IMPROVING EFFICACY AND SAFETY OF DRUGS IN PEDIATRIC POPULATION: NEW CHALLENGES

EDITED BY: Domenica Altavilla, Annalisa Capuano, Eloisa Gitto, Serafina Perrone and Francesco Salvo

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Topic Editors:
Domenica Altavilla, University of Messina, Italy
Annalisa Capuano, University of Campania Luigi Vanvitelli, Italy
Eloisa Gitto, University of Messina, Italy
Serafina Perrone, University of Parma, Italy
Francesco Salvo, Université de Bordeaux, France

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A Comparison of Intranasal Dexmedetomidine, Esketamine or a Dexmedetomidine-Esketamine Combination for Induction of Anaesthesia in Children: A Randomized Controlled Double-Blind Trial

Xinlei Lu[1,2], *Ling Tang*[1], *Haiyan Lan*[1], *Chunli Li*[1] *and Han Lin*[1]*

[1]*Department of Anesthesiology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China,* [2]*Department of Anesthesia, Hangzhou Hospital Affiliated with Zhejiang University School of Medicine, Hangzhou First People's Hospital, Hangzhou, China*

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Edited by:
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University of Trieste, Italy

***Correspondence:**
Han Lin
nanlinhannansh@qq.com

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Objective: To compare the efficacy of dexmedetomidine, esketamine or combined intranasal administration on the induction of inhalation anaesthesia in children.

Methods: Ninety children aged 1–6 years were randomly allocated into three equal groups to be premedicated with either intranasal dexmedetomidine 2 μg/kg (Group D), esketamine 1 mg/kg (Group S), or dexmedetomidine 1 μg/kg combined with esketamine 0.5 mg/kg (Group DS). The primary endpoint was the Induction Compliance Checklist (ICC) Scale. Secondary outcomes included the sedation success rate; the modified Yale Preoperative Anxiety Scale score; the time of reaching up to two points on the University of Michigan Sedation Scale (UMSS); Parental Separation Anxiety Scale; anaesthesiologist satisfaction with induction based on the visual analogue scale; emergence agitation scale score; and adverse effects.

Results: The children in the DS group showed a high degree of cooperation with inhalation anaesthesia induction, and their ICC score was significantly lower than that of the D and S groups ($p = 0.001$), but there was no difference between the D and S groups. The success rate of sedation was higher in Group DS (90%) than in Group D (70%) and Group S (53.3%) ($p = 0.007$). Anaesthesiologist satisfaction with induction was significantly higher in Group DS than in Groups D and S ($p = 0.001$). The incidence of emergence agitation and the Paediatric Anaesthesia Emergence Delirium (PAED) score in the DS group were lower than those in the D and S groups.

Conclusions: Preoperative intranasal administration of dexmedetomidine combined with esketamine can significantly improve the cooperation of children with inhalation anaesthesia masks. It is a sedation method that has a high success rate and reduces the incidence and degree of emergence agitation.

Keywords: dexmedetomidine, esketamine, intranasal administration, children, preoperative sedation

INTRODUCTION

Most children undergoing elective surgery are afraid of the hospital environment and the operating waiting area. It is estimated that approximately 60–70% of children show significant anxiety before surgery (Kain et al., 1996). High levels of preoperative anxiety may lead to poor inhalational anaesthesia induction in children and even induction difficulties. The use of violence during inhalation anaesthesia induction is not a suitable option. It may lead to increased demand for postoperative analgesia and emergence agitation or even cause adverse behavioural changes after surgery (Mason, 2017). Therefore, anaesthesiologists should adopt appropriate strategies to reduce the potential psychological trauma to children induced by inhalation anaesthesia. Preoperative sedation is one of the most commonly used methods to prevent and treat preoperative anxiety in children and improve cooperation with inhalation anaesthesia (Kain et al., 2004; Rosenbaum et al., 2009).

Dexmedetomidine is a specific and selective α-2-adrenergic receptor agonist (α 2/α 1 = 1,620). It acts on the locus coeruleus and produces sedation similar to physiological sleep. Dexmedetomidine has many characteristics, such as analgesia, sedation (Nelson et al., 2003), anti-sympathetic (Ueki et al., 2014), and anti-inflammation (Venn et al., 2001) effects and no respiratory inhibition. Nasal administration of dexmedetomidine is a common method of preoperative sedation (Baier et al., 2016); however, the drug may lead to bradycardia or hypotension in children (Lei et al., 2020).

Ketamine is a classic anaesthetic. Its main effect is noncompetitive antagonism to N-methyl-D-aspartate (NMDA) receptors. It has sedative and analgesic properties when used at subanesthetic doses. However, it also has some adverse side effects, including nausea, high blood pressure and tachycardia (Tsze et al., 2012; Scheier et al., 2017). Sedation can be given in a variety of ways, including nasal administration. Intranasal infusion of dexmedetomidine combined with ketamine is used for sedation in children (Yang et al., 2019), which has the advantages of stable haemodynamics. Compared to that with dexmedetomidine alone, the onset time is shorter, and it is not easy for patients to wake up. In addition, there is less postoperative agitation and reduced airway secretions compared to those with ketamine alone (Sun et al., 2020).

Esketamine (S (+)-ketamine) is the dextral enantiomer of ketamine. Esketamine is approximately twice as potent as racemic ketamine (Arendt-Nielsen et al., 1996). At the same time, compared with racemic ketamine, esketamine has the properties of a shorter recovery period, less postoperative pain, faster recovery of cognitive function (Himmelseher and Pfenninger, 1998), and lower occurrence of psychiatric side effects (Pfenninger et al., 2002).

Esketamine can counteract the bradycardic and hypotensive effects of dexmedetomidine. Therefore, the combined use of dexmedetomidine and esketamine may be beneficial to patients. However, the preoperative sedation of children with intranasal infusion of dexmedetomidine combined with esketamine or with esketamine alone has not been studied.

This study aimed to compare the effects of dexmedetomidine, esketamine or combined preoperative intranasal infusion on the induction of inhalation anaesthesia in children through a randomized controlled double-blind study to provide a theoretical basis for improving the comfort of anaesthesia in children.

MATERIALS AND METHODS

Research object

This study was a randomized controlled double-blind trial. We obtained approval from the Medical Ethics Committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Written informed consent was obtained from all parents/legal guardians. This trial was registered with the Chinese Clinical Trial Registry (registration number ChiCTR2000039445).

The selection criteria were as follows: 1) ASA I-II; 2) scheduled elective lower abdominal or perineal surgery with a surgery time of less than 2 h; and 3) age from 1 to 6 years. 4) The guardian of each child signed the informed consent form.

1.2 The exclusion criteria were as follows: 1) congenital heart disease (tetralogy of Fallot, patent ductus arteriosus, ventricular septal defect, etc.); 2) lung diseases (pulmonary infection, bronchial asthma, etc.); 3) known rhinitis or nasal deformities; 4) allergy to the drugs used in this subject; and 5) known difficult airway.

1.3 The elimination criteria were as follows: 1) laryngospasm during anaesthesia induction; 2) laryngeal spasm after removal of the laryngeal mask; and 3) use of opioids during surgery.

Research design

The sample size was assessed using NCSS-PASS software version 11.0. According to previous literature (Johansson et al., 2013), the success rate of nasal sedation with 2 μg/kg dexmedetomidine was 69.4%. The noninferiority cut-off value was 15%, $\alpha = 0.05$, $\beta = 0.2$, and the proportion of patients lost to follow-up was 0.05. The final sample size of this study was 90 patients.

Children who met the inclusion criteria were randomly assigned to three groups, D (n = 30), S (n = 30), and DS (n = 30), using the random number table generated by SPSS 26.0 (SPSS Inc. Chicago, IL, United States). Children were accompanied by their parents into the anaesthesia induction room 40 min before surgery. The child was fasted for more than 8 h, forbidden to have infant formula for more than 6 h, forbidden to have breast milk for more than 4 h, and forbidden to have clear liquids for more than 2 h.

The anaesthesia nurse used an LMA mucosal nebulizer (MAD140, Wolfe-Tory Medical, Inc. Utah, United States) to prepare nasal drops according to the random grouping. The liquid medicine was prepared by the anaesthesia nurse using the LMA® MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex; Item number: MAD140) according to the randomization. Black opaque tape was used to cover and block the volume of the drug solution and then hand it over

to the same anaesthesiologist. Group D received intranasal dexmedetomidine at a dose of 2 μg/kg, group S received 1 mg/kg esketamine, and Group DS received 1 μg/kg dexmedetomidine +0.5 mg/kg esketamine.

Before nasal administration, the anaesthesiologist cleaned the child's nasal passages with a disposable cotton swab.

Before medication, anaesthesia nurses used the modified Yale Preoperative Anxiety Scale (*m*-YPAS) to evaluate the degree of anxiety in children. The anaesthesiologist uses a mucosal atomization device (MAD) to spray the medicine over a wide range of surface areas of the nasal cavity and then gently massaged the alar nasi to facilitate fluid absorption.

The patient's parents, the attending anaesthesiologist, the surgeons, and data collection personnel were blinded to the group assignment.

After medication, the anaesthesiologist used the University of Michigan Sedation Scale (UMSS) to observe and record the time when the child reached a UMSS score of two points. At the same time, the respiratory conditions of the children, such as respiratory rhythm, mucocutaneous colour, and lip colour, were closely observed, and adverse reactions of the children after medication were recorded. If there is an adverse reaction after the administration, the adverse reaction shall be recorded in detail and the case shall be excluded.

