were generally elevated. The highest recorded value of the inflammatory markers for each patient was recorded, and the mean for these highest values was taken and noted to be elevated. Procalcitonin was elevated in 8 out of 9 patients, or 89%, even as none of the patients were documented to be bacteremic.

Table 4: Proportion of cardiac markers elevated, and highest level, MIS-C cases, July 2020 to January 2023 (n=36)

Cardiac marker	Result
Pro-BNP	**n = 28**
Elevated	22 (79%)
Highest level, mean	2,507 pg/ml
Troponin-I	**n = 21**
Elevated	8 (38%)
Highest level, mean	0.11 ng/ml
CPK-MB	**n = 14**
Elevated	3 (21%)
Highest level	17.9 ng/ml; 52.7 U/L

The pro-BNP was the cardiac marker that was most often elevated among those tested. The unit of measurement of CPK-MB was different for the two hospitals. The mean CPK-MB of 17.9 ng/ml in one hospital was high, as the figure was raised by two outlier measurements of 110 ng/ml and 23 ng/ml from two patients who had severe MIS-C illness, while the rest ranged from 0.4 to 4.5 ng/ml.

Table 5: 2-D echocardiography findings, MIS-C cases, July 2020 to January 2023 (n=36)

Finding	n (%)
Pericardial effusion	19 (50%)
Coronary artery dilatation/aneurysm	14 (39%)
Mitral regurgitation	13 (36%)
Peri-vascular brightness	9 (25%)
Tricuspid regurgitation	6 (17%)
Decreased ejection fraction	4 (11%)
Dilated left ventricle	1 (3%)

The 2-DEs were read by five pediatric cardiologists in the two hospitals. Pericardial effusion (50%), coronary artery ectasia or aneurysm (39%)

and mitral regurgitation (36%) were most commonly seen. A normal 2-DE result was seen in 8 (22%).

Table 6: Acute co-illnesses and complications during the admission (n=36)

Finding	n (%)
Pneumonia	11 (31%)
Myocarditis	10 (28%)
Hypotension	5 (14%)
ARDS	3 (8%)
Dengue fever	2 (6%)
New-onset severe reactive airway disease	2 (6%)
Ventricular arrhythmia	2 (6%)
Pleural effusion	2 (6%)
Rhinovirus PCR-positive bronchiolitis	1 (3%)
Secondary sepsis	0

Myocarditis was documented in 28%; hypotension was noted in 14%, with 8% requiring vasopressors. Acute respiratory distress syndrome (ARDS) occurred in 8%.

Table 7: Treatment and supportive measures given to MIS-C cases, July 2020 to January 2023 (n=36)

Corticosteroid	**89%**
Methylprednisolone 2 mg/kg/day	61%
Methylprednisolone 5 mg/kg/day	3%
Methylprednisolone 10 mg/kg/day	11%
Methylprednisolone 30 mg/kg/day	8%
Dexamethasone	6%
IVIg	**83%**
No IVIg given for:	
Mild disease, 2-DE normal	8%
Refused (DAMA, doctor's choice)	6%
No funds	3%
Others	
Antibacterials	31%
Epinephrine drip	8%
Enoxaparin	8%
Tocilizumab	8%
Mechanical ventilation	6%
Anti-tuberculosis medications	6%
Dobutamine drip	6%
Casirivimab-indevimab monoclonal antibody	3%

Corticosteroids (89%) and IVIG (83%) were most commonly used. All, but one, went home on oral prednisone over 2-4 weeks.

Table 8: Outcome of MIS-C cases, July 2020 to January 2023 (n=36)

Outcome	n (%)
Length of stay ≤5 days	31 (86%)
Discharged on prednisone	35 (97%)
Discharged on digoxin (lanoxin)	2 (6%)
Died	2 (6%)
HIV, severe wasting, shock, ARDS, MODS	
AML, on chemotherapy, died in another hospital after transfer from study hospital	
Home against advice, outcome unknown	1 (3%)

The hospital course was five days, or less, in 86%. The mortality rate was 6%, both occurring in severely immunocompromised individuals.

DISCUSSION

The total number of MIS-C cases seen by one infectious disease physician in the two hospitals was 36. This number is 47% of all the COVID-related cases referred to the author in the same time period (data not shown). In the first hospital, there were 25 cases, while in the second, there were 11. A Swedish population-based study determined a MIS-C rate of 6.8 Swedish children per 100,000-person-years.[8] The reported<20-year-old populations for the two cities in which the study hospitals are located are 181,038 and 39,962.[9-10] If the Swedish MIS-C rate is applied to the two local populations, the expected number of MIS-C cases over three years would be 36.9 children in the first city and 8.2 children in the second, or a total of 45 cases over three years. As there are three other hospitals in the two cities, and other infectious disease doctors in the two hospitals, the number of cases seen in this report (36) is not far from the 45 cases projected, extrapolating from the MIS-C rate reported in Sweden.

There were three cases seen in 2020, when the original Wuhan strain was in circulation; the number of cases increased to nine in 2021, when the alpha, beta and delta strains were seen. There were eight cases in early 2022, when the omicron strain entered the population, and the number spiked sharply to sixteen between September 2022 to January 2023, following the increase of circulating omicron BA.4

and BA.5 cases. Possible reasons for the last spike were the known high transmissibility of the omicron BA.4 and BA.5 sub-variants and the return of children back into schools in June 2022 for face-to-face education after a prolonged lockdown.[11]

The mean age of MIS-C cases was 6.4 years, and the median was 6 years. The mean age among patients (n=12) seen before the COVID-19 vaccine became available for children 5-12 years in October of 2021 was 5.3 years. In the U.S. and Europe, before childhood vaccination became available, the mean age for children who developed MIS-C was 8-9 years.[12-15] In a meta-analysis involving 2,275 MIS-C cases, the mean age was 9 years.[16] MIS-C has been compared to Kawasaki disease, due to the coronary artery involvement in both illnesses;[16-19]the mean age of MIS-C cases (6.4 years) in the present study is higher than the mean age of children with Kawasaki disease (2.8 years) in a previous report from one of the hospitals in the present study.[20]

A few unusual MIS-C occurrences were encountered. Two male siblings in one household developed MIS-C separately, with a one-month interval between each other, even as both acquired the acute COVID-19 illness at the same time. One six-year old male had MIS-C after having had Kawasaki disease four years before. One adolescent had two MIS-C events within a year. This patient was initially diagnosed with hemophagocytic lymphohistiocytosis (HLH), after which, a first MIS-C episode occurred. Shortly after recovering, disseminated tuberculosis (TB) (lymph node, lung, bone marrow) was diagnosed and treated for. Many months later, this patient was diagnosed with acute myelogenous leukemia (AML). The second MIS-C episode occurred after the AML diagnosis, and while undergoing chemotherapy. There have been reports that there may be genetic factors to explain the hyperinflammatory state seen in patients with MIS-C.[21-23]

There were more males (64%) with MIS-C in this study. In the U.S., among 4,107 MIS-C cases, 59.5% were male.[1]

There were 15 COVID-vaccine-eligible children who had MIS-C after COVID-19 vaccination program was started in November 2021. Of these, seven (47%) had not been vaccinated at the time of illness, while two (13%) had received only one vaccine dose. Six (40%) had received two COVID-19 vaccine doses before their MIS-C illness; one of the six had been treated for Kawasaki disease four years before the MIS-C illness occurred. The COMIRNATY monovalent COVID-19 vaccine, the same vaccine that these children received, has been reported to have a vaccine efficacy rate of 91% and 94% in preventing MIS-C.[24-25]

The top co-morbidity before the MIS-C admission was obesity (8%). One had AML during a second admission (this patient had two MIS-C episodes in 12 months), and one severely wasted patient had an undiagnosed human immunodeficiency virus (HIV) infection. Obesity, male sex and asthma are known to be risk factors for MIS-C.[4, 8, 26]

Fever was seen in all but one (97%), with the only afebrile case being a newborn. One 2-year old child presented with prolonged fever of 16 days and a generalized rash, and was managed at another hospital, before being transferred to the study hospital. In a local report, incomplete Kawasaki disease was reported to be a cause of fever of unknown origin in five cases; MIS-C shares many common findings with Kawasaki disease.[27] Otherwise, the present study found the tetrad of symptoms of vomiting, cough, abdominal pain and rash, to be seen in about half (42-53%) of the cases, while diarrhea and conjunctivitis were seen in one-third. Locally, in a report of 16 MIS-C cases, the most common symptoms were fever (100%), decreased appetite (75%), vomiting (56%), diarrhea (50%), abdominal pain (50%) and rash (44%), which are very similar to the present report.[4] In a meta-analysis which included 2,197 children with MIS-C, the most common symptoms were fever (100%), gastrointestinal symptoms (82%), abdominal pain (68%), erythema/rash (59%), non-purulent conjunctivitis (54%) and cough (41%).[16] In another meta-analysis that included 4,475 children with MIS-C, the most common symptoms were fever (91%),

not-specified gastrointestinal symptoms (52%), rash (50%), abdominal pain (49%), conjunctivitis (47%), vomiting (44%), respiratory symptoms (42%) and diarrhea (40%).[18] Compared to children with Kawasaki disease in a report from one of the hospitals in this study, the other classic findings in Kawasaki disease (rash, conjunctival injection, cervical lymphadenopathy, mouth and extremity changes) were more common among the Kawasaki disease patients, in comparison to those with MIS-C, but abdominal symptoms were more common among MIS-C cases (47-53%, vs. 34%).[20]

Two cases presented with very prominent abdominal pain, for which referral to surgery was necessary to rule out a surgical abdomen: one 5-year-old with vomiting, diarrhea and abdominal pain, was found to have small bowel wall thickening, most prominent at the ileum, with gall bladder wall thickening, splenomegaly, and a minimal pelvic effusion, on abdominal ultrasonography; the second case with marked abdominal pain was a COVID-19-vaccinated, 7-year-old male, who was found to have myocarditis and a moderate pericardial effusion, who required an epinephrine drip and mechanical ventilation. He recovered, and the myocarditis and pericarditis were thought to be the likely causes of the severe epigastric pain upon admission. Collectively, gastrointestinal symptoms were the predominant manifestation in MIS-C, after fever. Severe abdominal pain during MIS-C, to the point of surgical intervention, has been well-reported; the most common associated conditions found have been mesenteric lymphadenitis and ascites.[16] In a meta-analysis of 72 children with MIS-C who had acute abdomen, intra-operative findings showed mesenteric adenitis (32%),terminal ileitis/ileocolitis (26%), ascites (11%), and paralytic ileus (4%); laparotomy was done in 49% of acute abdomen cases, and was proven unnecessary in 51%.[28] Appendicitis and obstructive ileus were seen in 24%.[28]

One 17-day old neonate was readmitted after birth, for fever and vomiting, and was treated for MIS-C with myocarditis; the mother had COVID-19 illness at 34 weeks of gestation. This case was reported earlier.[6] One afebrile, 10-hour old, term neonate developed tachypnea, with radiography showing findings seen in transient tachypnea of the newborn. As the mother had COVID-19 illness at 34 weeks of gestation, the newborn was worked up, and showed elevated inflammatory parameters, and a 2-DE that revealed coronary artery dilatation and minimal pericardial effusion. One 3-month-old had MIS-C and PCAP-C, and responded well to treatment. In a systematic review of infants <6 months old who had MIS-C, only 18% of the neonates presented with fever, while cardiovascular dysfunction and respiratory symptoms were the predominant manifestations.[28] Among 84 infants <12 months (median age of 7.7 months) with MIS-C reported to the CDC's MIS-C national surveillance system, pneumonia (21%), hypotension (21%), coronary artery dilatation (14%), shock (13%), and myocarditis (6%) were most often reported. The authors concluded that infants appear to have a milder course of MIS-C than older children, with illness resolution after discharge.[19]

The criteria for Kawasaki disease, other than fever, were seen in only a minority of MIS-C cases (rash in 42%, conjunctivitis in 33%, extremity redness in 17%, mouth changes in 14% and cervical lymphadenitis in 6%). Difficulty of breathing was seen in 11% of MIS-C cases, which is an infrequent finding in Kawasaki disease.[20] In a meta-analysis of 4,475 MIS-C patients, the MIS-C cases were compared to those with Kawasaki disease in nine studies.[18] Children with MIS-C were less likely to develop conjunctivitis (OR 0.27), cervical adenopathy (OR 0.21) and rash (OR 0.44), in comparison with Kawasaki disease patients; while MIS-C cases were more likely to have gastrointestinal symptoms (OR 11.4), mitral regurgitation (OR 6.6), pericardial effusion (OR 1.74) and pleural effusion (OR 19.2).[18]

For the laboratory work-up, the white blood cell counts (WBC) were generally lower than those seen in cases of Kawasaki disease, with a mean of 6,700/mm^3. Platelet counts were less than 150,000/mm^3 in 39% of cases, which has been

reported to be an adverse prognosticator in MIS-C.[30] In the CDC's updated (December 2022) standardized case definition of MIS-C, a platelet count of less than 150,000/mm^3, and an absolute lymphocyte count of less than 1,000/mm^3, are the only abnormal hematologic markers that can be used as criteria for evidence of hematologic dysfunction.[31] The inflammatory markers, C-reactive protein (CRP), D-dimer and ferritin, were generally elevated; all of these markers were included in the 2020 CDC case definition for MIS-C, but in the updated CDC (December 2022) definition, only a CRP of >3 mg/dl is required to satisfy the laboratory evidence of inflammation.[7] Procalcitonin was elevated in 89% when it was requested, with none of these patients having a growth from a blood culture; procalcitonin has also been reported to be elevated in MIS-C.[4] In a meta-analysis of 787 MIS-C patients, MIS-C cases, when compared to non-severe COVID-19 cases, had lower absolute lymphocyte counts and higher CRP and D-dimer levels; severe MIS-C patients had higher WBC, absolute neutrophil count (ANC), CRP, D-dimer and ferritin levels, when compared with patients with non-severe MIS-C.[32]

Among the cardiac markers, the pro-BNP was the most often elevated (79%), while troponin-I and CPK-MB were elevated in 38% and 21%, respectively. In the latest December 2022 case definition of MIS-C, an elevated troponin is the only laboratory test that can be used as evidence of cardiac involvement (or a 2-DE showing coronary artery dilatation or aneurysm, or a left ventricular ejection fraction of <55%), which is one of five systems that have to be involved to make the diagnosis; the others are hypotension/shock, dermatologic manifestation(s), gastrointestinal symptom(s) and hematologic abnormalities.[31] In a meta-analysis involving 1,613 MIS-C cases, the cardiac marker that showed a significant difference between MIS-C patients and non-severe COVID-19 patients, and between severe MIS-C and non-severe MIS-C, was BNP.[34]

Pericardial effusion (50%), coronary artery dilatation or aneurysms (39%) and mitral regurgitation (36%) were the most common 2-DE findings. Most effusions were minimal, with only one case being moderate. This latter case was under consideration for pericardial fluid drainage during the hospital stay, but the effusion decreased with medical treatment, using a higher corticosteroid dose, fluid restriction and diuresis. A decreased ejection fraction was seen in four patients (11%); these cases were managed with vasopressors, while two were given oral digoxin (lanoxin). In a local report, the top 2-DE findings were pericardial effusion (60%), myocardial dysfunction (40%) and coronary arteritis (20%).[4] These findings are similar to those reported in the literature, with coronary artery abnormalities reported from 8% to 50%.[34]

Among acute co-illnesses seen during the admission for MIS-C, two (6%) had concomitant dengue fever. One COVID-unvaccinated, 15-year old had melena, transient hypotension and dengue shock syndrome, and MIS-C at the same time. He did not have evidence of myocarditis during the illness course, but during recovery, a 2nd degree atrio-ventricular heart block was detected, which resolved after three days. A 10-year old COVID-19-vaccinated patient had dengue with warning signs and MIS-C. He was dengue NS-1/IgM/IgG-positive, with a lowest WBC of 2,000/mm^3,lowest platelet count recorded of 40,000/mm^3, SGPT of 210 U/l and SGOT of 500 U/l; he had pericardial effusion and an ejection fraction of 55% on 2-DE. He recovered with standard treatment. There were two other MIS-C cases that tested dengue IgM-positive, but these were assessed to be false-positive results. One MIS-C case was admitted with a bronchiolitis picture, who tested positive for rhinovirus by PCR of nasal swab.