When the child's UMSS score reached two points, another anaesthesiologist was taken to the operating room, and the Parental Separation Anxiety Scale (PSAS) was recorded. If the UMSS score of the child did not reach 2 points for more than 40 min, it was considered a failure of sedation, and the satisfactory sedation time was recorded as 40 min.

Upon arrival in the operation room, all patients were monitored with peripheral pulse oximetry. To avoid the influence of monitoring stimulation on evaluation by the anaesthesia Induction Compliance Checklist (ICC) in children, electrocardiography and noninvasive blood pressure monitoring were temporarily withheld.

General anaesthesia was induced with 8% sevoflurane in 100% oxygen at 8 L/min. The anaesthesiologist used a suitable mask (reaching down to the chin and up to the bridge of the nose), gently covered the mouth and nose of the child, and used a pressure measuring valve to control the pressure of the mask to approximately 30 cm H_2O.

After the child lost consciousness, electrocardiography and noninvasive blood pressure monitoring were performed. At the same time, the sevoflurane concentration decreased from 8 to 4%, the oxygen flow decreased from 8 L/min to 4 L/min, and the concentration remained unchanged. After achieving an adequate depth of anaesthesia, an intravenous line was placed by the operating room nurse, and then the corresponding size laryngeal mask was placed by the anaesthesiologist. The anaesthesiologist evaluated and recorded the child's ICC score and anaesthesiologist induction satisfaction based on the visual analogue scale (VAS).

After the laryngeal mask was inserted, the corresponding regional anaesthesia was compounded according to our department's standard. If the child underwent inguinal surgery, an iliohypogastric nerve block with 0.15% ropivacaine +0.8% lidocaine at a dosage of 0.5 ml/kg was used. If the child underwent perineal surgery, caudal anaesthesia with 0.15% ropivacaine +0.8% lidocaine at a dosage of 0.75 ml/kgwas used.

Anaesthesia was maintained using sevoflurane in a 50% oxygen/air mixture at 2 L/min. During the operation, the child maintained spontaneous breathing. The sevoflurane inhalation concentration was adjusted according to the end-tidal carbon dioxide concentration (PetCO2). PetCO2 was maintained at 35–50 mmHg, and the end-tidal concentration of sevoflurane was maintained above 0.7.

At the end of surgery, the laryngeal mask airway was immediately removed, and an inlet pharyngeal airway was placed. Then, the child was sent to the Post Anaesthesia Care Unit (PACU) for observation. The emergence time (stopping sevoflurane until the first eye opening) was observed and recorded by PACU nurses, and the Paediatric Anaesthesia Emergence Delirium (PAED) score was recorded.

TABLE 1 | Comparison of the intranasal sedation success rate between the groups.

	Success rates
Group D (n = 30)	21 (70%)
Group S (n = 29)	16 (55.2%)
Group DS (n = 29)	27 (93.1%)#
χ^2	10.689
p Value	0.005

Data are presented as the number (percentage). group D, dexmedetomidine group; group S, esketamine group; Group DS, dexmedetomidine-esketamine group.
**p < 0.05, the dexmedetomidine group versus the esketamine group, **p < 0.05, the dexmedetomidine group versus the dexmedetomidine-esketamine group, #p < 0.05, the esketamine group versus the dexmedetomidine-esketamine group.*

Statistical analyses

All of the individual participant data were analysed using SPSS version 26.0 (IBM SPSS Inc. Armonk, United States). Descriptive statistics were obtained on all the study variables. Data are reported as the means (standard deviations), medians (interquartile ranges, IQRs), or frequencies (percentages) where appropriate. The Kolmogorov–Smirnov test was applied to determine whether continuous variables were normally distributed. Single factor variance analysis was adopted for the comparison of normally distributed quantitative data. Nonnormally distributed data were analysed using the Kruskal-Wallis test. Categorical variables were compared using the $\chi 2$ test or Fisher's exact test, where appropriate. The *p* value was set at 0.05 for statistical significance.

RESULTS

A total of 105 patients were assessed for eligibility. Finally, 90 patients were enrolled in this randomized study and were randomized into three groups of 30 children each. In Group D, 21 cases were successfully sedated, and 9 cases failed to be sedated. The success rate of sedation was 70%. In Group S, sedation was successful in 16 cases, and sedation failed in 14

TABLE 2 | Demographic data and preoperative anxiety score.

	Group D (n = 30)	Group S (n = 29)	Group DS (n = 29)	*p* value
Male/female (%)	80.0/20.0	96.6/3.4	93.1/6.9	0.085
Age (months)	41.80 (±18.95)	33.86 (±17.00)	40.17 (±19.14)	0.224
Weight (kg)	15.33 (±3.98)	15.14 (±4.55)	16.83 (±4.47)	0.309
m-YPAS	52.50 (45.35–67.00)	58.20 (48.30–73.20)	50.00 (46.60–69.10)	0.456

Data are presented as numbers (percentages), medians (IQRs) or means ± standard deviations; F, female; D, dexmedetomidine; DS, dexmedetomidine-esketamine; M, male; m-YPAS, the modified yale preoperative anxiety scale; S, esketamine.

TABLE 3 | Duration of anaesthesia and surgery.

	Duration of anaesthesia (min)	Duration of surgery (min)
Group D (n = 30)	35.50 (26.25–48.50)	25.50 (14.75–32.00)
Group S (n = 29)	37.00 (27.00–52.00)	25.00 (16.50–42.50)
Group DS (n = 29)	34.00 (30.00–56.00)	23.00 (16.00–38.50)
p Value	0.847	0.604

Data are presented as the median (IQR); D, dexmedetomidine; DS, dexmedetomidine-esketamine; S, esketamine.

TABLE 4 | Time to satisfactory sedation.

	Time to satisfactory sedation
Group D (n = 30)	23.50 (17.75–40.00)
Group S (n = 29)	28.00 (21.00–40.00)
Group DS (n = 29)	20.00 (15.00–24.00)#
p Value	0.001

Data are presented as the median (IQR). D, dexmedetomidine; DS, dexmedetomidine-esketamine; S, esketamine.
**p < 0.05, the dexmedetomidine group versus the esketamine group, **p < 0.05, the dexmedetomidine group versus the dexmedetomidine-esketamine group, #p < 0.05, the esketamine group versus the dexmedetomidine-esketamine group.*

TABLE 5 | Comparison of anaesthesia induction.

	PSAS scores	ICC scores	Anaesthesiologist satisfaction score
Group D (n = 30)	1.0 (1.0–2.0)	4.0 (2.75–6.0)	7.0 (6.0–8.0)
Group S (n = 29)	1.0 (1.0–2.5)	4.0 (2.0–6.0)	7.0 (6.0–9.0)
Group DS (n = 29)	1.0 (1.0–1.0)	2.0 (0.0–4.0)*#	9.0 (7.5–10.0)*#
p Value	0.155	0.001	0.001

Data are presented as the median (IQR); D, dexmedetomidine; DS, dexmedetomidine-esketamine; ICC, induction compliance checklist; PSAS, parental separation anxiety scale; S, esketamine.
**p < 0.05, the dexmedetomidine group versus the esketamine group, **p < 0.05, the dexmedetomidine group versus the dexmedetomidine-esketamine group, #p < 0.05, the esketamine group versus the dexmedetomidine-esketamine group.*

cases, of which 1 case had failed sedation and was excluded by the addition of opioids during the operation. The sedation success rate was 55.2%. In Group DS, 27 cases were successfully sedated, and 3 cases showed failed sedation. Among them, one child had failed sedation, and the operation time exceeded 2 h. The sedation success rate was 93.1%. After excluding two children, 30 cases in Group D, 29 cases in Group S, and 29 cases in Group DS were included in the data analysis. The success rate of sedation in Group DS was significantly higher than that in Group D and Group S($\chi 2 = 10.070, p = 0.006 < 0.05$). As shown in **Table 1**.

There were no significant differences between the groups in terms of patient sex, age, weight, preoperative anxiety score (*m*-YPAS), maintenance time of anaesthesia, or duration of surgery, as shown in **Tables 2**, **3**.

The time for the child to reach satisfactory sedation was recorded (UMSS score of two points). If the UMSS score of the child did not reach 2 points for more than 40 min, the satisfactory sedation time was recorded as 40 min. The time to satisfactory sedation in Group DS was significantly shorter than that in Group S ($p = 0.001 < 0.05$), but there was no significant difference between Group DS and Group D (**Table 4**).

There was no significant difference in the PSAS among the three groups. The ICC score of Group DS was significantly lower than that of Groups D and S ($p = 0.001 < 0.05$), while there was no difference between Groups D and S. There was a difference between the groups in the anaesthesiologist satisfaction score ($p = 0.001 < 0.05$); Group DS had a significantly higher score than Groups D and S, but there was no difference between Groups D and S, as shown in **Table 5**.

In terms of emergence time, the median emergence time from anaesthesia in Group S was 26.0 min, that in Group D was 35.0 min, and that in Group DS was 33.0 min. There was no significant difference between the three groups ($p = 0.163 > 0.05$). In terms of the quality of emergence, there was a difference in the incidence of emergence delirium between the groups. The incidence of emergence delirium in Group DS was significantly lower than that in Groups D and S ($p = 0.001 < 0.05$). There was a difference in the PAED score between groups ($p = 0.000 < 0.05$). The PAED score of Group DS was lower than that of Group D and Group S, as shown in **Table 6**.

Adverse events occurred in six children in Group S. Among them, three children had nausea, one child had increased secretions, one child had separation anxiety, and one child had abnormal irritability. The incidence of adverse events in Group S was significantly higher than that in Groups D and DS ($p = 0.01 < 0.05$), as shown in **Table 7**.

DISCUSSION

The results of this trial showed that children sedated with dexmedetomidine combined with esketamine before surgery had higher cooperation in anesthesia induction, higher success

TABLE 6 | Emergence time and emergence quality.

	Emergence time (min)	Occurrence of ED	PAED scores
Group D (n = 30)	35.00 (29.75–45.55)	40.0%	8.00 (4.75–12.00)
Group S (n = 29)	26.00 (19.50–41.50)	58.6%	12.00 (6.50–14.50)
Group DS (n = 29)	33.00 (24.00–46.50)	10.3%*#	5.00 (3.00–7.50)*#
p Value	0.163	0.001	0.000

Data are presented as numbers (percentages), medians (IQRs) or means ± standard deviations; D, dexmedetomidine; DS, dexmedetomidine-esketamine; ED, emergence delirium; PAED, paediatric anaesthesia emergence delirium; S, esketamine.

**p < 0.05, the dexmedetomidine group versus the esketamine group, **p < 0.05, the dexmedetomidine group versus the dexmedetomidine-esketamine group, #p < 0.05, the esketamine group versus the dexmedetomidine-esketamine group.*

TABLE 7 | Adverse events.

	Nausea	Increased secretions	Separation Phenomenon	Abnormal irritability	Total
Group D (n = 30)	0	0	0	0	0
Group S (n = 29)	3	1	1	1	6*
Group DS (n = 29)	0	0	0	0	0#
χ^2	—	—	—	—	13.100
p Value	—	—	—	—	0.01

Data are presented as numbers. D, dexmedetomidine; DS, dexmedetomidine-esketamine; S, esketamine.

**p < 0.05, the dexmedetomidine group versus the esketamine group, **p < 0.05, the dexmedetomidine group versus the dexmedetomidine-esketamine group, #p < 0.05, the esketamine group versus the dexmedetomidine-esketamine group.*

rate of sedation, higher satisfaction of anesthesiologists and lower incidence of agitation after anesthesia. Preoperative sedation with esketamine alone has a low success rate and a high incidence of side effects.

The results of this trial showed that children sedated with dexmedetomidine combined with esketamine before surgery showed greater cooperation with anaesthesia induction, a higher success rate of sedation, higher satisfaction of anaesthesiologists and a lower incidence of agitation after anaesthesia. Preoperative sedation with esketamine alone has a low success rate and a high incidence of side effects.

In our study, it was found that the Group DS [2.0 (0.0–4.0)] inhalation anaesthesia induction coordination score (ICC) was significantly lower than that of Group D [4.0 (2.75–6.0)] and Group S [4.0 (2.0–6.0)], $p = 0.001 < 0.05$. However, there was no significant difference between Group D and Group S. At the same time, compared with Group D and Group S, the satisfaction of anaesthesiologists in Group DS was higher.During the induction of inhalation anaesthesia, the anaesthesiologist puts the anaesthesia mask on the face of the child. Although we used a pressure valve to strictly control the pressure of the mask at approximately 30 cmH2O, the anaesthesia mask still caused compression or even pain in the child's cheek. This in turn caused the child to wake up from sedation, especially as dexmedetomidine alone produces mild sedation similar to physiological sleep (Baier et al., 2016). Therefore, when receiving anaesthesia mask inhalation induction, children will awaken from a sedative state and fear the induction of anaesthesia, resulting in a decrease in the degree of mask coordination. Esketamine is a noncompetitive antagonist of NMDA receptors that regulates nociceptive sensations. Previous studies have shown that esketamine has a good analgesic effect. Johansson Joakim et al. applied esketamine nasal administration to prehospital analgesia and found that the VAS score decreased from a median of 10 points (8–10) after nasal administration for 5 min to a median of 3 (2–4) points (Johansson et al., 2013). In our study, both dexmedetomidine combined with esketamine intranasal drops or esketamine alone yielded a certain degree of analgesia under the condition of satisfactory sedation, which may help to improve the cooperation of inhalation anaesthesia induction in children. As a result, Group DS using esketamine showed better inhalation anaesthesia induction coordination, lower ICC scores and higher anaesthesiologist satisfaction.

We recorded the time at which the child reached satisfactory sedation (UMSS score of two points). If the UMSS score of the child did not reach 2 points for more than 40 min, the satisfactory sedation time was recorded as 40 min. The time to satisfactory sedation in Group DS was significantly shorter than that in Group S ($p = 0.001 < 0.05$), but there was no significant difference between Group DS and Group D.Previous studies have shown that the plasma concentration of dexmedetomidine reaches the lowest effective sedative concentration after the nasal administration of 2 μg/kg dexmedetomidine for 10 min (Miller et al., 2018). However, the plasma concentration of children after the nasal administration of esketamine reached a peak within 18 ± 13 min (mean ± standard deviation) (Weber et al., 2004). Dexmedetomidine is a specific and selective α-2-adrenergic receptor agonist with a good sedative effect. In addition to being a noncompetitive antagonist of NMDA receptors, esketamine can also stimulate GABA receptors and mediate their anaesthetic effects. The combined application of the two drugs for preoperative sedation in children showed excellent sedative effects.A recent randomized controlled trial by Mang

Sun et al. in children with congenital heart disease requiring transthoracic echocardiography showed that intranasal dexmedetomidine combined with ketamine (DEX 2 μg/kg + KET 1 mg/kg) had a faster onset time but longer recovery time than intranasal dexmedetomidine alone (2 μg/kg) (Sun et al., 2020). In this study, the dose of esketamine used in Group S (ESKET 1 mg/kg) was small, and its anaesthetic effect was approximately equal to that of 2 mg/kg ketamine. At the same time, a previous study found that the nasal administration of 3 and 6 mg/kg ketamine cannot achieve sufficient sedation (Tsze et al., 2012).Finally, the success rate of sedation in Group S was low. However, if the UMSS score of the child did not reach 2 points for more than 40 min, we recorded a satisfactory sedation time of 40 min. This may be the reason that the time to satisfactory sedation in the DS group was significantly shorter than that in the S group ($p = 0.001 < 0.05$).Bin Qian et al. compared intranasal dexmedetomidine (2 μg/kg) and dexmedetomidine combined with ketamine (DEX 2 μg/kg + KET 2 mg/kg) for tonsillectomy in preschool children and found that the median onset time of sedation (median) (15 vs 24 min) was significantly faster in the combination group than in the dexmedetomidine-alone group (Qian et al., 2020). In our study, the time to satisfactory sedation in Group D (median) was 23.5 min, which was similar. The formula used in Group DS was DEX 1 μg/kg + ESKET 0.5 mg/kg, and the time to satisfactory sedation (median) was 20 min longer than that observed by Bin Qian et al. This may have been caused by the use of only 1 μg/kg dexmedetomidine in Group DS, which is smaller than the dose of dexmedetomidine used by Bin Qian et al. At the same time, this is also the reason why the time to satisfactory sedation in Group DS was shorter than that in Group D, though there was no significant difference. In our study, the time to satisfactory sedation for children in Group D was shorter than that in Group S, but there was no significant difference. This may have been caused by our small sample size; therefore, we need to further expand the sample size.

In this study, the success rate of sedation in Group S was only 55.2% lower than that in Group D (70%) and Group DS (93.1%). This may have been caused by the low bioavailability of the drug ketamine when administered intranasally (Andolfatto et al., 2013). The concentration of the esketamine preparation we used was 25 mg/ml, and thus, the volume of esketamine used per unit body weight was twice that of dexmedetomidine. A larger drug volume will reduce air exchange in the nasal cavity, causing discomfort or even suffocation in children. This will increase the difficulty of nasal administration or even the failure of the medication and cause swallowing with larger doses of drugs; however, the bioavailability of swallowed esketamine is very low (Grant et al., 1981). Similar to the findings in previous studies, multiple children indicated that the drug had a bitter aftertaste, which may also cause additional irritation, in turn resulting in failure of sedation (Weber et al., 2003; Fantacci et al., 2018). The success rate of sedation in the DS group was 93.1%. In the study of Yang Fei et al., 2 μg/kg dexmedetomidine combined with 1 mg/kg ketamine was used as a nasal sedation regimen, and the success rate was approximately 93% (Yang et al., 2019). The use of 1 μg/kg dexmedetomidine combined with 0.5 mg/kg esketamine in our DS group reduced the dosage of dexmedetomidine, which helped to reduce the incidence of delayed postoperative recovery and accelerated the transport rate in the day ward. This treatment was also useful when extended to outpatient imaging examinations and improved the economic efficiency of the hospital.

Esketamine is considered to be a racemic ketamine that can produce anaesthetic effects, while R (-)-ketamine is considered to produce adverse effects. Esketamine was developed to reduce the psychomimetic side effects of racemic ketamine, but a review showed that the improvement was not obvious (Engelhardt, 1997). Another side effect of ketamine and esketamine is excessive secretion, although the incidence of increased secretion of esketamine is lower than that of ketamine (Adams and Werner, 1997). In our study, a total of six patients (all in group S) experienced adverse effects. The incidence was 20.69%, which was similar to that in previous studies (Marhofer et al., 2001). Among the patients, three children developed nausea, one child had increased secretion, one child developed abnormal restlessness, and one child developed irritability.