Among complications during the hospital admission, pneumonia was the most common (31%), but these were mostly mild to moderate in severity. Only two patients were mechanically ventilated, one due to mild ARDS, myocarditis and a moderate pericardial effusion; and the other, due to severe anemia, ARDS, cardiogenic and respiratory failure, in a severely wasted, HIV-positive adolescent. The cases with pneumonia were treated with 2nd or 3rd generation cephalosporins, and/or azithromycin.

One case who had prominent abdominal pain and thickened small intestinal walls by ultrasonography, was treated with piperacillin-tazobactam. Otherwise, those with pneumonia were the only ones who received antimicrobials. Two cases (6%) presented with new-onset severe reactive airway disease, and were managed accordingly. Hypotension was seen in five cases (14%) for whom volume and intravenous vasopressors (8%) were given; two (6%) received oral digoxin (lanoxin), due to a decreased left ventricular ejection fraction. No patient developed a secondary healthcare-associated infection.

Treatment for MIS-C was mainly with intravenous (IV) corticosteroids (89%) in the acute phase, and intravenous immunoglobulin(IVIG) (83%). With defervescence, with or without improvement in inflammatory markers, the corticosteroid was stepped down to oral form.

One patient was transferred from outside of Metro Manila; he had received IVIG at the provincial hospital, but the fever recurred nine days after the IVIG was given. This patient was not given a corticosteroid during the first hospitalization, nor upon discharge. When he was admitted to the study institution, he received an IV corticosteroid, and promptly defervesced. Early in the course of the pandemic, when the appropriate corticosteroid doses and duration were not yet well-defined in MIS-C management protocols, two patients in the present study had recurrence of fever after the IV corticosteroid was abruptly stopped after 2-3 days; restarting the corticosteroid, and sending patients home on an oral corticosteroid over 2-4 weeks allowed a continued resolution of the illness, and non-recurrence of fever. In a local report, IVIG was used in 94%,and corticosteroids in 75%, of MIS-C cases.[4]

Hypotension was seen in 14%, for which volume infusion and vasoactive drugs(epinephrine, dobutamine, and dopamine) were used. A decreased ejection fraction was detected by 2-DE in 11%. In a French/Swiss MIS-C study of 35 children, cardiogenic shock with collapse was seen in 80%; left ventricular ejection fraction was found to be 30-50% at baseline in 72%, and <30% in 28%.[26] In the same study, the median delay between the first clinical symptom and heart failure symptoms was six days; 62% were mechanically ventilated, and upon PICU admission, 80% were in shock and needed vasopressors.[26] In a meta-analysis of MIS-C cases, 60% were reported to be hypotensive.[16] Fortunately, we did not see the above rates of cardio-respiratory decompensation in the present report.

Antibacterial(s) (31%) was/were only given for those with evidence of pneumonia, clinically or radiographically, and in one case who had evidence of ileitis. Mechanical ventilation, vasopressors, enoxaparin and tocilizumab were used in 8%, each. Mechanical ventilation was done in two (6%) patients who developed ARDS. Enoxaparin was used, in consultation with the hematology service, when intravascular thrombosis was deemed a risk. Tocilizumab was used in clinically ill patients whose serum interleukin-6 (IL-6) levels were elevated, for which a cytokine storm was suspected, as reported elsewhere.[35]

Unlike in Kawasaki disease, acetyl salicylic (ASA) was not routinely used, because of a concern for upper gastrointestinal bleeding, if corticosteroid and the former were used together; nevertheless, most patients were discharged on low-dose (3-5 mg/kg/dose once daily) ASA, together with an oral corticosteroid. There were no thromboembolic events seen in this report. In a study to evaluate the incidence and risk factors of thrombosis in hospitalized COVID-19 (n=715) and MIS-C (n=138) patients, 6.5% of the MIS-C cases had thrombosis; MIS-C patients aged 12 years and older had the highest thrombosis rate at 19%.[36] Seventy-one percent of thromboembolic events not present on admission, occurred in spite of prophylaxis. Multivariate analysis showed that age of 12 years or older, cancer, presence of a central venous catheter, and MIS-C were significantly associated with thrombosis, and mortality increased from 2.3% for all COVID-19 patients, to 28% for those with thromboembolic events.[36]

The length of stay was generally short, with 86% going home in five days, or less, as most responded well to the usual treatment of IVIG and corticosteroid. This combination is the current standard of treatment for MIS-C, and has shown better outcomes than IVIG alone.[37]

There were two mortalities (6%). One was an adolescent who had received one COVID-19 vaccine dose four weeks prior to the MIS-C illness. The patient had a 7-day fever course and was hypotensive, with premature ventricular contractions in bigeminy, on day 7 of illness upon presenting at the emergency room, necessitating vasopressors, and subsequently, mechanical ventilation. Severe myocarditis progressed to cardiogenic shock and cardio-respiratory arrest. An HIV antibody test was positive, taken a day before demise; this patient was not previously known to be ill with HIV. A second patient was an adolescent who was under treatment for HLH, which was complicated by MIS-C. After treatment for MIS-C, the patient developed disseminated TB within a month of the MIS-C illness, with draining, TB-GeneXpert-positive, supraclavicular lymphadenitis; lung and pleural disease, hepatosplenomegaly, and a bone marrow aspirate which showed granulomas. The course was further complicated by a diagnosis of AML, and while on chemotherapy, a second MIS-C illness occurred a year after the first one. The patient transferred to another hospital due to financial reasons, where death ensued during the course of the second MIS-C episode. Elsewhere, MIS-C mortality rates have been from 1.1 - 4%.[38-39] In a large, population-based U.S. report of 4,107 cases, the authors found that MIS-C outcomes worsened as the number of organ systems affected increased; the inpatient death rate was <1% if 0-2 systems were involved, but increased to 5.8% when 6-8 organ systems were affected.[1] In a local report, the MIS-C mortality was 19%.[4]

This report is limited by the non-inclusion of other MIS-C cases seen by other infectious disease doctors in the two hospitals. The generalizability of the findings from these two hospitals is unclear, because pediatric infectious disease physicians in a government hospital in the same city as one of the study hospitals here, as well as in two other large government hospitals where COVID cases are seen, do not appear to have encountered as many MIS-C cases in the last three years, as has been seen in this report. (personal communication)

CONCLUSION

MIS-C cases had a median age of 6 years, presented with fever in 97%, while one-half had abdominal pain, vomiting, diarrhea and/or rash. CRP, D-dimer, ferritin, LDH and procalcitonin were generally elevated, and thrombocytopenia was seen in 39%. The most common 2-DE abnormalities were pericardial effusion (50%), coronary artery dilatation or aneurysm (39%) and mitral regurgitation (36%). The main complications were pneumonia (31%), myocarditis (28%), hypotension (14%) and ARDS (8%); 6% were mechanically ventilated. Treatment was with corticosteroids (89%), IVIG (84%), fluids and vasopressors (8%), when needed. Most (94%) recovered, with appropriate treatment, and the hospital stay was five days, or less, in 86%.The two mortalities were in an adolescent with previously undiagnosed HIV infection who had severe wasting; and an adolescent undergoing chemotherapy for AML, who was also being treated for disseminated TB.

RECOMMENDATION

There is a need to create a greater awareness of MIS-C as a post-COVID complication in children. MIS-C presents like a common everyday illness, with fever in almost all, and only half will have gastroenteritis-like symptoms and a non-specific rash, which are childhood disease manifestations that many might not place much importance on. In such illnesses, it is important for the health care worker to determine if the patient, or anyone else in the household, has had a COVID-19 illness, whether documented or not, in the preceding 60 days. If such is present, a proper evaluation might be pursued because, similar to Kawasaki disease,[40-41] MIS-C may have the potential to become an important cause of acquired heart disease.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Encinosa W, Moon K, Figueroa J, et al. Complications, adverse drug events, high costs, and disparities in Multi-system Inflammatory Syndrome in children with COVID-19. JAMA Netw Open. 2023;6(1):e2244975. doi:10.1001/jamanetworkopen.2022.44975
2. Radia T, Williams N, Agrawal P, et al. Multi-system inflammatory syndrome in children and adolescents (MIS-C): A systematic review of clinical features and presentation. Paediatr Respir Rev 2021 Jun;38:51-57. doi: 10.1016/j.prrv.2020.08.001. Epub 2020 Aug 11.
3. Gottlieb M, Bridwell R, Ravera J, et al. Multi-system inflammatory syndrome in children with COVID-19. Am J Emerg Med 2021 Nov;49:14-152. doi: 10.1016/j.ajem.2021.05.076.
4. Rivera AC, Pantig FMT, Lazarte CCM, et al. SARS-CoV-2 infection in Filipino children: An interim report from the SALVACION registry. PIDSP Journal 2022;23(2):31-42.
5. Blasurca JR, Monge G, Ritona JAG, et al. Multi-system Inflammatory Syndrome in Children (MIS-C): A case series in a tertiary hospital. PIDSP Journal 2021;22(1):19-25.
6. Cano CU, Ignacio LAD, Rodriguez EV, et al. Neonatal Multi-system inflammatory Syndrome in a neonate (MIS-N) presenting as bowel obstruction: a case report. PIDSP Journal 2022;23(2):9-18.
7. 2020 CDC Case definition for MIS-C. National Center for Immunization and Respiratory Diseases.
8. Rhedin S, Lundholm C, Horne AC, et al. Risk factors for multisystem inflammatory syndrome in children - A population-based cohort of over 2 million children. Lancet Reg Health Eur 2022 Aug; 19: 100433. doi: 10.1016/j.lanepe.2022.100443
9. Makati City Profile - PhilAtlas [online]
10. San Juan City Profile - PhilAtlas [online]
11. Cao Y, Yisimayi A, Jian F, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. Nature 2022 Aug;608(7923):593-602. doi: 10.1038/s41586-022-04986-y.
12. Garcia-Salido A , de Carlos Vicente JC, Hofheinz SB et al. Crit Care.2020;24(1):666. doi:10.1186/s13054-020-03332-4.
13. Belot A. Eurosurveillance 2020 June;25(22).
14. Whittaker E, Bamford A. JAMA June 8, 2020. doi:10.1001/jama.2020.10369
15. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem inflammatory syndrome in U.S. children & adolescents. June 29, 2020. doi:10.1056/NEJMoa2021680
16. Santos MO, Goncalves LC, Silva PAN, et al. Multi-system Inflammatory Syndrome (MIS-C): a systematic review and meta-analysis of clinical characteristics, treatment and outcomes. J Pediatr (Rio J) 2022 Jul-Aug; 98(4):338-349. Doi: 10.1016/j.jped.2021.08.006
17. Bukulmez H. Current understanding of Multi-system Inflammatory Syndrome (MIS-C) following COVID-19 and its distinction from Kawasaki disease. CurrRheumatol Rep 2021 Jul 3;23(8):58. doi: 10.1007/s11926-021-01028-4.
18. Jiang L, Tang K, Irfan O, et al. Epidemiology, clinical features, and outcomes of Multisystem Inflammatory Syndrome in Children (MIS-C) and adolescents - a live systematic review and meta-analysis. CurrPediatr Rev 2022;10(2):19-30. doi: 10.1007/s40124-022-00264-1.
19. Godfred-Cato S, Tsang CA, Giovanni J, et al. Multisystem inflammatory syndrome in infants <12 months of age, United States, May 2020-January 2021. The Pediatric Infectious Disease Journal. July 2021;40(7):601-605.
20. Malimban J, Francisco-Mallari MR, Garcia RDJ. A comparative study of pediatric patients with complete vs. incomplete Kawasaki Disease in a private, urban, tertiary hospital: an eleven-year review. PIDSP Journal 2022;23(2):55-63.
21. Chou J, Platt CD, Habiballah S, et al. Mechanisms underlying genetic susceptibility to multisystem inflammatory syndrome in children (MIS-C). J Allergy Clin Immunol 2021 Sep;148(3):732-738.e1. doi: 10.1016/j.jaci.2021.06.024.
22. GavriilakiE,Tsiftsoglou SA, Tououmenidou T, et al. Targeted genotyping of MIS-C patients reveal a potential alternative pathway-mediated complement dysregulation during COVID-19 infection. Curr Issues Med Biol 2022 Jun 28;44(7):2811-2824. doi: 10.3390/cimb44070193.
23. Chen MR, Kuo HC, Lee YJ, et al. Phenotype, susceptibility, autoimmunity, and immunotherapy between Kawasaki disease and Coronavirus disease-19 associated Multisystem Inflammatory Syndrome in Children. Front Immunol 2021 Feb 26;12:632890. doi: 10.3389/fimmu.2021.632890. eCollection 2021.
24. Zambrano LD, Newhams MM, Olson SM, et al. Effectiveness of Pfizer-BioNTech vaccination against MIS-C among persons aged 12-18 years - U.S., July-December 2021. Morb Mort Wkly Rep 2022 Jan 14;71(2):52-56. doi: 10.15585/mmwr.mm7102e1.
25. Nygaard U, Holm M, Hartling UB, et al. Incidence and clinical phenotype of MIS-C after infection with the COVID-19 delta variant by vaccination status: a Danish nationwide prospective cohort study. Lancet Child Adolesc Health 2022 Jul;6(7):459-465. doi: 10.1016/S2353-4642(22)00100-6.
26. Belhadjer Z et al. Ciculation. 2020 Aug 4;142(5):429-436.
27. Garcia RDJ. Fever of unknown origin among children in two private, urban, tertiary hospitals: A 27-year retrospective collection of cases. PIDSP Journal 2021;22(1):63-71.
28. Rouva G, Vergadi E, Galanakis E. Acute abdomen in multisystem inflammatory syndrome in children: A systematic review. Acta Paediatr 2022 Mar;111(3):467-472. doi: 10.1111/apa.16178.