In terms of the quality of emergence, we found differences between the incidence of postoperative agitation and PAED scores among the three groups. The incidence of postoperative agitation in Group DS was significantly lower than that in Groups D and S ($p = 0.001 < 0.05$), and the PAED score of Group DS was lower than that of Groups D and S ($p = 0.000 < 0.05$). The preoperative use of esketamine alone for nasal sedation yields a higher incidence of postoperative agitation and higher PAED scores. This may be due to the psychomimetic side effects of ketamine and esketamine. In addition, esketamine can cause sympathetic stimulation, resulting in an increased plasma concentration of norepinephrine (Kienbaum et al., 2001). Although ketamine isomers cause less cognitive impairment than racemic ketamine at equivalent analgesic doses, esketamine can still cause a certain degree of cognitive impairment [38]. This may be the reason why the incidence of postoperative agitation and PAED scores in Group DS were significantly lower than those in Group S. Dexmedetomidine can reduce sympathetic tension and inhibit catecholamine release, and previous studies have shown that it has a good preventive effect on postoperative agitation (Pfenninger et al., 2002). Dexmedetomidine combined with esketamine has a higher preoperative sedation success rate, and esketamine has a certain analgesic ability. Our study showed that Group DS had lower ICC scores, higher mask cooperation, and less fear induction during the induction of anaesthesia. This may be the reason why the incidence of postoperative agitation and PAED scores in Group DS were significantly lower than those in Group D.

LIMITATIONS

Our study has several limitations. First, we do not have a family-centred care plan. Thus, although the preoperative anxiety state of parents may affect the preoperative anxiety level of children, we have not evaluated this aspect, which may affect the sedative effect of preoperative medication. Second, we did not set up different doses of dexmedetomidine in the esketamine test group. Consequently, we

cannot evaluate the dose-response relationship of dexmedetomidine combined with esketamine as a preoperative medication. Thirdly, the induction time may exceed 40 min, and some children need longer onset time. Therefore, it should be considered that the guardian will bring the child into the anesthesia induction room 1 hour in advance. Finally, the main result in our study is the success rate of sedation, so we use the success rate as the basis for sample size calculation. But other variables are still bias factors, which should be fully considered in future studies.

Therefore, it is necessary to conduct further research to resolve these limitations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XL and HLi: conceptualization, design of the study and project administration as Principals Investigators. XL and HLi wrote the first draft of the manuscript. HLa and LT: revision and validation of the manuscript. LT and CL: data curation and statistical analysis. All authors gave final approval of the submitted version.

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frontiers in Pediatrics

CASE REPORT
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Case Report: Severe Hypercalcemia Following Vitamin D Intoxication in an Infant, the Underestimated Danger of Dietary Supplements

*Alexandre O. Gérard[1,2], Audrey Fresse[2], Myriam Gast[3], Diane Merino[2], Pierre Gourdan[4], Audrey Laurain[1], Irène Margaritis[5], Pierre-Alexis Gauci[6], Fanny Huret[5], Nadège Parassol[2] and Fanny Rocher[2]**

[1] Department of Nephrology-Dialysis-Transplantation, Centre Hospitalier Universitaire de Nice, Nice, France, [2] Department of Pharmacology and Pharmacovigilance Center of Nice, Centre Hospitalier Universitaire de Nice, Nice, France, [3] Department of Pediatry, Hospital Center of Bastia, Bastia, France, [4] Department of Pediatry, Lenval Hospital - Centre Hospitalier Universitaire de Nice, Nice, France, [5] Nutrition Risk Assessment Unit, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France, [6] Department of Gynecology, Centre Hospitalier Universitaire de Nice, Nice, France

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Annalisa Capuano, University of Campania Luigi Vanvitelli, Italy

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***Correspondence:**
Fanny Rocher
pharmacovigilance@chu-nice.fr

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Vitamin D supplementation is routinely introduced in infants, according to medical guidelines. However, vitamin D overdose can result in life-threatening hypercalcemia. We report the case of a 3-month-old infant who suffered from severe hypercalcemia. Upon detailed questioning of the parents, a vitamin D administration error has been identified. Indeed, the parents had followed the advice of their midwife. They substituted the prescribed medicinal vitamin D by a dietary supplement, different in concentration and dosing, without performing the dose conversion needed. In fact, many different medications and dietary supplements with vitamin D exist, offering various concentrations and units of measurement. This case highlights the pivotal role of therapeutic education. Broadly, there is a need for harmonization of the regulation and labeling of dietary supplements and medications containing vitamin D.

Keywords: vitamin D, overdose, intoxication, dietary supplement, hypercalcemia, pharmacovigilance, misuse, case report

INTRODUCTION

Vitamin D is a fat-soluble hormone either synthetized endogenously or from external vitamin intakes. Vitamin D bears pleiotropic functions (1, 2), primarily on phosphocalcic and bone homeostasis, crucial in infants (3). The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) (4) and the European Food Safety Authority (EFSA) have revised the nutritional guidance for vitamin D. The considered Adequate Intake (AI) for infants < 6 months is 10 μg or 400 IU per day. In this context, according to guidelines recommendations, vitamin D supplementation is routinely initiated in infants (5) under medical control. The Institute of Medicine (IOM) and the EFSA have also defined Upper tolerable Levels (UL) of vitamin D according to the age (6, 7), even if the threshold for acute toxicity is still unclear (3). Based on the risk of growth perturbation and hypercalcemia, the vitamin D UL for children < 6-months, is 25 μg or 1,000 IU per day (4, 7, 8). We report a case of vitamin D overdose in an infant, caused by the erroneous substitution of the prescribed supplementation by a dietary supplement.

CASE DESCRIPTION

In August 2020, a 3-month-old infant was referred to the pediatric emergency department for severe anorexia, vomiting, and weight loss. After an uncomplicated pregnancy (36 weeks and 5 days of amenorrhea), he was born at term, weighing 2.3 kg. Since, he had been exclusively breastfed and had not presented any medical problem.

At the time of admission, the infant weighted 4.5 kg. He had no fever, his heart rate was 109 beats per minute, his blood pressure was 107/85 mmHg, and his oxygen saturation was 99%. Blood pressure was later reassessed several times, and was found normal or at the upper limit of normal for the age. Clinical examination was unremarkable except a global hypotonia and the presence of moderate dark circles around the eyes. Natremia was 139 mmol/L, kalemia 4.4 mmol/L, alkaline reserve 21 mmol/L, hemoglobin 9.1 g/dL, leukocytes 11.11 G/L, platelets 471 G/L, and C-reactive protein (CRP) 13 mg/L with a negative procalcitonin. Blood gases analysis and liver function tests were within normal range. Natriuresis was < 20 mmol/L for a kaliuresis of 33 mmol/L, suggestive of hypovolemia. Hemoculture, stool bacterial culture and virology, as well as lumbar puncture results were all negative. Lumbar puncture had been performed because the infant presented with global hypotonia and severe behavioral changes, in the absence of an obvious diagnosis. Immunoglobulin E (IgE) antibodies to cow milk were negative. Cranial ultrasonography and brain Magnetic Resonance Imaging (MRI) were normal. Five days after admission, an abdominal ultrasound confirmed 3 days later revealed some deposits in the bladder, which were deemed clinically not significant.

Because of a suspicion of urinary infection, probabilistic intravenous antibiotic therapy was initiated, then discontinued 3 days later as no inflammatory syndrome nor bacteria in urinary analysis (including mycobacteria in a context of sterile leukocyturia) were found. Breastfeeding was supplemented with infant milk and intravenous hydration.

Calcemia was assessed for the first time 12 days after admission to hospital and was at 3.08 mmol/L (normal values: 2.15–2.55 mmol/L) with an albuminemia of 41 g/L (normal values: 34–42 mg/L). Parathormone (PTH) was below the limit of quantification (normal values: 18–88 ng/L), whereas calcidiol was above the upper limit of quantification (normal values: 30–400 ng/mL) and calcitriol was 200 pg/mL (normal value < 182 pg/mL). Phosphate was 1.8 mmol/L (normal values: 1.6–2.4 mmol/L). Serum creatinine was 23 μmol/L (normal values: 15–37 μmol/L) while urea was 2.3 mmol/L (normal values: 1.8–6.4 mmol/L). Urinary calcium was 2.75 mmol/L with a creatininuria of 1 mmol/L.

Electrocardiogram was normal and no arrhythmia was documented on the monitoring. The infant was kept hydrated and calcemia progressively decreased to reach 2.78 mmol/L in mid-September. Bisphosphonates were not deemed necessary. The child was finally discharged with a weight of 4.8 kg and with a close monitoring of his calcemia recommended. Throughout a follow-up consultation, 2 weeks later, calcemia was 2.6 mmol/L and the child weighed 5.2 kg. On last follow-up consultation 3 months after the onset of symptoms, he weighed 5.6 kg and was clinically asymptomatic. Renal ultrasound was suggestive of calcic deposits in the urinary tract.

Upon detailed questioning of the parents, a chronic overdose of vitamin D was identified. In order to provide the recommended daily dose for breastfed newborn, the maternity medical staff initially had prescribed 4–5 drops per day of ZymaD®, a brand name containing cholecalciferol dosed at 10,000 International Unit (IU)/mL, one drop containing 300 IU of vitamin D (9). Once at home, a caring midwife suggested to the parents to replace the initially prescribed vitamin D supplementation by a so-called "natural" vitamin D-based dietary supplement (DS). She indeed considered that "classical" drugs including vitamin D may contain endocrine disrupters, as well as preservatives, and believed that "natural" vitamin D, marketed as DS should be healthier. She was also convinced that DS would be associated with less abdominal pain in newborn. The midwife suggested several DS, available on the Internet, and let the parents pick one of them. She recommended the parents to maintain "the same dose" as the one initially prescribed (for ZymaD®).

Thereupon, the parents bought on the Internet the DS Sunday Natural® brand of D3 10,000 IU (+ vitamin K2). This product is deemed to contain 10,000 IU *per* drop (and not *per* mL), and the manufacturer's recommendation consists of one drop every 10 days. However, as initially prescribed with ZymaD®, 4–5 drops per day were administered. This switch between two non-equivalent drugs, with different dosing, consequently exposed the infant to 40,000–50,000 IU per day, which represents 50-fold the Upper tolerable Level (UL) recommended. Such exposition conducted to a symptomatic severe vitamin D overdose, with a 15-day hospital stay and potential sequelae such as nephrocalcinosis and lithiasis.

DISCUSSION

This case highlights the pivotal role of health practitioners in limiting the risk of drug and dietary supplement misuse, even for so-called innocuous "vitamins." Because of different regulatory specific domains, blurred frontiers between recommended use and potentially harmful errors may lead to serious health hazards. This situation may become more and more frequent, especially considering the current hype surrounding vitamin D.

Vitamin D overdose is suspected when hypercalcemia coexists with calcidiol > 150 ng/mL (10) and low plasmatic levels of parathormone (11, 12). The clinical manifestations result from the subsequent acute hypercalcemia: confusion, polyuro-polydipsia and dehydration, anorexia, various transit troubles, cardiac rhythm and conduction disorders. In this case, the infant likely presented severe symptomatic hypercalcemia, as it was > 3 mmol/L despite hydration, the 12th day of hospitalization. Indeed, he probably suffered from a delay in diagnosis, as hypercalcemia was not suspected until the 2nd week of hospitalization. Early assessment of calcemia would have dramatically changed the management of this patient, preventing diagnostic delay and unwarranted investigations.

Acute intoxication with calcitriol lasts a few days only, because of its short half-life. Conversely, calcidiol has a high affinity for its transport binding protein, hence a circulating half-life of 2–3 weeks (13), and accumulates in adipose tissue (14) and liver (15). Therefore, intoxication with calcidiol can last for months.

Vitamin D intoxications have been reported in patients consuming large doses of vitamin D-containing supplements, either voluntarily or as medication and dietary supplement errors. The latter pertain to the multiple brands, types of packaging and formulations of dietary supplements, resulting in prescription or administration errors (11, 13, 16, 17).

In this case, the lack of communication and/or comprehension between the parents and the midwife resulted in an error in drug administration. It took roots in substituting the medicinal vitamin D by a dietary supplement of different dosing. The risk is further increased by the plethora of alternate formulas available on the Internet, with various concentrations and expression of dosages. Up to 28 pharmaceutical brands of vitamin D are available in France, not to mention the DS and the various multivitamin complexes. The labeling of vitamin D products (drugs and dietary supplements) suffers from a lack of harmonization, misleading the consumer and exposing to hazardous titrations.

Besides, products containing vitamin D are framed by two distinct regulations, regarding their qualification as drug or dietary supplement. Indeed, the sole notification to the competent authorities without any objection within 2 months allows the manufacturer to put a dietary supplement on the market. Manufacturers are free to mislead the consumer to benefit from the aura of the purported naturalness of their DS, maintaining confusion with equivalent drugs. However, vitamin D content of unlicensed DS is believed to vary widely from labeled claims, as DS are manufactured under less stringent quality standards (18). Yet, patients consuming DS are seldom suspicious toward those products, considered innocuous and branded as healthy, while they consider drugs with Marketing Authorization Holders as dubious. Indeed, the increasing use of DS is partly due to the presence in drugs of antioxidant excipients such as butylhydroxytoluene (BHT), considered harmful by parents and by some health professionals. Anyhow, some drugs are exempt of BHT, but are not reimbursed in most cases (e.g., Deltius®).

The error risk when dispensing or administering medications is further increased during the postpartum period, upon returning home. Mother and child both have their own prescriptions, sometimes including the same pharmacological classes or presentations (e.g., vitamin D, vaccines) exposing to potential errors. Parents are receptive to marketing arguments insisting on the naturalness of the products intended for their child.

Our case highlights the risk of vitamin D overdose, particularly in the pediatric population. The COVID-19 pandemic sparked interest regarding the potential protective effect of vitamin D and there is elsewhere growing hype regarding its potential role in cancer or immunity *inter alia* (12). This may lead more and more patients to the temptation of automedication and/or misuse of products containing vitamin D. Indeed, more than three quarters of reports about vitamin D intoxication have been published from 2010 (17). In this setting conducive to mistakes, actions should be taken to minimize as much as possible all add-on preventable sources of errors, insomuch as most cases of vitamin D intoxication could be easily preventable (17).

Whatever the drug, there is a need for patients to be adequately informed and instructed on how to administrate the appropriate dosage. Anyhow, keeping in mind the risk of vitamin D intoxication, early assessment of calcemia is pivotal to the diagnostic approach of unexplained behavioral change and anorexia in infants. The present report and two others fostered ANSES to issue a warning to recommend drugs containing vitamin D over DS (19, 20). Broadly, there is a real need for harmonization of the regulation, dosing and recommendations for use of dietary supplements.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.816965/full#supplementary-material

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The Effects of Different Doses of Alfentanil and Dexmedetomidine on Prevention of Emergence Agitation in Pediatric Tonsillectomy and Adenoidectomy Surgery

*Yan-zhuo Zhang, Xiong-li Wei, Bin Tang, Yuan-yuan Qin, Min Ou, Xiao-hong Jiang, Yu-feng Tan and Mao-ying Ye**

Department of Anesthesiology, Liuzhou Workers Hospital/The Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou, China

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Judith Ann Smith, University of Texas Health Science Center at Houston, United States

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***Correspondence:**
Mao-ying Ye
yemaoying625@163.com

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Background: Emergence agitation (EA) is a common problem often observed in children after sevoflurane anesthesia, which can be prevented by dexmedetomidine and alfentanil. This study aims to compare the effectiveness of dexmedetomidine alone and with different doses of alfentanil in preventing EA in children under sevoflurane anesthesia.

Materials and Methods: In a double-blind trial, 80 children (ASA I or II, 3–7 years old) undergoing tonsillectomy alone and adenotonsillectomy with sevoflurane anesthesia were randomly assigned into four groups: the control group, dexmedetomidine (DEX) group, dexmedetomidine plus 10 μg/kg alfentanil group (DEX + Alf1), and dexmedetomidine plus 20 μg/kg alfentanil group (DEX + ALf2). The incidence of EA was assessed with the Aono's scale, and the severity of EA was evaluated with the Pediatric Anesthesia Emergence Delirium (PAED) scale. The time of tracheal extubation and time of wake were recorded. Postoperative pain and complications such as nausea and vomiting, cough, laryngospasm, and bradycardia were recorded.

Results: The incidence of EA was 50% in the control group, 25% in the DEX group, and 5% in the DEX + Alf1 group, and it never happened in the DEX + Alf2 group. The Aono's scale, the PAED scale, and the FLACC scale in the control group and the DEX group were significantly more than those in the DEX + Alf1 group and the DEX + Alf2 group after the tracheal extubation ($p < 0.05$). The time of tracheal extubation of the control group and the DEX group were significantly shorter than those in the DEX + Alf1 group and the DEX + Alf2 group ($p < 0.05$). The awakening time of the DEX + Alf2 group is significantly longer than those in other groups ($p < 0.05$). The case of postoperative nausea and vomiting in the DEX + Alf1 group was fewer than those in the other groups ($p < 0.05$). And, the cases of cough and laryngospasm and bronchospasm in the DEX + Alf1 group and the DEX + Alf2 group were significantly less than those in the control group and the DEX group after the tracheal extubation ($p < 0.05$).

Conclusion: The combined administration of alfentanil and dexmedetomidine can reduce EA in children undergoing tonsillectomy alone and adenotonsillectomy with sevoflurane anesthesia. Dexmedetomidine plus 10 μg/kg alfentanil seems to be more appropriate than other dose combinations as it reduced EA and postoperative nausea and vomiting but did not prolong the time to awake.

Keywords: emergence agitation, tonsillectomy, adenoidectomy, alfentanil, dexmedetomidine

INTRODUCTION/BACKGROUND

Sevoflurane is a popular anesthetic for children used worldwide because of its low pungency, rapid onset, and fast recovery properties. However, it is associated with a higher incidence of emergence agitation (EA) (as high as 80%) (Sato et al., 2010). EA in children is usually a short-lived phenomenon with no after-effect. And, it is potentially dangerous because it can make the children fall out of bed and remove the surgical dressings and intravenous catheters, which increased the extra medical cost (Dahmani et al., 2014; Driscoll et al., 2017; Choi et al., 2018; Abbas et al., 2019).