29. De Rose DU, Pugnaloni F, Cali M, et al. Multisystem Inflammatory Syndrome in neonates born to mothers with SARS-CoV-2 infection and in neonates and infants younger than 6 months with acquired COVID-19: A systematic review. Viruses 2022 Apr 2;14(4):750. doi: 10.3390/v14040750.
30. Delshad M,Safaroghi-Azaz A, Pourbagheri-Sigaroodi A, et al. Platelets in the perspective of COVID-19; pathophysiology of thrombocytopenia and its implications as prognostic and therapeutic opportunity. Int Immunopharmacol 2021 Oct;99:107995. doi: 10.1016/j.intimp.2021.107995
31. Standardized surveillance case definition for MIS-C, December 2022. American Academy of Pediatrics.https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/multisystem-inflammatory-syndrome-in-children-mis-c-interim-guidance/
32. Zhao Y, Yin L, Patel J, et al. The inflammatory markers of multisystem inflammatory syndrome in children (MIS-C) and adolescents associated with COVID-19: A meta-analysis. L Med Virol 2021 Jul;93(7):4358-4369. doi: 10.1002/jmv.26951.
33. Zhao Y, Patel J, Huang Y, et al. Cardiac markers of multisystem inflammatory syndrome in children (MIS-C) in COVID-19 patients: A meta-analysis. A J Emerg Med 2021 Nov;49:62-70. doi: 10.1016/j.ajem.2021.05.044.
34. Gottlieb M, Bridwell R, Ravera J, et al. Multi-system inflammatory syndrome in children with COVID-19. Am J Emerg Med 2021 Nov;49:14-152. doi: 10.1016/j.ajem.2021.05.076.
35. Toniati P, Piva S, Cattalini M. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmune Rev. 2020 Jul; 19(7): 102568. Published online 2020 May 3. doi: 10.1016/j.autrev.2020.10256
36. Whitworth H, Sartain SE, Kumar R, et al. Rate of thrombosis in children and adolescents hospitalized with COVID-19 or MIS-C. Blood 2021 Jul 15;138(2):190-198. doi: 10.1182/blood.2020010218.
37. Ouldali N. Association of IVIG plus methylprednisolone vs. IVIG alone with the course of MIS-C. JAMA; Feb 1, 2021. (online)
38. Levin MN. Childhood multi-system inflammatory syndrome - a new challenge in the pandemic. N Engl J Med 2020;383:393-395.
39. Miller AD, Yousaf AR, Bornstein E, et al. MIS-C during SARS-CoV-2 delta and omicron variant circulation - U.S., July 2021-January 2022. Clin Infect Dis 2022 Jun;ciac471. doi: 10.1093/cid/ciac471.
40. Medaglia AA, Siracusa L, Gioe C. et al. Kawasaki disease recurrence in the COVID-19 era: a systematic review of the literature. Ital J Pediatr 2021 Apr 19;47(1):95. doi: 10.1186/s13052-021-01041-4.
41. Elakabawi K, Ling J, Jiao F, et al. Cardiol Res 2020 Feb;11(1):9-14. doi: 10,14740/cr993. Epub 2020 Jan 26.

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 52-63 July-December 2023
Artienda AO, Padua JRO. SARS-CoV-2 RT-PCR cycle threshold value and its association with disease severity and mortality among hospitalized pediatric covid-19 patients. https://doi.org/10.56964/pidspj20232402007

ORIGINAL ARTICLE

SARS-COV-2 RT-PCR CYCLE THRESHOLD VALUE AND ITS ASSOCIATION WITH DISEASE SEVERITY AND MORTALITY AMONG HOSPITALIZED PEDIATRIC COVID-19 PATIENTS

Aubrey O. Artienda, MD and Jay Ron O. Padua, MD
Philippine Children's Medical Center

ABSTRACT

Objective: This study determined the association of SARS-CoV-2 RT-PCR cycle threshold (Ct) value with disease severity and mortality among hospitalized pediatric COVID-19 patients.

Methodology: This is a retrospective cohort study of patients aged 0-18 years with SARS-CoV-2 RT-PCR-confirmed COVID-19 from 1-September-2020 to 31-August-2022. The cohort was divided into those with high (>30), medium (> 20) and low (</= 20) Ct values. Association between Ct values and disease severity was determined using Chi-square test and association between Ct values and mortality was determined using logistic regression.

Results: There were 236 patients included with male predominance. Median age was 7 years. Most belonged to the 0-5 years age group. Most were severe to critical COVID-19 cases. Median day of illness on swab collection was 4 days. Majority presented with symptoms such as fever (54%), cough (22%) and dyspnea (22%). Eighty-four percent had co-morbidities, of which majority were cancer and neurologic diseases. Median Ct value was 30.81. Fifty-four percent had high Ct values. The median age of patients with a high Ct value was significantly lower than other cohorts. The median day of illness of patients with low Ct value was significantly shorter than other cohorts. There was no significant difference across the terciles in terms of presence of co-morbidities. Majority of patients for each cohort had high Ct values. There was no significant association between Ct value and COVID-19 disease severity on admission. Nearly fifty percent had critical disease and the all-cause mortality rate was 21.61%. There was no significant association between Ct value and mortality.

Conclusions: Ct value was not associated with disease severity and all-cause mortality after controlling for confounders. A look into medical interventions, emergence of variants, and other factors that may affect the clinical presentation, disease course, severity and outcome are recommended in future studies.

KEYWORDS: *COVID-19, Cycle Threshold Value, Disease Severity, Mortality, Outcome, Pediatric Patients*

Correspondence:
Dr. Aubrey O. Artienda
Email: aubreyartienda@yahoo.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 52-63 July-December 2023
Artienda AO, Padua JRO. SARS-CoV-2 RT-PCR cycle threshold value and its association with disease severity and mortality among hospitalized pediatric covid-19 patients. https://doi.org/10.56964/pidspj20232402007

INTRODUCTION

Coronavirus Disease-2019 (COVID-19) remains a global health concern since the World Health Organization (WHO) declared it a pandemic in March of 2020[1]. As part of the emergency response, countries around the world proposed a series of interim guidelines in line with the WHO's advice regarding disease detection, testing and management.[2] Various efforts to understand this novel disease have driven the medical society to explore on diagnostics for Coronavirus-2 Severe Acute Respiratory Syndrome (SARS-CoV-2), with the hope of improving outcomes among COVID-19 confirmed cases. Disease complexity is manifested through a spectrum of illness severity states—from an asymptomatic or mild infection to severe and critical condition.[3] As the disease continues to evolve, knowledge on pathogenesis and subsequently, developments in diagnostic testing and test interpretation also remain to be partial. It is being explored if the ability to predict disease severity and outcome through these diagnostics would significantly benefit treatment and management decisions.

The Reverse Transcription Polymerase Chain Reaction for SARS-CoV-2 (SARS-CoV-2 RT-PCR) is the gold standard and is the most reliable test for the diagnosis of COVID-19.[4,5] It detects the viral ribonucleic acid semi-quantitatively by providing an indirect measure of the viral load found in the sample.[6] The kits for RT-PCR are designed to recognize a target gene.[6] Once a target gene has been detected, a cycle threshold value (CT value) is recorded which reflects the number of amplification cycles necessary for the recognition of the target gene.[7] An inverse relationship between the Ct value and viral load is observed—the lower the amplification necessary for the machine to detect the virus, the higher the viral load, and vice versa[7]. A low Ct value indicates a high concentration of SARS-CoV-2 genetic material or viral load. Conversely, a high Ct value indicates a low concentration of viral genetic material or viral load. It is uncertain, however, whether this semi-quantitative capability of the SARS-CoV-2 RT-PCR can be maximized.

Several studies have been done to investigate the significance of the Ct value and its association with disease severity and outcome among COVID-19 patients. However, to date, there are limited local studies on Ct value and its association with disease severity and outcome among pediatric patients with COVID-19 and morbidity & mortality from SARS-CoV-2 infection remain to be a concern especially among those with co-morbidities.

This study aimed to determine the association of SARS-CoV-2 RT-PCR Ct value with disease severity and mortality among pediatric COVID-19 patients admitted at the Philippine Children's Medical Center (PCMC). The demographic profile and clinical characteristics of the study population, their Ct values, the association between the Ct value and COVID-19 disease severity, and the association between the Ct value and all-cause mortality during admission were also studied.

MATERIALS AND METHODOLOGY

This is a retrospective cohort study of pediatric patients aged 0 to 18 years with confirmed COVID-19 admitted at the Philippine Children's Medical Center from September 1, 2020 to August 31, 2022. The study period coincided with the start of operations of the COVID-19 Testing Laboratory of the Pathology Division of PCMC.

Patients included in the study were those with positive SARS-CoV-2 RT-PCR result done by the COVID-19 Testing Laboratory as a requirement for admission, regardless of symptomatology. Three FDA-approved SARS-CoV-2 RT-PCR kits were available in the laboratory during the study period, namely the Maccura SARS-CoV-2 Fluorescent PCR, iPonatic 2019-nCoV Kit by Sansure Biotech and Sansure Biotech Novel Coronavirus Nucleic Acid Diagnostic Kit.

These kits used the same principle of a real-time reverse-transcription PCR system, where specific primers and fluorescent probes are used to target the ORF1ab, E and N genes for the Maccura kit; and ORF1ab, N gene, and internal standard gene

fragments of SARS-CoV-2 for the two Sansure Biotech kits. All kits follow the same set cycle parameters of reverse transcription, pre-denaturation, annealing, extension, fluorescence collection and instrument cooling. The thermal cycles are defined as the cycle threshold when the fluorescent signal exceeds the background fluorescence, which is a semi-quantitative measure of viral genetic material in samples. A standard RT-PCR assay runs a maximum of 40 thermal cycles. The interpretation of Ct value of the target gene (ORF1ab) has a cut-off of less than or equal to 38 for the Macurra kit, and less than or equal to 40 for the Sansure kits. Those over this set cut-off values are released as SARS-CoV-2 RNA not detected and interpreted as negative, while those within the cut-off values are released as SARS-CoV-2 RNA detected and interpreted as positive result.

The specimens submitted were nasopharyngeal swab (NPS) and oropharyngeal swab (OPS), NPS alone or OPS alone. Collection of specimen from both NPS and OPS is the standard technique. However, for patients prone to bleeding due to underlying conditions, only OPS was sent. For intubated patients or for those with contraptions that do not permit access to the oral cavity, only NPS was sent. Specimens were immediately submitted to the COVID-19 Testing Laboratory and further evaluated by the laboratory analysts to ensure integrity and adequacy prior to testing. Specimen collection procedures complied with those set by the PCR kit manufacturers.

The definitions provided by the Interim Guidelines on the Screening, Assessment and Clinical Management of Pediatric Patients with Suspected or Confirmed Coronavirus Disease-2019 of the Philippine Pediatric Society and Pediatric Infectious Disease Society of the Philippines were used to categorize cases. Those with mild disease were symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia[3]. Moderate disease included those with clinical signs of non-severe pneumonia such as absence of fast breathing, difficulty of breathing, chest indrawing or desaturation less than 90% on room air[3]. Severe disease included those with clinical signs of pneumonia such as cough or difficulty of breathing plus at least one of the following: central cyanosis or oxygen saturation less than 90%, poor intake, lethargy and unconsciousness, or convulsions. Lastly, critical disease included patients who presented with acute respiratory distress syndrome, sepsis, septic shock, acute thrombosis or multi-system inflammatory syndrome in children.[3]

Patient outcomes were classified as survival (discharged improved) or mortality for those who succumbed to death regardless of cause while admitted.

Due to the finite number of eligible patients in PCMC, the researcher employed a total enumeration technique, a type of purposive sampling design wherein all eligible patients were enrolled in the study. PASS 15 software was used to calculate the sample size requirement to achieve 80% statistical power. Parameters were based on the published study by Klinger, *et al.*[8] Specifying an odds ratio of 2.93, probability of mortality among low Ct value patients equal to 22%, and alpha set at 0.05, a minimum of 66 patients were required to achieve 80% statistical power.

There was no randomization and intervention employed. The study was approved by the ethics committee of PCMC prior to its implementation. Data gathering was done by chart review and there was no direct encounter with patients or guardians hence a waiver of consent was applied.

There were 360 patients who tested positive from September 2020 to August 2022 based on the records of the Infection Prevention and Control Committee of PCMC. This list was submitted to the COVID-19 Testing Laboratory for retrieval of Ct values. Further information was gathered for the 360 patients from the database of the Section of Pediatric Infectious Diseases and supplemented by chart review. Eleven cases were still admitted at the end of the data collection period, hence these were excluded in the study. From the 349 remaining subjects, only 236 had retrievable records with complete data, including age, gender, symptoms (if

any), day of illness on the day of swab, disease severity, co-morbidities (if any) and outcome. From 236 subjects, 170 were tested using the Maccura kit, 59 using the iPonatic kit by Sansure Biotech and 7 using the 2019-nCoV Nucleic Acid Diagnostic kit by Sansure Biotech.

The cohort was divided into terciles based on Ct values for the SARS-CoV-2 -specific target (ORF1ab). High Ct value included those with Ct value more than 30, medium included those with more than 20 and low included those with less than or equal to 20.

Data were recorded by the researcher in a data collection form and encoded in Microsoft Excel. Stata MP version 17 software was used for data processing and analysis. Continuous data were presented as median/interquartile range due to the non-normal data distribution. Categorical data were presented as frequencies and percentages. Kruskal Wallis test was used to compare continuous variables by Ct value, while Chi-Square test and Fisher's Exact test were used for categorical variables. Comparison of characteristics by mortality status was performed using Mann-Whitney U test for continuous variables, and Chi-Square and Fisher's Exact test were used for categorical variables.

The association between Ct value and disease severity was determined using Chi-square test. Logistic regression analysis was performed to determine the association between Ct value and mortality, and in case of sparse data, Firth's bias correction was applied. Confounder selection utilized a cut-off of $p<0.20$ and change-in-estimate criterion of 10%,[9] and p values ≤0.05 were considered statistically significant.

RESULTS

A total of 236 pediatric patients were included in the study. Table 1 shows the baseline demographic and clinical characteristics of patients. The median age was 7 years old (range: 1 day to 18 years old), and most patients belonged to the 0 to 5 years age group, with an interquartile range (IQR) of 1 to 3 years. Majority were males. More than half were severe to critical COVID-19 cases. The median day of illness at the time of swab collection was 4 days (range: 0 to 56 days; IQR: 2 to 7 days).

Table 1. Baseline demographic and clinical characteristics of patients (n=236)

	n (%)
Age (in years), median	7 [IQR: 1-3]
0-5 years old	106 (45)
6-10 years old	54 (23)
11-15 years old	47 (20)
16-18 years old	29 (12)
Gender	
Male	145 (61)
Female	91 (39)
COVID-19 severity on diagnosis	
Asymptomatic	22 (9)
Mild	69 (29)
Moderate	24 (10)
Severe	60 (25)
Critical	61 (26)
Day of illness at the time of swab	
Days, median	4 [IQR: 2-7]
Symptoms	
With	214 (91)
Without	22 (9)
Co-morbidities	
With	199 (84)
Without	37 (16)

Majority of patients had symptoms and more than half had fever followed by cough (22%), dyspnea (22%), vomiting (18%), and seizures (17%). The most common organ systems involved were the respiratory (55%), neurologic (25%), gastrointestinal (17%) and hematologic (16%) systems. Thirty-eight percent of patients had multi-organ involvement.

Table 2. List of specific symptoms of pediatric COVID-19 patients (n=236)

Symptoms	n (%)
With	214 (91)
Without	22 (9)
Specific symptoms	
Fever	128 (54)
Cough	54 (22)
Dyspnea	53 (22)
Vomiting	43 (18)
Seizure	41 (17)
Bleeding	27 (11)
Anorexia	24 (10)

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 52-63 July-December 2023
Artienda AO, Padua JRO. SARS-CoV-2 RT-PCR cycle threshold value and its association with disease severity and mortality among hospitalized pediatric covid-19 patients. https://doi.org/10.56964/pidspj20232402007

Table 2 continued. List of specific symptoms of pediatric COVID-19 patients (n=236)

Specific symptoms	
Abdominal pain	21 (9)
Colds	19 (8)
Headache	15 (6)
Diarrhea	14 (6)
Body pains	11 (5)
Edema	11 (5)
Easy fatigability	11 (5)
Pallor	8 (3)
Poor activity/ decreasing sensorium	8 (3)
Rashes	7 (3)
Irritability	6 (3)
Weight loss	3 (1)
Cyanosis	3 (1)
Dysuria	3 (1)
Oliguria	2 (1)
Sore throat	2 (1)
Apnea	1 (1)
Oral sores	1 (1)

Eighty-four percent of patients had co-morbidities and most common were cancer (24%) and neurologic (16%) diseases as listed in Table 3.

Majority of patients (72%) were tested for RT-PCR using the Maccura kit, the first utilized in the hospital, followed by the iPonatic (25%), and Sansure kit (3%).

Table 3. List of specific co-morbidities of pediatric COVID-19 patients (n=236)

Co-morbidities	n (%)
With	199 (84)
Without	37 (16)
Specific co-morbidities	
Cancer	57 (24)
Neurologic	37 (16)
Co-infection	24 (10)
Gastrointestinal	21 (9)
Renal	17 (7)
Congenital anomaly	16 (7)
Hematologic	13 (6)
Cardiac	8 (3)
Respiratory	7 (3)
Others	11 (5)

The median Ct value was 30.81 [IQR: 20.48-35.81, range of 10.13-44.29]. Fifty- four percent had high Ct values, 23% had medium Ct values and the remaining 23% had low Ct values. Table 4 compares the patient characteristics by Ct value.

The median age significantly differ by Ct values. The median age of patients with high Ct value was significantly lower than those with low (p=0.0229) and medium (p=0.0084) Ct values. There was no significant difference between the low and medium Ct value groups (p=0.3617). The age groups also showed significant differences by Ct value. A higher proportion of patients with high Ct values belong to the 0 to 5 year age group compared to those with low and medium Ct values.

The median day of illness at the time of swab was significantly different by Ct values. The median day of illness of patients with low Ct values was significantly shorter compared to those with medium Ct (p=0.0007) and high Ct values (p=0.0118). There was no significant difference in the median day of illness between those with medium and high Ct values (p=0.0608).

Table 4. Demographic profile, clinical characteristics and SARS-CoV-2 RT-PCR Ct Values of pediatric COVID-19 patients (n=236)

	Ct value [n (%)]			
	Low (n=55)	**Medium (n=53)**	**High (n=128)**	***p* value**
Age (years), median	9 [IQR:1-14]	9 [IQR: 4-14]	5 [IQR: 0.79-10]	0.0232*[a]
0 to 5	22 (40)	18 (34)	66 (52)	0.042*[b]
6 to 10	9 (16)	14 (26)	31 (24)	
11 to 15	14 (26)	16 (30)	17 (13)	
16 to 18	10 (18)	5 (10)	14 (11)	
Gender				
Male	30 (55)	33 (62)	82 (64)	0.475[b]
Female	25 (45)	20 (38)	46 (36)	
Day of illness at the time of swab				
Days, median	3 [IQR: 1-4]	5 [IQR: 3-9]	4 [IQR: 2-7]	0.0052*[a]
Symptoms				
With	52 (95)	52 (98)	110 (86)	0.020*[b]
Without	3 (5)	1 (2)	18 (14)	
Co-morbidities	0	0	3 (2)	0.422[c]
With	47 (85)	43 (81)	109 (85)	0.768[b]
Without	8 (15)	10 (19)	19 (15)	

Table 4. Demographic profile, clinical characteristics and SARS-CoV-2 RT-PCR Ct Values of pediatric COVID-19 patients (n=236)

Organ systems involved during the clinical course				
With	46 (84)	48 (91)	107 (84)	0.455[b]
Without	9 (16)	5 (9)	21 (16)	

[a]Kruskal Wallis test was used. Significant results further analyzed using Dunn's test; [b]Chi square test was used; [c]Fisher's exact test was used

The presence of symptoms significantly differ by Ct value as summarized in Table 5. A higher proportion of patients with symptoms had low to medium Ct values compared to other groups. Across all symptoms, only sore throat was significantly different by Ct value. A higher proportion of cases with medium Ct values experienced sore throat than other groups.

Table 5. Specific symptoms and Ct values (n=236)

Specific symptoms, %yes	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Headache	4 (7)	0	11 (9)	0.067[c]
Irritability	2 (4)	1 (2)	3 (2)	0.862[c]
Body pains	1 (2)	2 (4)	8 (6)	0.462[c]
Poor activity/ decreasing sensorium	1 (2)	1 (2)	6 (5)	0.704[c]
Easy fatigability	1 (2)	1 (2)	9 (7)	0.278[c]
Anorexia	1 (2)	7 (13)	16 (13)	0.064[b]
Vomiting	9 (16)	12 (23)	22 (17)	0.633[b]
Weight loss	0	2 (4)	1 (1)	0.176[c]
Colds	5 (9)	4 (8)	10 (8)	0.949[c]
Dyspnea	14 (25)	14 (26)	25 (20)	0.499[b]
Apnea	1 (2)	0	0	0.458[c]
Cyanosis	1 (2)	1 (2)	1 (1)	0.594[c]
Diarrhea	6 (11)	2 (4)	6 (5)	0.223[c]
Sore throat	0	2 (4)	0	0.050*[c]
Oral sores	0	1 (2)	0	0.225[c]
Cough	14 (25)	17 (32)	23 (18)	0.106[b]
Bleeding	9 (16)	7 (13)	11 (9)	0.286[b]
Fever	36 (65)	31 (58)	61 (48)	0.067[b]
Seizure	10 (18)	9 (17)	22 (17)	0.983[b]
Abdominal pain	2 (4)	7 (13)	12 (9)	0.187[c]
Pallor	3 (5)	3 (6)	2 (2)	0.141[c]
Rashes	1 (2)	1 (2)	5 (4)	0.777[c]
Edema	1 (2)	3 (6)	7 (5)	0.602[c]
Oliguria	0	0	2 (2)	1.000[c]
Dysuria	0	0	3 (2)	0.422[c]

There was no significant difference across the terciles in terms of presence of co-morbidities. However, when specific co-morbidities were analyzed, significant differences were observed for co-infections as seen in Table 6. A higher proportion of cases with co-infection had high Ct values.

Table 6. Specific co-morbidities and Ct values of pediatric COVID-19 patients (n=236)

Specific co-morbidities, %yes	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Neurologic	12 (22)	4 (8)	21 (16)	0.118[b]
Cancer	13 (24)	15 (28)	29 (23)	0.708[b]
Hematologic	5 (9)	3 (6)	5 (4)	0.326[c]
Respiratory	2 (4)	2 (4)	3 (2)	0.673[c]
Co-infection	1 (2)	4 (8)	19 (15)	0.022*[b]
Congenital anomaly	4 (7)	1 (2)	11 (9)	0.270[c]
Renal	3 (5)	4 (8)	10 (8)	0.891[c]
Gastrointestinal	5 (9)	5 (9)	11 (9)	1.000[b]
Cardiac	3 (5)	2 (4)	3 (2)	0.494[c]
Others	2 (4)	4 (8)	5 (4)	0.553[c]

There was no significant difference across the three groups in terms of sex and specific organs involved during the course of COVID-19 illness.

As to disease severity, more than half of patients for each COVID-19 category have high Ct values (Table 7). There was no significant association between Ct value and COVID-19 severity on admission.

Table 7. Association between Ct value and COVID-19 severity (n=236)

COVID-19 severity on admission	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Asymptomatic/ Mild	24 (26)	17 (19)	50 (55)	
Moderate/ Severe	20 (24)	21 (25)	43 (51)	0.679[a]
Critical	11 (18)	15 (25)	35 (57)	

[a]Chi-square test was used

As to mortality, 51 patients died, with an all-cause mortality rate of 21.61% (95% CI: 16.80-

27.35%). Incidence of mortality by COVID-19 severity is as follows: asymptomatic/mild: 9.89% (95% CI: 5.18-18.07%), moderate/severe: 14.29% (95% CI: 8.23-23.66%), critical: 49.18% (95% CI: 36.70-61.76%).

Table 8 presents the association between Ct value and mortality. There was no significant association between Ct value and mortality even after controlling for the confounding effect of age. Other potential confounders have been screened and eliminated by simple logistic regression or univariable analysis. A crude odds ratio is generated and *p* value <0.20 is considered as a potential confounder and is entered into the multiple logistic regression model together with Ct value to generate an adjusted OR. Change-in-estimate (CIE) criterion is used to check if these are true confounders. The crude OR of the Ct value is compared to the adjusted OR. After analyses of possible confounders, only age was considered.

Table 8. Association between Ct value and mortality (n=236)

Ct value	CRUDE OR (95% CI)[a]	*p* value	ADJUSTED OR (95% CI)[b]	*p* value
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.15 (0.53-2.49)	0.726	1.30 (0.58-2.90)	0.528
Low	1.21 (0.57-2.59)	0.615	1.17 (0.54-2.56)	0.687

Ref: Reference category; [a] Simple logistic regression analysis; [d]Multiple logistic regression analysis controlled for the confounding effect of age group

Table 9 presents the association between Ct value and mortality in patients by COVID-19 disease severity. Even after controlling for the confounding effect of age, there was no significant association observed between Ct value and mortality among asymptomatic/mild and moderate/severe cases. There was also no significant association observed between Ct value and mortality among critical COVID-19 cases. Furthermore, no significant confounder was recorded, thus, multiple logistic regression analysis was not performed.

Table 9. Association between Ct value and mortality by COVID-19 severity (n=91)

Ct value	CRUDE OR (95% CI)[a]	*p* value	ADJUSTED OR (95% CI)[b]	*p* value
Asymptomatic/Mild				
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.53 (0.25-9.23)	0.641	2.46 (0.43-14.12)	0.312
Low	1.64 (0.34-8.00)	0.539	1.91 (0.36-10.06)	0.443
Moderate/Severe				
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.09 (0.27-4.49)	0.903	1.28 (0.30-5.26)	0.762
Low	1.15 (0.28-4.76)	0.843	1.03 (0.25-4.33)	0.964
Critical				
High	*Ref*	*Ref*	-	-
Medium	1.04 (0.32-3.40)	0.945	-	-
Low	1.97 (0.52-7.52)	0.321	-	-

Ref: Reference category; [a] Simple logistic regression analysis with Firth's bias correction; [b]Multiple logistic regression analysis with Firth's bias correction

DISCUSSION

Our study showed that pediatric COVID-19 patients admitted in PCMC, were comprised mostly of children 0 to 5 years old, with a median age of 7, and predominantly males. This profile is similar to a retrospective study which looked into epidemiological characteristics of pediatric COVID-19 patients in China.[10] In terms of disease severity, however, they have observed more asymptomatic, mild or moderate cases,[10] while our study observed more severe to critical cases. This may be attributed to a skewed population of admitted cases with co-morbidities (84%) as PCMC is a tertiary multi-

specialty referral hospital. This trend in demographics and clinical characteristics was reflected in the study of Gonzales-Ritona in the same institution.[11] Nevertheless, a larger number of studies still support the finding that the pediatric population generally present with milder disease course compared to their adult counterparts.[10,12-13]

As to clinical presentation, fever and cough were the most commonly seen symptoms in our study. This is comparable to studies done previously in children abroad. [12-15] This also conforms with the latest case bulletin released by the Surveillance and Analysis of COVID-19 in Children Nationwide (SALVACION) of the Pediatric Infectious Disease Society of the Philippines (PIDSP). From this registry, the most common symptoms identified in Filipino children were fever at 57.6% followed by cough at 45.2%.[16] Additionally, other symptoms found in our study were dyspnea, vomiting and seizures. These symptoms were also reflected in the SALVACION registry and other pediatric databases.[15-16]

The organ systems most often involved during the course of COVID-19 in this study were the respiratory, neurologic, and gastrointestinal systems with more than a quarter of patients having multi-organ involvement. It is known that in those who acquired COVID-19, the respiratory system is most commonly affected.[17-18] The SARS-CoV-2 binds to angiotensin converting enzyme-2 receptors which are most abundant in the lungs.[17] However, these receptors are also present elsewhere in the body including the heart, vascular endothelial cells, brain, kidneys, intestines, liver, pharynx, and other tissues, thus, directly injuring these other organs as well.[15,17] This explains the multi-organ involvement in COVID-19. Neurological presentations may be due to accompanying fever or the direct viral invasion of the central nervous system since SARS-CoV-2 binds to the ACE-2 receptors found in the cerebral vascular endothelium.[15,17] Coagulopathy leading to infarction is also a plausible mechanism.[15] As for gastrointestinal manifestations, besides the greater expression of ACE-2 receptors in the liver and intestinal tract, it was suggested that the fecal-oral route in disease transmission accounted for the higher incidence of gastrointestinal symptoms in some studies.[15,19.]