Pharmacological prophylactic interventions were a good method to prevent EA. Some research studies showed that propofol (Jalili et al., 2019), α_2-agonist (Tsiotou et al., 2018; Tan et al., 2019), and μ-opioid agonists (Choi et al., 2011; Tan et al., 2016) were effective in preventing EA. Dexmedetomidine (DEX), a selective α_2-adrenoceptor agonist, significantly reduces the incidence of EA in children after sevoflurane anesthesia (Bilgen et al., 2014; Yang et al., 2020). Sato M (Sato et al., 2010) found that intravenous 0.3 μg/kg DEX after induction of anesthesia reduced EA from 68 to 24% under sevoflurane anesthesia. Alfentanil, μ-opioid receptor agonists, can decrease the incidence of EA in children after sevoflurane anesthesia (Bilgen et al., 2014). Kim et al. (2009) showed that administration of alfentanil at the dose of 10 μg/kg and 20 μg/kg after induction of anesthesia reduced EA in children after sevoflurane anesthesia from 71 to 34%. Choi et al. (2016) revealed that 10 μg/kg alfentanil can reduce the EA from 64 to 32% in children receiving sevoflurane anesthesia. Even if both DEX and alfentanil were associated with reducing the incidence of the unsettling behavior after surgery, up to one in four children may present negative behaviors after the awakening of anesthesia.

The sedation caused by DEX with minimal respiratory depression made it a safer option when used in combination with opioids. We hypothesized that alfentanil and DEX have a synergistic effect, and the co-administration of DEX and alfentanil can decrease the EA to a satisfactory degree that is lower than using one drug alone in children after sevoflurane anesthesia.

In this study, we aimed to evaluate the effects of DEX alone and the co-administration of DEX and two different doses of alfentanil to prevent the unsettling behavior after sevoflurane anesthesia in children undergoing tonsillectomy and adenoidectomy.

MATERIALS AND METHODS

The study was approved by the institutional ethics committee and the Chinese clinical trial registry with written informed consent (the ethics number: ChiCTR-2000040530). After signing the informed consent with the parents of all children, 89 children aged between 3 and 7 years old with an ASA physical status I or II, who were scheduled for either a tonsillectomy alone or both an adenoidectomy and tonsillectomy, were enrolled in this prospective, randomized, double-blind, controlled study. The inclusion criteria were the children with tonsils enlargement more than II degrees, repeated infection of tonsils, or snoring. The excluded criteria were the children with asthma, cardiac disease, abnormal upper airway, obstructive sleep apnea syndrome (OSAS), developmental delay, or a history of the upper respiratory tract infection in the preceding 4 weeks.

The children were randomly divided into four groups according to the random number table: The children in the control group received normal saline intravenously for 10 mins from the induction; the children in the DEX group were given intravenously 0.4 μg/kg dexmedetomidine for 10 mins from the induction; the children in the DEX + Alf1 group were administered intravenously with 0.4 μg/kg dexmedetomidine for 10 mins from the induction and alfentanil (10 μg/kg) at the induction of anesthesia; the children in the DEX + Alf2 group were administered intravenously with 0.4 μg/kg dexmedetomidine for 10 minutes from the induction and alfentanil (20 μg/kg) at the induction of anesthesia.

The primary outcome of our study is if DEX and DEX-added alfentanil can decrease the degree of EA. The second outcome of our study is which combination of drugs inhibits EA the best and has the least side effects.

In the operating room, we monitored the children with noninvasive blood pressure (NIBP), electrocardiography (ECG), pulse oximetry (SpO_2), and the bispectral index (BIS). The children were inducted with 3% sevoflurane with 5 L/min oxygen, lidocaine 1 mg/kg, propofol 2–2.5 mg/kg, atracurium 0.3 mg/kg, and study drugs which were prepared with another anesthesiologist. The anesthesiologist responsible for anesthesia and observation did not know which drug it was. 3 min later, we performed the tracheal intubation. After the induction of anesthesia, we intravenously administrated 2 mg/kg tramadol and 0.1 mg/kg dexamethasone for postoperative analgesia and preventing nausea and vomiting after surgery. We maintained the anesthesia with 2–3% sevoflurane mixed in 2 L/min 50% oxygen.

The concentration of sevoflurane was adjusted so that the BIS value is in the range of 40–60. During the surgery, we injected 3 ml 0.5% lidocaine and 1:200,000 epinephrine mixture into the mucosa surrounding each tonsillar fossa for local anesthesia and vasoconstriction. The mean blood pressure (MAP), heart rate (HR), and SpO_2 were recorded on arrival in the operating room (baseline), intubation time (T0), and 1 min (T1), 5 min (T5), 10 min (T10), 20 min (T20), and 30 min (T30) after intubation and at tracheal extubation (E0), 5 min after tracheal extubation (E5) and 10 min after tracheal extubation (E10). Tracheal extubation was performed when the patients breathed spontaneously, moved, and coughed.

The time of anesthesia was defined from the administration of DEX or saline until the tracheal tube was extubated. The time of tracheal extubation was defined from the end of the surgery and the discontinuation of sevoflurane until the tracheal tube was extubated. The time of awakening was defined from the end of the surgery and discontinuation of sevoflurane until the children acted on command. The incidence of EA was rated on the following Aono's (Aono et al., 1997) four-point scale: 1 = calm; 2 = not calm but could be easily consoled; 3 = moderately agitated or restless and not easily calmed; and 4 = combative, excited or disoriented, and thrashing around. Scores of one and two were considered nonproblematic behavior, and scores of three and four were considered EA. The severity of EA was evaluated with the Pediatric Anesthesia Emergence Delirium (PAED) scale (Sikich and Lerman, 2004), which consists of five items: 1) the child makes eye contact with the caregiver, 2) the child shows purposeful actions, 3) the child is aware of his or her surroundings, 4) the child is restless, and 5) the child is inconsolable. Items 1–3 are scored as follows: 4 = not at all, 3 = just a little, 2 = quite a bit, 1 = very much, and 0 = extremely. Items 4 and 5 are scored as follows: 0 = not at all, 1 = just a little, 2 = quite a bit, 3 = very much, and 4 = extremely. When the comprehensive score was greater than 15, we considered that severe agitation appeared. Postoperative pain was assessed with the Face, Legs, Activity, Cry, Consolability scale (FLACC) (Redmann et al., 2017) at 10-min intervals from admission in the PACU for 30 min. When the FLACC scale of children was more than 4, 5 μg/kg alfentanil was administered intravenously. The incidence and severity of EA and pain were measured at E0, E5, and E10. The adverse reactions after surgery such as nausea and vomiting, cough, laryngospasm and bronchospasm, and bradycardia were recorded at E0, E5, and E10.

Statistical analyses were performed with SPSS 23.0. Demographic data such as the age, gender, weight, type of surgery, and duration of anesthesia and surgery were compared with unpaired Student's t-tests. Differences in the incidence of EA and severe EA among the groups were analyzed using a χ^2 test with the Fisher's exact test correction compared in the three timepoints. Intra and postoperative hemodynamic and respiratory variables in the same subjects were compared with the Bonferroni test after repeated measures of analysis of variance. $p < 0.05$ was considered to be statistically significant.

RESULTS

A total of 89 children were enrolled in our study, and out of them, 9 children were excluded. Two children with asthma, 1 child with abnormal upper airway, two children with OSAS, and 4 children having a history of upper respiratory tract infection were excluded. In total, 80 children finished this study, with 20 children in each group. The demographic data such as the age, gender, surgery type, weight, duration of surgery, and anesthesia showed no significant differences among the four groups ($p > 0.05$) (**Table 1**).

DEX alone treatment reduced the occurrence rate of EA from 50% in the control group to 25% in the DEX group. Co-administered 10 μg/kg alfentanil further decreased the incidence to 5% in the DEX + Alf1 group, and co-administered 20 μg/kg alfentanil decreased the incidence to 0% in the DEX + Alf2 group, respectively (**Table 2**). There was no difference in the time of tracheal extubation between the DEX group and the control group ($p > 0.05$), while the time of tracheal extubation was prolonged by administered alfentanil from 12.08 ± 3.69 min in the DEX group to 15.24 ± 4.68 min in the DEX + Alf1 group and 16.06 ± 4.76 min in the DEX + Alf2 group ($p < 0.05$), respectively. There was no difference in the time of tracheal extubation between the DEX + Alf1 group and the DEX + Alf2 group ($p > 0.05$). The time of awakening in the DEX + Alf two group was significantly longer than those in the other groups ($p < 0.05$) (**Table 2**).

The Aono's scores and the scale of PAED in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly less than those in the control group at E0, E5, and E10 ($p < 0.05$). The Aono's scores and the scale of PAED in the DEX + Alf1 group and the DEX + Alf2 group were significantly less than those in the DEX group at E0, E5, and E10 ($p < 0.05$) (**Table 3**) (**Table 4**). There was no severe EA in the DEX + Alf1 group and the DEX + Alf2 group. The severe EA in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly lower than that in the control group at E0, E5, and E10 ($p < 0.05$). The severe EA in the DEX + Alf1 group and the DEX + Alf2 group were significantly lower than that in the DEX group at E0, E5, and E10 ($p < 0.05$) (**Table 5**).