When the Ct value is taken into account, we found that age significantly differed across the Ct value terciles, and that younger patients have higher Ct values which indicate lower viral loads compared to children five years and older. Published studies about this association have conflicting findings. In support of our study, one research with a small study population concluded that older patients tend to have higher viral loads.[20] Several postulates on host factors were explored in relation to this including the capacity to form antibodies and the maturity of the immune system. Children often contract viral respiratory infections hence produce more antibodies against viruses compared to adults.[10] Because of this, the immune systems of children are thought to respond to viral infections better.[10] Moreover, since children's immune systems are still underdeveloped, they may react to diseases differently from adults.[10] Due to this immaturity , the binding ability of their ACE-2 receptors may be less efficient compared to adults, therefore, children tend to have less sensitivity to SARS-CoV-2.[18] Also, since there is inefficient binding to receptors , the virus becomes incapable of replication,[18,21] hence, younger children have lower viral load. However, contrary to our study, one small-scale research found that children younger than 5 years of age have low Ct values, hence have higher viral loads.[22] Several other large-scale studies in children and adults found no association between age and viral load.[23-25]

Another significant finding in our study was that the Ct value for patients whose specimens were collected on the third day of illness, were lower than those collected later in the course of the disease. This is congruent with the findings of Zou, *et al.,* wherein higher viral loads were detected soon after symptom onset.[26] This information coincides with the phase of the disease and may aid clinicians in ascertaining whether the patient is in the first phase of illness (about the first week) wherein viremia is expected, or if the patient is in the second phase of illness when

viremia starts to declines and inflammation occurs.[27] This is relevant for clinicians in making treatment decisions, *i.e.* whether to give anti-viral medications, anti-interleukin 6 or steroids when a patient is expected to enter a cytokine storm or during a macrophage activation syndrome.[24]

Pediatric patients in our study who had symptoms had lower Ct values compared to asymptomatic patients. The finding of Roversi, *et al.* strongly suggested that the lack of overt symptomatology in children may be associated with a higher Ct value.[28] However, a number of studies still found that Ct values are comparable between symptomatic and asymptomatic patients, supporting the potential transmission of COVID-19 from asymptomatic children.[29-31]

As for co-morbidities, our study found that patients with co-infections had high Ct values hence a lower viral load. Co-infections play an important role in reducing or augmenting disease severity.[32] In our study, majority of co-infections were viral in etiology. The lower viral load represented by higher Ct values may be explained by viral interference.[33] This phenomenon occurs when one virus influences the replication of the other.[33] The mechanism is also mediated by various factors such as interferons, defective interfering particles, production of trans-acting proteases, cellular factors, and non-specific double-stranded RNA.[34] The host's immune system also affects the outcome of viral co-infections.[33] After exposures to antigens, naive T cells convert into activated effector T cells and later on into long-term memory T cells.[34] Memory responses created to act upon one infection may influence the performance of the immune response to a subsequent secondary infection which is known as heterologous immunity.[34-35] Several immune cells are involved in heterologous immunity and these may result in either a protective or immunopathological response.[35]

Few studies showed an association between Ct values and disease severity.[36-39] In a study by Maltezou, *et al.*, high viral load was inversely correlated with COVID-19 severity across the cohort and in the subgroup of hospitalized patients, even after adjusting for several patient characteristics.[36] Their findings could be useful in the identification of those patients at risk for severe illness or mortality.[36] However, our study did not show significant association between SARS-CoV-2 RT-PCR Ct value and COVID-19 disease severity as well as mortality. This is similar to some larger studies done among pediatric patients.[40-44] A possible explanation for the conflicting results with the association of Ct value and disease severity is the variability in definition of disease severity in different studies[42] In our study, the disease severity classification was based on international and local interim guidelines that evolved throughout the study period encompassing various surges of different variants of SARS-CoV-2.

As for the lack of association of Ct value with mortality, interventions, such as antiviral drugs, immunomodulators, corticosteroids and even vaccination could have had an impact on disease outcome.[42,45] These interventions have been used in some patients during the latter part of the pandemic and those who acquired the disease earlier may not have received them. In our study, the different interventions were not accounted for, hence further studies are recommended to explore their effect on Ct values and disease outcomes.

There were various limitations in our study. This is a retrospective research hence the information gathered depended on what was available on patients' records. As PCMC is a tertiary referral hospital catering mostly to pediatric patients with co-morbidities and those needing specialty care, the results of this study cannot be generalized to other institutions. Moreover, while the study period covered the emergence of different SARS-CoV-2 variants and subvariants, the potential impact of these viral mutations on disease severity and outcome were not included in the analysis. Additionally, among patients with co-morbidities, the causal relationship between COVID-19 and mortality was not explored and established. Lastly, since information about COVID-19 is still evolving, the interventions offered are changing as well. It may be

useful to account for various interventions done on patients that may have affected their clinical presentation, altered the clinical course or affected the severity and outcome of the disease.

CONCLUSION

This study showed no significant association between SARS-CoV-2 RT PCR cycle threshold value with disease severity and in-hospital all-cause mortality among hospitalized pediatric COVID-19 patients. However, younger pediatric patients particularly those five years and below have higher Ct values which indicate lower viral loads. The Ct values of pediatric patients are significantly lower when specimens were collected in the first 3 to 5 days of illness compared to 5 days beyond symptom onset. Pediatric patients with symptoms also had lower Ct values compared to other groups. Lastly, we found that patients with co-infections had higher Ct values.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

This is to acknowledge the following for their contribution: Dr. Ma. Lucila Perez for technical review and assistance; Dr. Maria Anna Bañez, Dr. Fatima Gimenez, Dr. Mary Ann Bunyi and Dr. Jaime Santos for participating in the review of this paper; Dr. Jacqueline Rochelle Acuña and Dr. Elmer Hilary Balbin for the assistance in data gathering.

REFERENCES

1. World Health Organization [Internet]. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 (2020 March 11). Retrieved May 5, 2021. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
2. McIntosh K. Coronavirus disease 2019 (COVID-19): Epidemiology, virology, and prevention. In A. Bloom (Ed.), UpToDate. (2021 June 9). Retrieved July 8, 2021. Available from: https://www.uptodate.com/contents/covid-19-epidemiology-virology-and-prevention#topicContent
3. Philippine Pediatric Society and Pediatric Infectious Disease Society of the Philippines. (2021 February 6). Interim Guidelines on the Screening, Assessment and Clinical Management of Pediatric Patients with Suspected or Confirmed Coronavirus Disease 19. 2022 January 08. Version 6. 13-16
4. Padua Jr., R. Diagnostic Testing Strategies to Manage COVID-19 Pandemic: Proposed by the Philippine Society of Pathologists, Inc. (2020). PJP, 5(1), 5-8. Available from: https://doi.org/10.21141/PJP.2020.08
5. Sethuraman N, et al. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA [Internet]. 2020 (2020 May 6).;323(22):2249–2251. Available from: doi:10.1001/jama.2020.8259
6. Stites EC, Wilen CB. The Interpretation of SARS-CoV-2 Diagnostic Tests, Med. [Internet] (2020). Available from: https://www.sciencedirect.com/science/article/pii/S2666634020300167
7. Bustin SA, Mueller R. Real-time Reverse Transcription PCR (qRT-PCR) and Its Potential Use in Clinical Diagnosis. [Internet]. (2005). Available from: https://pubmed.ncbi.nlm.nih.gov/16171460/
8. Faico-Filho KS, et al. Is Higher Viral Load in SARS-CoV-2 Associated with Death? The American Society of Tropical Medicine and Hygiene. [Internet]. (2020).
9. Greenland, S., & Pearce, N. Statistical foundations for model-based adjustments. Annual review of public health, [Internet]. (2015). 36, 89–108. Available from https://doi.org/10.1146/annurev-publhealth-031914-122559
10. Yuanyuan Dong, MD, et. Al. Epidemiology of COVID-19 Among Children in China. [Internet]. (2020). Available from: http://publications.aap.org/pediatrics/article-pdf/145/6/e20200702/1080196/peds_20200702.pdf
11. Gonzales-Ritona J. Clinical Presentation and Outcome of Pediatric COVID-19 Patients admitted in Philippine Children's Medical Center (PCMC): The First 100 Cases. [Internet]. (April 2021). Available from: http://www.pcmc.gov.ph/images/RF2021/Poster_Fellows/GONZALES-RITONAJENNEELYNA.pdf
12. Gotzinger F, Begoña S, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. The Lancet, Child and Adolescent Health. [Internet]. (2020 September). Available from: https://doi.org/10.1016/S2352-4642(20)30177-2
13. Ranabothu, S., Onteddu, S., Nalleballe, K., et al. Spectrum of COVID-19 in children. Acta paediatrica [Internet]. (2020). (Oslo, Norway : 1992), 109(9), 1899–1900. Available from: https://doi.org/10.1111/apa.15412
14. Zimmermann P, Curtis N. COVID-19 in Children, Pregnancy and Neonates: A Review of Epidemiologic and Clinical Features. The Pediatric Infectious Disease Journal

[Internet]. (2020 June). - Volume 39 - Issue 6 - p 469-477. Available from: DOI: 10.1097/INF.0000000000002700

15. Sayed M.A., Abdelhakeem M. Typical and Atypical Clinical Presentation of COVID-19 infection in children in the top of pandemic in EL-Minia governorate (two center experience). Mediterr J Hematol Infect Dis. [Internet]. (2022). 14(1): e2022002. Available from: DOI: http://dx.doi.org/10.4084/MJHID.2022.002
16. Chiong-Rivera A, Pantig F, Maramba-Lazarte C, et al. Surveillance and Analysis of COVD-19 in Children Nationwide (SALVACION), Case Bulletin #5. [Internet]. (2002). Available from: http://www.pidsphil.org/home/wp-content/uploads/2022/05/Salvacion20casebulletin205.pdf
17. Jain U. Effect of COVID-19 on the Organs. Cureus, [Internet]. (2020). 12(8), e9540. Available from: https://doi.org/10.7759/cureus.9540
18. Fang F, Lu X. Facing the pandemic of 2019 novel coronavirus infections: the pediatric perspectives [Internet]. (2020 February 2]. 58(0):E001
19. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J. [Internet]. 2020. 39:355–368. Available from: doi: 10.1097/INF.0000000000002660.
20. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS- CoV-2: an observational cohort study. Lancet Infect Dis. [Internet]. (2020). 20:565–74. Available from: DOI: 10.1016/S1473-3099(20)30196-1
21. Pierce C.A., Sy S., Galen B., et al. Natural mucosal barriers and COVID-19 in children. JCI Insight. 2021;6:eabd5487.
22. Heald-Sargent T, Muller WJ, Zheng X, et al. Age-related differences in nasopharyngeal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) levels in patients with mild to moderate Coronavirus Disease 2019 (COVID-19). JAMA Pediatr. [Internet]. (2020). 174:902–3. Available from: DOI: 10.1001/jamapediatrics.2020.3651
23. Mahallawi WH, Alsamiri AD, Dabbour AF, et al. Association of Viral Load in SARS-CoV-2 Patients With Age and Gender. Front. Med. [Internet]. (2021). 8:608215. Available from: DOI: 10.3389/fmed.2021.608215
24. Jacot D, Greub G, Jaton K, Opota O. Viral load of SARS-CoV-2 across patients and compared to other respiratory viruses. Microbes Infect. [Internet]. (2020). 22:617–21. Available from: DOI: 10.1016/j.micinf.2020.08.004
25. Baggio S, L'Huillier AG, Yerly S, et al. SARS-CoV-2 viral load in the upper respiratory tract of children and adults with early acute COVID-19. Clin Infect Dis. [Internet]. (2020). 6:ciaa1157. Available from DOI: 10.1101/2020.07.17.20155333
26. Zou, L., Ruan, F., Huang, M., et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. The New England journal of medicine, [Internet]. (2020); 382(12), 1177–1179. Available from: https://doi.org/10.1056/NEJMc2001737
27. Tay M.Z., Poh C.M., Renia L., et al. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. (2020);20(6):363–374.
28. Roversi, M., Coltella, L., Piccioni, L. et al. Relationship between viral load and symptoms in children infected with SARS-CoV-2. Pediatr Res. [Internet]. (2022). Available from: https://doi.org/10.1038/s41390-022-02293-4
29. Qian Wu, Lixia Shi, Haomin Li, et al. Viral RNA Load in Symptomatic and Asymptomatic COVID-19 Omicron Variant-Positive Patients", Canadian Respiratory Journal, [Internet] (2022). vol., Article ID 5460400, 6 pages, 2022. Available from: https://doi.org/10.1155/2022/5460400
30. Ra SH, Lim JS, Kim G, et al. Upper respiratory viral load in asymptomatic individuals and mildly symptomatic patients with SARS-CoV-2 infection. Thorax [Internet]. . (2021 May). 2021;76:61-63.
31. Zuin M, Gentili V, Cervellati C, Rizzo R, Zuliani G. Viral Load Difference between Symptomatic and Asymptomatic COVID-19 Patients: Systematic Review and Meta-Analysis. Infect Dis Rep.[Internet]. (2021 July). 13(3):645-653. Available from: doi: 10.3390/idr13030061. PMID: 34287354; PMCID: PMC8293148.
32. Asner SA, Science ME, Tran D, Smieja M, Merglen A, Mertz D. Clinical disease severity of respiratory viral co-infection versus single viral infection: a systematic review and meta-analysis. PLoS One [Internet]. 2014.9:e99392. Available from: DOI: 10.1371/journal.pone.0099392.
33. Kumar N, Sharma S, Barua S, et al. Virological and immunological outcomes of coinfections. Clin Microbiol Rev. [Internet]. (2018) 31:17. Available from: DOI: 10.1128/CMR.00111-17
34. Salas-Benito JS, De Nova-Ocampo M. Viral interference and persistence in mosquito-borne flaviviruses. J Immunol Res [Internet]. 2015:873404. Available from: DOI: 10.1155/2015/873404.
35. Sharma S, Thomas PG. The two faces of heterologous immunity: protection or immunopathology. J Leukoc Biol [Internet]. (2014). 95:405–416. Available from: DOI: 10.1189/jlb.0713386.
36. Maltezou, H. C., Raftopoulos, V., Vorou, R., et al. Association Between Upper Respiratory Tract Viral Load, Comorbidities, Disease Severity, and Outcome of Patients With SARS-CoV-2 Infection. The Journal of infectious diseases, [Internet]. (2021). 223(7), 1132–1138. Available from: https://doi.org/10.1093/infdis/jiaa804

37. Souverein, D. Stralen, K. Lelyveld, S. et al. Initial Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load Is Associated With Disease Severity: A Retrospective Cohort Study, Open Forum Infectious Diseases, [Internet]. (2022 July). Volume 9, Issue 7. Available from: https://doi.org/10.1093/ofid/ofac223
38. Shah, V., Farah, W., Hill, J. Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis, Open Forum Infectious Diseases, [Internet]. (2021 September). Volume 8, Issue 9, ofab453. Available from: https://doi.org/10.1093/ofid/ofab453
39. Rabaan, A.A. et al. Viral Dynamics and real-time RT-PCR CT values correlation with disease severity in COVID-19, Diagnostics (Basel, Switzerland). MDPI. [Internet]. (2021). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232180/
40. AlBahrani, S., Alghamdi, M., Zakary, N. et al. Initial viral cycle threshold values in patients with COVID-19 and their clinical significance. Eur J Med Res [Internet]. (2022). 27, 101. Available from: https://doi.org/10.1186/s40001-022-00729-5
41. Camargo JF, Lin RY, Komanduri KV. Lack of correlation between the SARS-CoV-2 cycle threshold (Ct) value and clinical outcomes in patients with COVID-19. J Med Virol. [Internet]. 2021;93:6059–62. Available from: https://doi.org/10.1002/jmv.27171.
42. Yilmaz A, Marklund E, Andersson M, et al. Upper respiratory tract levels of severe acute respiratory syndrome coronavirus 2 RNA and duration of viral RNA shedding do not differ between patients with mild and severe/critical coronavirus disease 2019. J Infect Dis [Internet]. 2021; 23:15–18.
43. Argyropoulos KV, Serrano A, Hu J, et al. Association of initial viral load in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with outcome and symptoms. Am J Pathol 2020; 190:1881–7.
44. Rhoads D, Peaper DR, She RC, et al. College of American Pathologists (CAP) Microbiology Committee Perspective: Caution Must Be Used in Interpreting the Cycle Threshold (Ct) Value. Clin Infect Dis. [Internet]. (2020). Available from: https://doi.org/10.1093/cid/ciaa1199.
45. Zheng A, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejang province, China, January–March 2020: retrospective cohort study. BMJ [Internet]. 2020; 369:m144

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

ORIGINAL ARTICLE

CLINICAL PROFILE AND TREATMENT OUTCOMES OF ACUTE CHOLANGITIS IN CHILDREN IN A TERTIARY GOVERNMENT HOSPITAL IN THE PHILIPPINES: A FIVE-YEAR RETROSPECTIVE STUDY

Arianne L. Calimlim-Samson, MD, FPPS, DPIDSP, Carmina A. delos Reyes, MD, FPPS, FPIDSP,
Germana Emerita V. Gregorio, MD, MSc, PhD
Philippine General Hospital

ABSTRACT

Background: Acute cholangitis (AC) in children is a rare but life-threatening infection. Symptoms vary from mild to severe disease. There are no local published data on pediatric AC.

Objective: To determine the clinical, biochemical, ultrasonographic, microbiologic features, and treatment outcome of pediatric patients with definite AC.

Methodology: Cross-sectional study using medical records of pediatric patients diagnosed with definite AC based on the Modified Tokyo Guidelines of 2018 admitted from January 2016 to June 2021.

Results: Twenty-seven patients aged 0 to 18 years old (10.06 $\pm$ 7.34), predominantly male (51.85%) were included. Choledocholithiasis (22%) and post-Kasai biliary atresia (22%) were the common underlying biliary conditions. Fever (88.89%) was the most frequent presenting symptom. Majority were classified as moderate AC (40.74%). Leukocytosis (mean $16x10^9$/L), elevated inflammatory markers (93.33% with CRP >12mg/L and 100% with serum procalcitonin >0.25ng/mL), hyperbilirubinemia (total bilirubin 192.54±126.87umol/L) and elevated alanine transferases (mean 59 IU/L) were noted. Twenty-one out of 27 cases (87%) had a negative blood culture. Only 4 patients underwent bile culture, of which two (50%) grew *Klebsiella pneumoniae* resistant to empiric antibiotics. Dilated biliary ducts were observed on abdominal ultrasound in 92.59% of patients. Ampicillin-sulbactam (29.63%) was the most commonly utilized antibiotic. Discharge rate was high (88.89%).

Conclusions: AC affects all pediatric age groups but clinical presentations vary. Drug resistant organisms are a significant concern but despite this, favorable outcomes have been documented.

KEYWORDS: *Cholangitis, Children, Choledocholithiasis*

Correspondence:
Dr. Arianne L. Calimlim-Samson
Email: ariannecalimlimsamson@gmail.com

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

INTRODUCTION

Acute cholangitis (AC), also known as ascending cholangitis, is a life-threatening disease affecting the biliary tree caused by a bacterial infection. The diagnosis is described by clinical presentation, laboratory results, and imaging studies implying infection and biliary obstruction.[1] In children, acute cholangitis is rare. Symptoms may be mild like fever, abdominal pain or jaundice to severe forms like shock. It occurs most often with specific diseases such as biliary atresia, biliary obstructions, or a sequela of previous biliary surgeries.[2] Severe AC may be a result of delayed diagnosis and treatment, significantly increasing mortality and morbidity rates. The leading causes of death are septic shock and multiple organ failure. Mortality rate of AC in children may reach 10-30%.[3]

Standard criteria for diagnosing and treating acute cholangitis in pediatrics have not been well established and only derived from a draft of the Tokyo Guidelines, an international consensus meeting of specialists for the management of acute cholecystitis and cholangitis held in Tokyo, Japan in the year 2006. The criteria for diagnosis and severity grading of AC were amended and reintroduced in 2013. Its validation through application in clinical practice was established in 2018. The updated guidelines determined the conditions that should be present to identify acute cholangitis in patients, which includes evidence of systemic inflammation, presence of cholestasis, and occurrence of biliary disorder based on history, histology or imaging.

As of this writing, no substantial description of demographic and clinical profile of pediatric acute cholangitis is available in the country. This study can initiate a local database on this condition and help identify further gaps in knowledge that can be addressed in future researches. The clinical and laboratory profile can inform clinicians on how acute cholangitis presents among pediatric patients and increase early recognition and thus early treatment as well, for improved outcomes. Ultimately, providing quality healthcare for Filipino children.

MATERIALS AND METHODOLOGY

Study Design

This was a retrospective, cross-sectional institution-based study conducted at the Philippine General Hospital, Department of Pediatrics, Divisions of Pediatric Gastroenterology, Hepatology and Nutrition and Infectious and Tropical Diseases .

Study Population and Setting

All pediatric patients 0 to 18 years old with a diagnosis of definite or recurrent acute cholangitis and severity classification as defined using the Modified Tokyo Guidelines 2018 (Table 1 and 2) admitted from January 1, 2016 to June 30, 2021 were included in this study. Suspected cases of acute cholangitis or cases that did not satisfy the diagnostic criteria were excluded.

Table 1. Diagnostic Criteria for Acute Cholangitis in Children (Modified from Tokyo Guidelines 2018)

A. Systemic inflammation
Persistent fever with no other focus of infection with laboratory data demonstrating evidence of any of the following laboratory response:
A-1. Increased white blood cell count
A-2. Increased quantitative C-reactive protein
B. Cholestasis
B-1. Appearance of jaundice or Increased intensity of jaundice in a child who is previously icteric
B-2. Presence of pale or acholic stools in a child with previously normal colored stool
B-3. Laboratory data: abnormal liver function tests (increased bilirubin and ALT levels) or deterioration in the levels of liver function tests
C. Biliary disorder based on history, histology or imaging (ultrasound of liver and hepatobiliary tree, CT scan of abdomen, magnetic resonance cholangiopancreatography)
C-1. History of biliary surgery
C-2. Evidence of biliary pathology on liver biopsy
C-3. Biliary dilatation on imaging
C-4. Evidence of the etiology on imaging (bile sludge, stricture, stone, mass, etc.)
Diagnosis
Suspected diagnosis: 1 item in A + 1 item in either B or C
Definite diagnosis: 1 item each in A, B and C
Recurrent Cholangitis: occurrence of 1 or more episodes of acute cholangitis within a 6-month period

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

Table 2. Severity Assessment Criteria for Acute Cholangitis in Children (Modified from Tokyo Guidelines 2018)

Mild Acute Cholangitis
If the acute cholangitis does not meet the criteria of Severe or Moderate acute cholangitis at initial diagnosis
Moderate Acute Cholangitis
If associated with at least any three of the following conditions: 1. High grade fever (T>39C) with no other focus of infection 2. Abnormal white blood cell count for age[a] 3. Appearance of jaundice (TB > 5mg/dl) or increased in intensity of jaundice in a child who was previously icteric 4. Presence of irritability
Severe Acute Cholangitis
If the patient fulfills criteria for moderate acute cholangitis AND ANY of the following signs of organ dysfunction 1. Cardiovascular dysfunction: hypotension <5th percentile for age or systolic BP < 2 SD below normal for age) despite administration of isotonic intravenous fluid bolus ≥ 60ml/kg in 1 hour and/or inotropic support (dopamine or dobutamine >5ug/kg/min, or epinephrine or norepinephrine at any dose) 2. Neurological dysfunction: disturbance of consciousness (Glascow coma scale ≤11 or acute change in mental status with a decrease in GCS ≥3 points from abnormal baseline) 3. Respiratory dysfunction: PaO2/FiO2 < 300 in the absence of cyanotic heart disease or preexisting lung disease, or need for >50% FiO2 to maintain saturation ≥ 92% 4. Renal dysfunction: Serum creatinine ≥ 2 times upper limit of normal for age or 2-fold increase in baseline creatinine 5. Hepatic dysfunction: PT-INR >1.5 unresponsive to Vitamin K 6. Hematological dysfunction: platelet count <100,000/mm

[a]1 to 23 months >14,000/mm^3, 2 to 9 years >12,000/mm^3, 10 to 18 years >10,500/mm^3

Data Collection

The list of all pediatric patients who fulfilled the inclusion criteria was generated from the database of the pediatric divisions of gastroenterology and infectious disease of all patients diagnosed with acute cholangitis or ascending cholangitis from January 1, 2016 to June 30, 2021.

Using a standardized data collection sheet, the patient's age and sex were logged in the demographic profile. Their clinical manifestations, onset of symptoms, severity classification, recurrence of infection, and use of prophylaxis were noted in the clinical profile. Predisposing condition, underlying biliary diseases, as well as previous biliary procedures were documented. Determination of biochemical features included white blood cell count, inflammatory markers such as C-reactive protein and serum procalcitonin, serum bilirubin, and alanine transaminase. Isolates from blood and bile cultures were noted. Imaging findings, specifically dilatation of bile duct, presence of bile duct stones or calculi, strictures, obstructive lesions and stenosis, from abdominal ultrasound, abdominal computed tomography (CT) scan, abdominal magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), and endoscopic ultrasound were noted. The antibiotics used as well as the outcome of the patients were documented. Clinical outcomes identified include the following: discharged, mortality, with complications (morbidity), and home against medical advice.

Statistical Analysis

Descriptive statistics were used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for categorical variables. Shapiro-Wilk test was used to determine the normality distribution of continuous variables. Continuous quantitative data that met the normality assumption were summarized using mean and standard deviation (SD), while those that did not, were described using median and range. All valid data were included in the analysis. Missing data were neither replaced nor estimated. The null hypothesis was rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

Ethical Considerations

This study was approved by the Philippine General Hospital Expanded Hospital Research Office (EHRO) Technical Review Panel and the University of the Philippines Manila Research Ethics Board (UPMREB) prior to data collection. This study adhered to the ethical considerations and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, World Health organization

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012, and National Ethics Guidelines for Health Research.

A waiver of informed consent was requested from the Ethical Panel since the research presents no more than minimal risk and the waiver or alteration will not severely affect the rights and welfare of the participants. In accordance with the National Ethical Guidelines of Health and Health-related Research 2017, the research cannot be carried out without the waiver and the review of medical records and its anonymity will be maintained. Since this is a retrospective study using medical records, it is prudent to state that a written informed consent was obtained from the parents/guardian upon admission. Data was solely collected by the primary investigator. All subject information was anonymized via identification code numbers and kept confidential. A master list linking the code number and subject identity was kept separately from the research data. Only members of the research team have access to the list. Computerized study information were stored on a secured network with password access and saved in a USB storage device. This and the data collection forms were kept in filing cabinets under lock and key and accessed only by the investigator. The data will be securely stored for at least five years from the date of final publication and will be destroyed thereafter. The risk to privacy is minimal in this study, however in case of a breach, the matter will be forwarded immediately to the Philippine General Hospital data privacy officer.

The investigators declare that there is no conflict of interest nor were they associated with any sponsor or compensation in the conduct of this study.