The FLACC scale and the case of rescue alfentanil in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly less than those in the control group ($p < 0.05$). The FLACC scale and the case of rescue alfentanil in the DEX + Alf1 group and the DEX + Alf2 group were significantly less than those in the DEX group ($p < 0.05$). The cases of nausea and vomiting in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly lower than those in the control group ($p < 0.05$). And, the case of nausea and vomiting in the DEX + Alf1 group was significantly lower than those in the DEX group and the DEX + Alf2 group ($p < 0.05$) (**Table 6**). Furthermore, DEX plus 10 μg/kg alfentanil seems to be more appropriate than other dose combinations because it has a lower incidence of postoperative nausea and vomiting and shorter awakening time. Administration of alfentanil at the doses of 10 μg/kg and 20 μg/kg with DEX significantly reduced cases of cough and laryngospasm and bronchospasm ($p < 0.05$) (**Table 6**)

During the late stage of surgery and the period of extubation, the HR and the MAP increased gradually; DEX alone and a

TABLE 1 | Demographic and surgical characteristics (n = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
Age (year)	4.53 ± 1.32	4.81 ± 1.09	5.13 ± 1.29	5.11 ± 1.23
Gender (M/F)	10/10	12/8	11/9	9/11
Weight (kg)	21.35 ± 9.69	21.60 ± 5.12	23.15 ± 9.31	22.69 ± 9.83
Tonsillectomy/Adenotonsillectomy	2/18	1/19	1/19	2/18
Anesthesia duration (min)	61.01 ± 11.38	62.01 ± 11.99	65.99 ± 10.87	66.01 ± 12.03
Surgery duration (min)	37.02 ± 5.80	38.04 ± 6.01	37.16 ± 5.76	37.48 ± 6.71

DEX, dexmedetomidine; Alf, alfentanil; M, male; F, female.

TABLE 2 | EA and the time of extubation and awaken (mean ± SD, n = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
Emergence agitation	10 (50%)	5 (25%)	1 (5%) *	0 (0) *
Time of extubation (min)	11.15 ± 3.49	12.08 ± 3.69	15.24 ± 4.68$^{*\#}$	16.06 ± 4.76$^{*\#}$
Time of awake (min)	14.95 ± 3.57	14.86 ± 3.89	15.61 ± 4.59	19.25 ± 4.38$^{*\#\Delta}$

$^{*}p < 0.05$, Significant difference compared with the control group; #$p < 0.05$, Significant difference compared with the DEX, group; Δ$p < 0.05$, Significant difference compared with the DEX + Alf1 group; DEX, dexmedetomidine; Alf, alfentanil.

TABLE 3 | Aono'S Four-Point Scale of different groups (mean ± SD, n = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
E0	3.20 ± 0.77	2.20 ± 0.41*	1.50 ± 0.51$^{*\#}$	1.30 ± 0.47$^{*\#}$
E5	2.95 ± 0.60	1.90 ± 0.55*	1.40 ± 0.50$^{*\#}$	1.20 ± 0.41$^{*\#}$
E10	2.70 ± 0.47	1.70 ± 0.66*	1.10 ± 0.31$^{*\#}$	1.00 ± 0.00$^{*\#}$

$^{*}p < 0.05$, Significant difference compared with the control group; #$p < 0.05$, Significant difference compared with the DEX, group; DEX, dexmedetomidine; Alf, alfentanil.

TABLE 4 | Scale of PAED in the different groups (mean ± SD, n = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
E0	12.49 ± 2.60	12.07 ± 2.13*	10.05 ± 1.72$^{*\#}$	10.11 ± 1.68$^{*\#}$
E5	10.29 ± 4.48	8.12 ± 4.15*	6.59 ± 2.54$^{*\#}$	6.90 ± 3.69$^{*\#}$
E10	7.65 ± 4.08	5.89 ± 3.46*	4.33 ± 2.31$^{*\#}$	4.24 ± 3.48$^{*\#}$

$^{*}p < 0.05$, Significant difference compared with the control group; #$p < 0.05$, Significant difference compared with the DEX group; DEX, dexmedetomidine; Alf, alfentanil.

TABLE 5 | Case of patients who suffered severe agitation (n = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
E0	5	3*	0$^{*\#}$	0$^{*\#}$
E5	3	2*	0$^{*\#}$	0$^{*\#}$
E10	2	1*	0$^{*\#}$	0$^{*\#}$

$^{*}p < 0.05$, Significant difference compared with the control group; #$p < 0.05$, Significant difference compared with the DEX, group; DEX, dexmedetomidine; Alf, alfentanil.

combination of alfentanil at the doses of 10 μg/kg and 20 μg/kg significantly inhibited the increase of the HR and the MAP ($p < 0.05$). The HR and the MAP in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly less than that in the control group at T10, T20, T30, E0, E5, and E10 ($p < 0.05$) (**Figure 1** and **Figure 2**).

DISCUSSION

This study demonstrated that intravenous administration of DEX 0.4 μg/kg alone or combined with intravenous different doses of alfentanil from induction of anesthesia could reduce EA in children with sevoflurane anesthesia undergoing adenotonsillectomy surgery. In our study, the Aono's score and the PAED scale of the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly less than that of the control group. The Aono's score and the PAED scale of the DEX + Alf1 group and the DEX + Alf2 group were significantly less than that of the DEX group; therefore, co-administration of DEX and alfentanil has a better effect to prevent EA than DEX alone. And, the time of awakening in the DEX + Alf2 group was longer than that in the DEX + Alf1 group, and the case of nausea and vomiting was more in the DEX + Alf2 group than that in the DEX + Alf1 group significantly. So intravenous administration of 0.4 μg/kg DEX with 10 μg/kg alfentanil was the appropriate dose because it can prevent EA after sevoflurane anesthesia, do not prolong the awakening time, and do not increase postoperative nausea and vomiting.

EA is a common phenomenon with children after sevoflurane anesthesia. A lot of risk factors may be considered during the development of EA, for example, pain, age, different types of surgery and inhaled anesthetics with fast emergence, and anesthetic techniques such as sevoflurane (Choi et al., 2019). In some research studies, the prophylactic use of analgesics successfully reduced EA after sevoflurane anesthesia, showed that pain may be one of the reasons for EA (Lynch et al., 1998; Kosar et al., 2014). On the other hand, post-anesthetic EA has been observed when the pain was effectively controlled (Weldon et al., 2004) or in the absence of pain (Cravero et al., 2000). In our study, The FLACC scale and the cases of rescue alfentanil in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group were significantly less than those in the control

TABLE 6 | Pain score and adverse reaction in different groups (mean ± SD, *n* = 20).

	Control	DEX	DEX + Alf1	DEX + Alf2
FLACC scale	5.20 ± 2.25	3.20 ± 1.03*	1.60 ± 1.27*#	1.40 ± 1.35*#
Case of rescue alfentanil	15	7*	3*#	2*#
Case of nausea and vomiting	4	2*	0*#	1*Δ
Case of cough	5	2*	0*#	0*#
Case of laryngospasm or bronchospasm	3	1*	0*#	0*#
Case of bradycardia	2	1	1	2

*p < 0.05, Significant difference compared with the control group; #p < 0.05, Significant difference compared with the DEX group; Δp<0.05, Significant difference compared with the DEX + Alf1 group; DEX, dexmedetomidine; Alf, alfentanil.

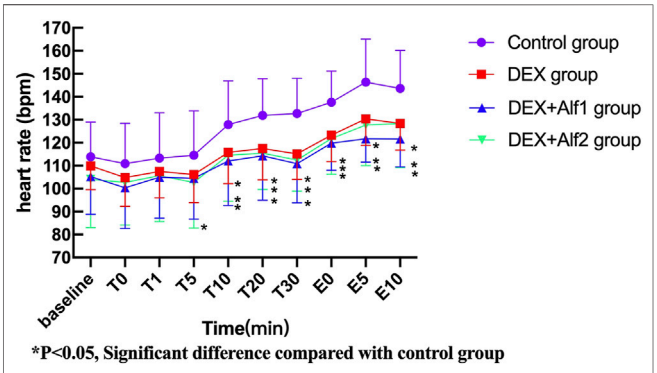

FIGURE 1 | Heart rate of patients during anesthesia among groups. *p < 0.05, Significant difference compared with the control group.

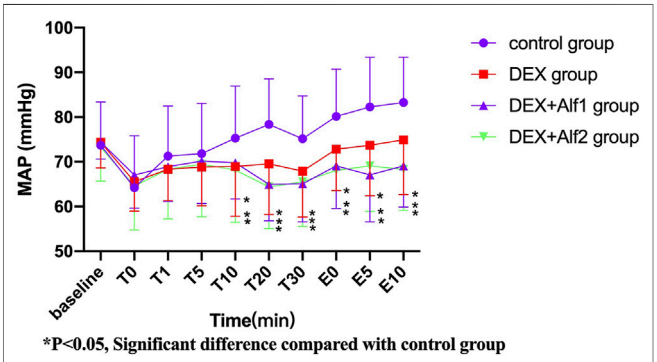

FIGURE 2 | MAP of patients during anesthesia among groups. *p < 0.05, Significant difference compared with the control group.

group. The differences of the FLACC scale and the Anno's scale and the PAED scale between the groups were similar. Then, in our study, the result showed that pain is one of the major reasons for post-anesthetic EA. Children aged less than or equal to 7 years old (preschool children) are more likely to undergo EA (Przybylo et al., 2003). The patients have emergence delirium and not pain, maybe related to the inhaled anesthetics with fast emergence and anesthetic techniques as sevoflurane, the age, and the type of the surgery. In this study, we enrolled 3–7-year-old children who were more likely to suffer from EA, and in our study, the incidence of EA is 50% which is similar to the 44% in Przybylo's research (Przybylo et al., 2003) at roughly the same age children. There is some relationship between EA and the type of surgery such as head and neck surgery, tonsils, thyroid, and middle ear surgery (Przybylo et al., 2003; Sato et al., 2010). In our study, the children we enrolled underwent tonsillectomy and adenoidectomy surgery. The age and the type of surgery subject the children at a high risk of EA.