RESULTS

Table 3. Demographic and clinical profile of patients with AC (n=27)

	All (n=27)	<5 years old (n=9)	≥5 years old (n=18)
Age, years (Mean ± SD)	10.06±7.34	-	-
Sex (Frequency [%])			
Male	14 (51.85)	4 (44.44)	10 (55.56)
Female	13 (48.15)	5 (55.56)	8 (44.44)
Clinical manifestation			
Fever	24 (88.89)	9 (100)	15 (83.33)
Jaundice	23 (85.19)	8 (88.89)	15 (83.33)
Abdominal Pain	18 (66.67)	1 (11.11)	17 (94.44)
Pale/acholic stool	8 (29.63)	7 (77.78)	1 (5.56)
Lethargy	7 (25.93)	2 (22.22)	5 (27.78)
Irritability	5 (18.52)	4 (44.44)	1 (5.56)
Chills/Rigor	1 (3.70)	0	1 (5.56)
Confusion	1 (3.70)	0	1 (5.56)
Abdominal pain, fever, and jaundice	12 (44.44)	1 (11.11)	11 (61.11)
Cardiovascular dysfunction			
Hypotension despite intravenous fluid bolus and/or inotropic support	3 (11.11)	0	3 (16.67)
Neurologic dysfunction			
GCS<11	1 (3.70)	0	1 (5.56)
Acute change in mental status with a decrease in GCS ≥3 points from abnormal baseline	1 (3.70)	0	1 (5.56)
Respiratory dysfunction			
PaO2/FiO2 < 300 in the absence of cyanotic heart disease or preexisting lung disease	0	0	0
Need for >50% FiO2 to maintain saturation ≥ 92%	1 (3.70)	0	1 (5.56)
Renal dysfunction			
Serum creatinine ≥ 2 times upper limit of normal for age	2 (7.41)	2 (22.22)	0
2-fold increase in baseline creatinine	2 (7.41)	2 (22.22)	0
Hepatic dysfunction			
(PT-INR >1.5 unresponsive to Vitamin K)	4 (14.81)	2 (22.22)	2 (11.11)
Hematologic dysfunction			
(Platelet count <100,000/mm)	4 (14.81)	2 (22.22)	2 (11.11)
Onset of symptom			
≤1 week	6 (22.22)	4 (44.44)	2 (11.11)
>1 week	21 (77.78)	5 (55.56)	16 (88.89)
AC severity on admission			
Mild	10 (37.04)	5 (55.56)	5 (27.78)
Moderate	11 (40.74)	2 (22.22)	9 (50)
Severe	6 (22.22)	2 (22.22)	4 (22.22)
Recurrent acute cholangitis	2 (7.41)	1 (11.11)	1 (5.56)
Prophylaxis[b]	1 (3.70)	1 (11.11)	0

[b]Cotrimoxazole prophylaxis was given to only one patient out of two with a recurrent episode of acute cholangitis

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

Twenty-seven pediatric patients with definite acute cholangitis were included in the analysis and divided into two groups: < 5 years old (n=9) and ≥ 5 years old (n=18) (Table 3). The youngest was 17 days old and the oldest was 18 years old. The average age of the patients was 10.06 ± 7.34 years. Patients were predominantly male (51.9% vs 48.2%). Overall, the three most common clinical manifestations were fever (88.89%), jaundice (85.19%), and abdominal pain (66.67%). Acholic stools were seen in eight (29.63%) patients. Other clinical manifestations seen were lethargy, irritability, chills/rigor and confusion. Multiorgan dysfunction such as cardiovascular, respiratory, renal, hepatic, and hematologic signs were also noted. The onset of symptoms was over a week from admission in 78% of the patients. Severity of AC in these patients varied from mild (37.04%), moderate (40.74%), to severe (22.22%). Two patients with biliary atresia and disseminated tuberculosis had recurrent AC, one of whom received prophylaxis with cotrimoxazole.

Table 4. Predisposing conditions of patients with AC (n=27)

	All (n=27)	<5 years old (n=10)	≥5 years old (n=17)
	Frequency (%)		
Conditions			
Choledocholithiasis	6 (22.22)	0	6 (35.29)
Post Kasai in Biliary Atresia*	6 (22.22)	5 (50)	1 (5.88)
Cholelithiasis	4 (14.81)	0	4 (23.53)
Choledochal cyst	3 (11.11)	1 (10)	2 (11.76)
Hepatobiliary tuberculosis	3 (11.11)	0	3 (17.65)
Biliary Atresia with no surgery	2 (7.41)	2 (20)	0
Gallbladder hydrops	1 (3.70)	1 (10)	0
Biliary ascariasis	1 (3.70)	0	1 (5.88)
Necrotizing enterocolitis	1 (3.70)	1 (10)	0
History of biliary disease			
Acute cholecystitis	2 (7.41)	1 (11.11)	1 (5.56)
Acute pancreatitis	0	0	0
Previous biliary procedures			
Kasai**	4 (14.81)	3 (33.33)	1 (5.56)
Open tube cholecystostomy	1 (3.70)	0	1 (5.56)
Roux-en-Y choledochocystojejunostomy	1 (3.70)	0	1 (5.56)
Cholecystectomy	0	0	0
Biliary stent placement	0	0	0

*Post Kasai procedure during the time of AC diagnosis and admission
**Previous Kasai procedure months or years prior to the time of AC diagnosis and admission

Of the 27 patients, there were six who had choledocholithiasis; six biliary atresia patients who had Kasai portoenterostomy procedure during the AC diagnosis and admission; four with cholelithiasis; three with choledochal cyst; three with hepatobiliary tuberculosis; two biliary atresia patients with no surgical intervention; and one patient for each case of gallbladder hydrops, biliary ascariasis, and sepsis from necrotizing enterocolitis (Table 4). Two patients also had a history of acute cholecystitis. Previous biliary procedures included four patients who had Kasai portoenterostomy months/years prior to AC diagnosis, one with open tube cholecystostomy and one with Roux-en-Y choledochocystojejunostomy.

Table 5. Biochemical and Microbiologic features of patients with AC (n=27)

	All (n=27)	<5 years old (n=9)	≥5 years old (n=18)
WBC [n=27]	Mean ± SD; Median (Range)		
10^9/L	16 (2.4-46.6)	15 (2.4-46.6)	16.55 (10.75-35.4)
CRP mg/L (n=13)	Frequency (%)		
<6	1 (6.67)	1 (20)	0
>12	12 (93.33)	4 (80)	8 (100)
Procalcitonin ng/mL (n=12)	Frequency (%)		
>0.25	12 (100)	5 (100)	7 (100)
ALT (n=27)	Mean ± SD; Median (Range)		
IU/L	59 (13-402)	53 (16-402)	74.5 (13-240)
Bilirubin umol/L (n=27)	Mean ± SD		
Total	192.54±126.87	201.46±151.97	188.57±118.78
Direct	163.58±116.92	167.49±141.89	161.85±108.65
Indirect	28.95±17.81	33.98±21.02	26.72±16.36
Blood culture done	24 (88.89)	9 (100)	15 (83.33)
No isolates	21 (87.50)	7 (77.78)	14 (93.33)
With positive isolates	3 (12.50)	2 (22.22)	1 (6.67)
Salmonella B sp.	1 (33.33)	1 (50)	0
Gram negative bacilli	1 (33.33)	0	1 (100)
Acinetobacter baumannii	1 (33.33)	1 (50)	0
Serratia marcescens	1 (33.33)	1 (50)	0
Bile culture done	4 (14.81)	0	4 (22.22)
No isolates	2 (50)	-	2 (50)
With positive isolates	2 (50)	-	2 (50)
Klebsiella pneumoniae	2 (100)		2 (100)

The average white blood cell count for those <5 years old was 15x10^9/L while for those >5 years old was 16.55x10^9/L. These values were particularly high based on the subjects' age. Meanwhile, the average

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

serum alanine transferase for all patients was likewise elevated at 59 IU/L. Increased level of C-reactive protein (>12mg/L) was likewise seen in all 13 subjects requested with the biomarker except for one patient aged less than 5 years old. Procalcitonin was requested amongst 12 subjects and all showed elevated levels >0.25ng/ml. The mean values of total (192.54±126.87 umol/L), direct (163.58±116.92 umol/L) and indirect (28.95±17.81 umol/L) bilirubin were all elevated in both age groups. Laboratory data indicative of inflammation (e.g., leukocytosis and an elevated C-reactive protein [CRP] level), and evidence of biliary stasis (e.g., hyperbilirubinemia, elevation of biliary enzymes and liver enzymes) are frequently seen in patients with acute cholangitis, and such laboratory findings support the diagnosis.

All patients had blood culture requested on their chart, but only 24 were found to have results available on record. Only three patients were identified to have isolates. One patient had a mixed growth of *Serratia marcescens* and *Acinetobacter baumannii*, another with a *Salmonella B sp.* and unspecified gram-negative bacilli.

On the other hand, all patients with bile cultures were >5 years old, of whom, two had positive isolate of *Klebsiella pneumoniae*. Both empiric antibiotics used for these isolates were resistant based on their sensitivity pattern.

Table 6. Radiologic findings in patients with AC (n=27)

	All (n=27)	<5 years old (n=9)	≥5 years old (n=18)
	Frequency (%)		
Abdominal Ultrasound done	25 (92.59)	8 (88.89)	17 (94.44)
No findings	3 (12)	2 (25)	1 (5.88)
Dilation of the bile duct	13 (52)	3 (37.50)	10 (58.82)
Bile duct stones	2 (8)	0	2 (11.76)
Others	7 (28)	3 (37.50)	4 (23.53)
Abdominal CT Scan done	9 (33.33)	1 (11.11)	8 (44.44)
No findings	0	0	0
Dilation of the bile duct	9 (100)	1 (100)	8 (100)
MRC cholangiopancreatography done	2 (7.41)	0	2 (11.11)
No findings	0	-	0
Low-diameter strictures	2 (100)	-	2 (100)
Large common bile duct stones	0	-	0
Others	2 (100)	-	2 (100)

Table 6 continued. Radiologic findings in patients with AC (n=27)

	All (n=27)	<5 years old (n=9)	≥5 years old (n=18)
	Frequency (%)		
Abdominal MRI done	0	0	0
ERC done	6 (22.22)	0	6 (33.33)
No findings	0	-	0
Asymmetrical dilation of bile ducts	2 (33.33)	-	2 (33.33)
Presence of calculi	2 (33.33)	-	2 (33.33)
Presence of obstructive lesions and stenosis	1 (16.67)	-	1 (16.67)
Others	1 (16.67)	-	1 (16.67)
Endoscopic Ultrasound done	1 (3.70)	0	1 (5.56)
No findings	0	-	0
Biliary duct dilation	0	-	0
Small stones	0	-	0
Malignancy	0	-	0
Pseudocyst	1 (100)	-	1 (100)

Only 25 subjects underwent abdominal ultrasound while the remaining two subjects had no abdominal imaging requested. Amongst the 25 subjects with abdominal ultrasound (92.59%), 52% had dilatation of the bile duct and 12% had bile duct stones. Among the nine (33.33%) patients who underwent abdominal CT scan, all were found to have dilatation of the bile duct but none with high-attenuated nodules. Only two (7.41%) underwent MRCP with low-diameter strictures noted for both. Only one (3.7%) patient had endoscopic ultrasound and was found to have pseudocyst; while there were six (22.22%) who underwent ERCP and their findings included asymmetrical dilatation of the bile ducts (33.33%), presence of calculi (33.33%), and obstructive lesions and stenosis (16.67%) [Table 6].

Table 7. Antibiotics given to patients with AC (n=27)

Antibiotics	Frequency (%)
Ampicillin-Sulbactam	7 (25.93)
Ceftriaxone and Metronidazole	6 (22.22)
Cefoxitin and Metronidazole	4 (14.81)
Meropenem	4 (14.81)
Piperacillin-Tazobactam	4 (14.81)
Ampicillin-Sulbactam, shifted to Meropenem	1 (3.70)
Ampicillin-Sulbactam, shifted to Piperacillin-Tazobactam	1 (3.70)
Cefoxitin	1 (3.70)
Cefoxitin and Metronidazole, shifted to Ceftazidime and Metronidazole	1 (3.70)
Ceftriaxone	1 (3.70)
Colistin and Gentamycin	1 (3.70)
Oxacillin and Metronidazole	1 (3.70)

Pediatric Infectious Disease Society of the Philippines Journal
Vol 24 No 2, pp. 64-74 July-December 2023
Calimlim-Samson AL, delos Reyes CA, Gregorio GEV. Clinical Profile and Treatment Outcomes Of Acute Cholangitis in Children in a Tertiary Government Hospital in the Philippines: A Five-Year Retrospective Study.
https://doi.org/10.56964/pidspj20232402008

The most common antibiotic given to patients was ampicillin-sulbactam (25.93%), followed by a combination of ceftriaxone and metronidazole (22.22%) [Table 7]. Depending on the course of the patient's hospital stay, initial antibiotics given could have been shifted to much broader coverage, thus some may have been on 1 or more antibiotics throughout their hospital stay. Average duration of antibiotic use was 4 to 7 days.

Most (88.89%) of the patients were discharged improved after treatment. There were two mortalities who were both classified as severe AC. The causes of death included multiorgan dysfunction and septic shock, respectively. One patient went home per request.

DISCUSSION

In 2007, the Tokyo guidelines defined acute cholangitis as an unusual type of biliary infection among children. In fact, acute cholangitis in the pediatric population has only about 0.13-0.22% incidence compared with the adults. Standards for diagnosis and treatment were only draft guidelines. [3] In this study, the clinical profile and treatment outcome of acute cholangitis in children were presented.

The clinical profile was disaggregated by age groups < 5 years and > 5 years based on the causes of AC depending on the age predilection. Cholangitis mostly occurs in children with specific diseases such as biliary atresia, pancreaticobiliary malfunction, or previous biliary surgeries like post Kasai procedure. [3] In this study, cholangitis from obstructive biliary conditions were higher in children >5 years old (66.6%) compared to cholangitis from biliary atresia and post-operative biliary procedures in children <5 years old (33.3%). This was consistent with the review of 693 cases conducted by Friesen and Roberts in which pediatric cholangitis are distributed to ages as follows: 9.8% (1 year old or younger), 4.5% (1–5 years old), 14.5% (6–10 years old), and 71.5% (age, 11–20 years). [4]

Clinical diagnosis of acute cholangitis is classically based on the Charcot triad of fever, jaundice, and abdominal pain. However, its excellent specificity is counteracted by its poor sensitivity. [5] In a summary of literature by Kiriyama, et al, fever and abdominal pain are the most frequently observed clinical manifestations of cholangitis involving adults. [6] In our study, fever was reliably the most common symptom while jaundice was observed more frequently than abdominal pain due to the usual causes of AC amongst the subjects. Pale or acholic stools, lethargy and irritability were also habitually noted for similar reasons.

The incidence of Charcot's triad is reported in not more than 72% of adult patients with acute cholangitis. On the other hand, more severe form such as Reynolds' pentad is extremely rare, about only 3.5%–7.7% of this population. [6] Our study likewise showed the same results having 44% children presenting with the triad of fever, abdominal pain, and jaundice while an average of 1 to 3 patients had multiple organ dysfunction. By severity of AC upon admission, mild and moderate AC predominate our study population compared to severe or recurrent forms of AC typically from post-Kasai procedures.

Laboratory findings supporting the diagnosis of AC typically indicate inflammation and evidence of biliary stasis.[6] Several published studies reported that in acute cholangitis despite its cause, evident leukocytosis, elevated levels of biomarkers such as C-reactive protein and serum procalcitonin, hyperbilirubinemia and elevation of biliary and liver enzymes were noted.[7-11] In this study, most of the children with AC regardless of its severity had increased white blood cell count, C-reactive protein, serum procalcitonin, serum alanine transferase and with direct hyperbilirubinemia.

The two key microbiological tests for acute cholangitis are hemoculture and bile culture. [6] Blood cultures usually show a low positive rate. [2] Wang et al. reported only 17% yield among 150 hemocultures of adult patients with biliary tract infections. [12] Specifically for episodes of AC due to biliary stent

obstruction in adults, a recent retrospective multicenter series showed 40% positive hemocultures. [13] On the other hand, Zhang et al. in 2018 illustrated only 11% positive blood cultures among 27 pediatric patients with definite AC of which, *Escherichia coli* and *Klebsiella pneumoniae* were the primary isolates. These data mentioned are consistent with the present study's low yield of 12.5% positive hemocultures. However, culture-negative result predominates our patient population at 88.89%. Varied gram-negative bacteria were isolated such as *Acinetobacter baumannii, Serratia marcescens,* and *Salmonella B sp.*

Studies have shown that cultures of bile collected through the duodenum have a high positive rate. This diagnostic is superior to blood cultures and can provide valuable evidence for clinical diagnosis of AC. [2] Yu et al. analyzed 128 patients with recurrent cholangitis after operation for biliary atresia and results showed that the positive identification rate was only 34.3% for blood culture but was as high as 100% for bile culture. [14] Among the four pediatric patients with AC in our study that underwent bile culture, two children showed no isolates while the other two subjects yielded *Klebsiella pneumoniae.* This finding is consistent with the 2018 Tokyo guidelines identifying *Escherichia coli* and Klebsiella spp. as the two main bacteria in bile which are responsible for most cases of acute cholangitis.[15] Although the isolates of *Klebsiella pneumoniae* from bile culture collected in this study were resistant to the empiric antibiotics started, the outcome of these patients remained to be favorable especially after targeted therapy was initiated. Of note, bile collection is an invasive procedure for children which could be the reason of its limitation of use in this study.

Highlighting biliary tract dilatation or an obstruction in the biliary tract is a key diagnostic element in AC. Several imaging modalities may be requested: abdominal ultrasound, abdominal computerized tomography (CT), abdominal magnetic resonance imaging (MRI) with or without endoscopic retrograde cholangiopancreatography (ERCP). Ultrasound is the primary imaging modality for assessment of patients with suspected acute cholangitis. It is often the first-line diagnostic test, as it facilitates search for biliary tract dilatation. [16] This study clearly showed that abdominal ultrasound was the common initial diagnostic imaging requested: 25 patients out of the 27 pediatric subjects. Majority of the findings showed dilatation of the bile duct followed by presence of bile duct stones. But while the results are relatively simple to visualize in abdominal ultrasound, findings may be inadequate in the event of acute obstruction. According to a meta-analysis conducted in 2015, abdominal ultrasound has low sensitivity (73%) for the detection of common bile duct stones. And as regards obstacles other than choledocholithiasis, its performances have been even less impressive. [17]

Abdominal and pelvic CT with and without contrast injection presents several advantages. It is more sensitive and specific than ultrasound in AC diagnosis regardless of the cause. Moreover, it facilitates search for complications and excludes alternative etiologies of abdominal pain. [18] Out of the 25 patients in our study, 9 subjects underwent abdominal CT scan and demonstrated bile duct dilatation. No dynamic CT imaging was done among these patients, as was emphasized as a need in the revised Tokyo guidelines. That is, in patients with acute cholangitis, a temporary uneven deep staining is frequently observed in the arterial phase of dynamic CT for the liver; furthermore, this deep staining disappears with improvement of the cholangitis. [6]

Magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sonography (EUS) are the most sensitive techniques to correctly determine the underlying cause and level of biliary obstruction in patients with acute cholangitis. [19] In this study, patients who underwent MRCP, ERCP and EUS showed strictures, presence of calculi and stenosis as well as findings of pseudocyst were noted respectively. By discovering these

abnormalities in the biliary tract, AC may be diagnosed by imaging.

The primary goal of antimicrobial therapy in acute cholangitis is to limit both the systemic septic response and local inflammation and to prevent complications like intrahepatic abscess formation. While drainage of the obstructed biliary trees has been recognized as the mainstay of management for patients with AC, antimicrobial therapy may allow patients to have elective source control procedures rather than emergency operation. [20] Antibiotic choice for coverage should be according to local ecology and resistance, subjective conditions of the patient (renal and hepatic functions, allergies) as well as the severity of the specific case of AC. [16] Based on the Tokyo guidelines 2018, agents appropriate for use can be grounded on antimicrobial class definitions. As regards community-acquired forms of AC without severity grade, the schema is based on 3rd-generation cephalosporin (ceftriaxone, cefotaxime) associated with an anaerobic agent (metronidazole); penicillin-based therapy with ampicillin-sulbactam if with <20% local resistance rate; carbapenem-based therapy with ertapenem or fluoroquinolone-based therapy such as ciprofloxacin and levofloxacin with an anaerobic agent (metronidazole) in cases involving biliary-enteric anastomosis. [20] In our study with majority of mild to moderate case severity of AC, monotherapy ampicillin-sulbactam predominated as the most started antibiotic. This was followed by ceftriaxone/cefoxitin with metronidazole as frequently used antimicrobials. Interestingly, several patients were shifted from initial ampicillin-sulbactam to broader carbapenems or penicillin-based therapy. These cases could be attributed to non-improving status of the subjects or a probable healthcare-associated AC.

In initially severe, healthcare-associated forms of AC, preferred antimicrobial treatments include broad-spectrum cephalosporin (cefepime) with an anaerobic agent; an association piperacillin + tazobactam; carbapenem-based therapy using meropenem or ertapenem; monobactam-based therapy with aztreonam with metronidazole. [20] Our study featured patients started with piperacillin + tazobactam or meropenem antibiotic regimens which could be attributable to severe or recurrent forms of AC. A study by Wong et al. in 2004 reported that patients with post- Kasai cholangitis prompted the use of wider coverage, higher generation antibiotics such as meropenem due to the challenge of antibiotic resistance. [21]

The duration of antimicrobial therapy is controversial. The 2018 Tokyo Guidelines suggested 4 to 7 days after identification of the source of infection. [20] On the other hand, the French Infectious Disease Society (SPILF) has proposed a reduction of antimicrobial therapy duration to 3 days. [22] In 2011, Kogure, et al conducted a study testing the cessation of antimicrobial therapy for AC once body temperature has been lower than 37°C for 24h after bile duct drainage. It concluded that the median duration of therapy was 3 days without relapse over the following 4 weeks. [23] Duration of 5 days antimicrobial therapy following drainage appears sufficient according to Sokal, et al in 2019. [16] For our study, average of 4-7 days antibiotic duration was recorded with no note of relapse. There were two recurrent cases of AC of which only one was given prophylactic cotrimoxazole. A systematic review done by Decharun, et al in 2016 showed contradictory recommendations for the use of prophylactic antibiotics to reduce incidence of AC in patients of post-Kasai procedure. [24]

The prognosis for AC depends on the timing of biliary drainage, administration of antibiotics, comorbidities of the patient and severity of the case. The overall mortality rate of AC is less than 10% after biliary drainage. In the pre-ERCP era, severe acute cholangitis was associated with a mortality of more than 50%. [25] Based on a study in 2013, poor prognostic factors include old age, high fever, leukocytosis, hyperbilirubinemia and hypoalbuminemia. Patients with comorbidities like malignancy, liver abscess and coagulopathy also carry greater risk. [26] For our study, 88.89% of the patients had good outcomes. Reasons may be largely because

most of the subjects are young, no comorbidities and only had mild to moderate cases of AC. Antibiotic coverage and duration of therapy were adequately given. And although invasive, ERCP was performed in very few cases for biliary drainage.

Procalcitonin has been proposed by some authors as a predictive indicator of severity and therefore of urgent biliary decompression. A value greater than 0.5 has an 18% mortality rate. [27] However, in our series, procalcitonin determination did not stand out as a strong prognostic factor and needs to be validated by more studies.

Out of the 6 severe forms of AC, two subjects succumbed to death due to multiorgan dysfunction and septic shock which could be attributable to absence of an emergent biliary drainage. The causes of death for these patients are in congruence with the report of Tokyo guidelines identifying multiple organ failure with irreversible shock as the major basis of mortality.[5]

This study had certain limitations as this involved data collection through chart review. It has a small sample size done in a single-center retrospective design. Sources of bias may be present due to varying ways of documentation. A number of patients were excluded in the data analysis owing to incomplete or missing charts. Suspected cases of AC were not included in the study as well.

CONCLUSION

Acute cholangitis in children remains to be unusual with only few studies describing the disease and its outcome. It is different from adult investigations due to the varied causes of pediatric biliary tract infections and anomalies. In this study, AC cases in children are mostly mild to moderate in severity, favorably responded to empiric antibiotics but possibly of better conclusion especially if adequately managed with prompt biliary drainage. However, generally, the subjects had good treatment outcome.

RECOMMENDATION

Existing studies about AC in children remain to be inadequate as of this writing. It is important to conduct further studies with multidisciplinary approach. Collaboration between gastroenterologists, infectious disease specialists, and surgeons is essential. Subsequent prospective clinical study with a larger population may be advisable to yield additional information and to eliminate the confounders encountered in this study.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Alizadeh MAH. Cholangitis: Diagnosis, Treatment and Prognosis. J Clin Transl Hepatol. 2017;5(4):404-413.
2. Zhang T, Zhou T, Deng Z, et al. Clinical Characteristics and Effectiveness of Anti-infective Therapy of Acute Cholangitis in Children. Int J Clin Exp Med. 2018;11(4):4114-4119.
3. Yasuda H, Takada T, Kawarada Y, et al. Unusual cases of acute cholecystitis and cholangitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007;14(1):98-113.
4. Friesen C, Roberts C. Cholelithiasis: clinical characteristics in children. *Clin Pediatr.* 1989; 28:194–298.
5. Kimura Y, Takada T, Kawarada Y, Nimura Y, Hirata K, Sekimoto M, Yoshida M, Mayumi T, Wada K, Miura F, Yasuda H, Yamashita Y, Nagino M, Hirota M, Tanaka A, Tsuyuguchi T, Strasberg SM, Gadacz TR. Definitions, pathophysiology, and epidemiology of acute cholangitis and cholecystitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007;14(1):15-26.
6. Kiriyama S, Kozaka K, Takada T, Strasberg SM, Pitt HA, Gabata T, et al. Tokyo Guidelines 2018: diagnostic criteria and severity grading of acute cholangitis (with videos). J Hepatobiliary Pancreat Sci. 2018;25(1):17–30.
7. Gigot JF, Leese T, Dereme T, Coutinho J, Castaing D, Bismuth H. Acute cholangitis: Multivariate analysis of risk factors. Ann Surg. 1989; 209:435-438
8. Boey JH, Way LW. Acute cholangitis. Ann Surg. 1980; 191:264-270
9. Saharia PC, Cameron JL, Clinical management of acute cholangitis. Surg Gynecol Obstet. 1976; 142:369-372
10. Ker CG, Chen JS, Lee KT, Sheen PC, Wu CC. Assessment of serum and bile levels of CA19-9 and CA125 in cholangitis and bile duct carcinoma. J Gastroenterol Hepatol. 1991; 6:505-508
11. Silangcruz K, Nishimura Y, Czech T, Kimura N, Yess J. Procalcitonin to Predict Severity of Acute Cholangitis and Need for Urgent Biliary Decompression: Systematic Scoping Review. *Journal of Clinical Medicine*. 2022; 11(5):1155
12. Wang PX, Zhou XY, Chen J, Lou JG. Analysis of 150 cases of biliary tract infection in children. Zhejiang Prev Med. 2007;

16:60
13. Gomi H, Takada T, Hwang T-L et al. Updated comprehensive epidemiology, microbiology, and outcomes among patients with acute cholangitis. J Hepato-Biliary-Pancreat Sci 2017; 24:310–318
14. Yu MN, Liu G, Huang LM, Wang W, Zhang HL, Qin SL, Duan L, Zhang J, Yu F. Recurrent cholangitis after hepatico-portoenterostomy for biliary atresia: analysis of bacterial spectrum and sensitive antibiotics. J Clin Pediatr Surg. 2012; 11:187-189
15. Gomi H, Solomkin JS, Schlossberg D et al. Tokyo Guidelines 2018: antimicrobial therapy for acute cholangitis and cholecystitis. J Hepato-Biliary-Pancreat Sci 2018; 25:3–16
16. Sokal A, Sauvanet A, Fantin B, et al. Acute cholangitis: Diagnosis and management. Journal of Visceral Surgery. 2019; 156:515 - 525
17. Gurusamy KS, Giljaca V, Takwoingi Y et al. Ultrasound versus liver function tests for diagnosis of common bile duct stones. Cochrane Database Syst Rev 2015:CD011548
18. Kim SW, Shin HC, Kim HC. Diagnostic performance of multidetector CT for acute cholangitis: evaluation of a CT scoring method. Br J Radiol 2012; 85:770–777
19. Gallix BP, Aufort S, Pierredon MA, Garibaldi F, Bruel JM. Acute cholangitis: imaging diagnosis and management. J Radiol. 2006; 87:430-440
20. Gomi H, Solomkin JS, Schlossberg D et al. Tokyo Guidelines 2018: antimicrobial therapy for acute cholangitis and cholecystitis. J Hepato-Biliary-Pancreat Sci 2018; 25:3–16
21. Wong KK, Fan AH, Lan LC, Lin SC and Tam PK. Effective antibiotic regime for postoperative acute cholangitis in biliary atresia-an evolving scene. J Pediatr Surg 2004; 39: 1800-1802
22. Wintenberger C, Guery B, Bonnet E, Castan B, Cohen R, Diamantis S, et al. Proposal for shorter antibiotic therapies. Médecine Mal Infect 2017; 47:92–141
23. Kogure H, Tsujino T, Yamamoto K et al. Fever-based antibiotic therapy for acute cholangitis following successful endoscopic biliary drainage. J Gastroenterol 2011; 46:1411–1417
24. Decharun K, Leys CM, West KW, Finnell SM. Prophylactic Antibiotics for Prevention of Cholangitis in Patients with Biliary Atresia Status Post-Kasai Portoenterostomy: A Systematic Review. Clinical Pediatrics 2016; 55(1):66-72
25. Zhang WZ, Chen YS, Wang JW, Chen XR. Early diagnosis and treatment of severe acute cholangitis. World J Gastroenterol 2002; 8:150–152
26. Buyukasik K, Toros AB, Bektas H, Ari A, Deniz MM. Diagnostic and therapeutic value of ERCP in acute cholangitis. ISRN Gastroenterol 2013; 2013:191729
27. Suwa Y, Matsuyama R, Goto K, Kadokura T, Sato M, Mori R, Kumamoto T, Taguri M, Miyasho T, Endo I. IL-7 and procalcitonin are useful biomarkers in the comprehensive evaluation of the severity of acute cholangitis. J Hepatobiliary Pancreat Sci. 2017; 24(2):81-88