The mechanism of DEX prevention of EA for children undergoing tonsillectomy and adenoidectomy may have sedative, analgesic, and anxiolytic properties (Olutoye et al., 2010; Zhang et al., 2019). Meanwhile, both a single dose administered and continuous intravenous infusion of DEX showed that can reduce EA after sevoflurane anesthesia in children (Yang et al., 2020). In our study, the continuous infusion of DEX for 10 minutes during induction decreased EA from 50 to 25% without prolonging awakening time. Kim found that the 50% effective dose and 95% effective dose of DEX to prevent EA in children undergoing tonsillectomy or adenoidectomy after desflurane anesthesia is 0.25 μg/kg or 0.38 μg/kg (Kim et al., 2015). And, the 0.3–0.5 μg/kg bolus dose of DEX has no hemodynamic effects in children (Guler et al., 2005; Ibacache et al., 2015). Although many research studies showed that DEX could be safely used in children, a recent study found that DEX caused some hemodynamic changes in children (Jooste et al., 2010). In our study, we choose a 0.4 μg/kg dose of DEX; it has a good effect of preventing EA and does not show any side effects.

Several research studies demonstrated that intravenous alfentanil 10 μg/kg and 20 μg/kg could decrease the incidence of EA (Aono et al., 1997; Kim et al., 2009; Choi et al., 2016). In our study, we selected these two doses combined with DEX. The mechanism of alfentanil to prevent EA after sevoflurane anesthesia may be related to its analgesic and slightly sedative effect (Choi et al., 2016). In our study, the difference of the FLACC scale and Anno's scale and PAED scale between the groups was similar. This supported the analgesic effect of alfentanil to prevent EA. One of the side effects of opioids is postoperative nausea and vomiting. In our study, although we use the prophylactic dexamethasone, the case of nausea and vomiting in the DEX + Alf2 group was more than that in the DEX + Alf1 group. Dexamethasone can decrease postoperative edema and improve subsequent oral take after tonsillectomy because it has anti-inflammatory effects (Yiu et al., 2017).

Alfentanil can depress the respiratory depression; DEX also has minimal effect on depressing the respiratory depression. In

our study, the children had not experienced respiratory depression during extubation and in the PACU. And, the children did not show hypoxemia when they came back to the ward. The MAP was stable during extubation and in the PACU in the DEX group, the DEX + Alf1 group, and the DEX + Alf2 group. The sedative effects of DEX and alfentanil make the patients' heart rate to not increase very high. The combination of DEX and alfentanil reduced the case of nausea and vomiting, cough, laryngospasm, and bronchospasm and did not increase the case of bradycardia after surgery. Thus, the combination of DEX and alfentanil has the synergistic effect to prevent EA after sevoflurane anesthesia and has no severe side effects.

CONCLUSION

Co-administration of alfentanil and DEX can reduce EA in children after sevoflurane anesthesia. DEX plus 10 μg/kg alfentanil seems to be more appropriate than other dose combinations with 20 μg/kg alfentanil as it reduced EA and postoperative nausea and vomiting but did not prolong the time to awake.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Liuzhou workers hospital/ Chinese clinical trial registry. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Y-zZ, M-yY, and X-lW designed experiments and carried out the experiment; BT, Y-yQ, and MO collected the data; X-hJ and Y-fT analyzed experimental results; Y-zZ and M-yY wrote the manuscript.

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SYSTEMATIC REVIEW
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Adverse Drug Reactions in Pediatric Oncohematology: A Systematic Review

Kristopher Amaro-Hosey[1,2,3], *Immaculada Danés*[2,3,4]* *and Antònia Agustí*[2,3,4]

[1]Clinical Pharmacology Service, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [2]Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain, [3]Vall d'Hebron Research Institute, Barcelona, Spain, [4]Clinical Pharmacology Service, Vall d'Hebron University Hospital, Barcelona, Spain

Introduction: Adverse drug reactions (ADR) are an important cause of morbidity and mortality in pediatric patients. Due to the disease severity and chemotherapy safety profile, oncologic patients are at higher risk of ADR. However, there is little evidence on pharmacovigilance studies evaluating drug safety in this specific population.

Methods: In order to assess the incidence and characteristics of ADR in pediatric patients with oncohematogical diseases and the methodology used in the studies, a systematic review was carried out using both free search and a combination of MeSH terms. Data extraction and critical appraisal were performed independently using a predefined form.

Results: Fourteen studies were included, of which eight were prospective and half focused in inpatients. Sample size and study duration varied widely. Different methods of ADR identification were detected, used alone or combined. Causality and severity were assessed frequently, whereas preventability was lacking in most studies. ADR incidence varied between 14.4 and 67% in inpatients, and 19.6–68.1% in admissions, mainly in the form of hematological, gastrointestinal and skin toxicity. Between 11 and 16.4% ADR were considered severe, and preventability ranged from 0 to 74.5%.

Conclusion: ADR in oncohematology pediatric patients are frequent. A high variability in study design and results has been found. The use of methodological standards and preventability assessment should be reinforced in order to allow results comparison between studies and centers, and to detected areas of improvement.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=96513, identifier CRD42018096513.

Keywords: pharmacovigilance, adverse drug reactions, pediatrics, hematology, oncology, neoplasms, systematic review

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Eloisa Gitto,
University of Messina, Italy

Reviewed by:
Ashwin Karanam,
Pfizer, United States
Evelyne Jacqz-Aigrain,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

***Correspondence:**
Immaculada Danés
id@icf.uab.cat

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INTRODUCTION

Adverse drug reactions (ADR) have been defined by the World Health Organization (WHO) as "a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for modification of physiological function" (WHO, 1972).

ADR are an important cause of morbidity and mortality in patients of all ages, including pediatric population, and are considered a public health problem worldwide (Impicciatore et al., 2001;

Clavenna and Bonati, 2009; Thiesen et al., 2013; Durrieu et al., 2014; Ramos et al., 2021). Children are more susceptible to ADR owing to insufficient standardized information, unlicensed and off-label use, unavailability of pediatric formulations, and physiological peculiarities inherent to age (Ramos et al., 2021).

Different systematic reviews and meta-analysis including ADR observational studies have found an incidence of ADR in pediatric inpatients ranging 0.6–16.8%, from 1.8 to 2.09% leading to hospital admission and 1.0–1.46% in outpatient setting (Impicciatore et al., 2001; Clavenna and Bonati, 2009; Smyth et al., 2012). In addition, ADR prevention in outpatients remains a public health and a patient safety challenge (Lombardi et al., 2018). A systematic review including 102 articles assessed preventability in only 19, which ranged from 7 to 98%. This high variability was explained due to a high heterogeneity in study designs, methods and settings (Smyth et al., 2012).

Risk factors for ADR in children are poorly characterized (Bellis et al., 2013; Lombardi et al., 2018). Age on admission, number of drugs, off-label drug use, and oncology diagnosis and treatment have been described as ADR risk factors (Bellis et al., 2013; Thiesen et al., 2013). Moreover, one of these studies stated the risk in oncology patients and found an increased risk for ADR (OR = 1.89 [95% CI 1.36–2.63]) (Thiesen et al., 2013).

Chemotherapy toxicity is a common cause of morbidity and mortality in most pediatric cancer patients, and a frequent cause of mid and long term sequel (Conyers et al., 2018). Even though drugs used in cancer diseases are described as a risk factor of ADR occurrence, and that ADR are frequent in oncology and hematology hospitalization wards, there are very few studies that have quantified or analyzed any of these aspects in pediatric population.

Oncohematological diseases have a high impact on children and their families, and on their quality of life. Improving the knowledge of ADR incidence, characteristics and preventability can be useful to compare results between studies and centers and to detect improvement areas, as a way to offer quality healthcare. Our aim was to perform a systematic review in order to describe the incidence and characteristics of ADR in pediatric oncology and hematology patients, to describe the methodology used in the included studies and, if possible, to identify preventive actions in order to minimize ADR occurrence.

METHODS

Study Design

A systematic review of observational studies that evaluated the prevalence, incidence and/or characteristics of ADR in pediatric oncohematology was performed. This study was conducted in accordance with the recommendations of the Joanna Briggs Institute (Munn et al., 2015) for systematic reviews of observational epidemiologic studies that evaluate prevalence and incidence data, and the PRISMA recommendations for systematic reviews (Tricco et al., 2018). This study was registered (CRD42018096513) at PROSPERO systematic review database.

Systematic Literature Search

A systematic literature search was carried out in PubMed from inception to 31st December 2020, both using free search and the combination of different MeSH terms ("Pediatrics," "Neoplasms," "Hematology," "Antineoplastic agents," "Drug-related side effects and adverse reactions," "Iatrogenic disease," "Prevention and control," "Medical oncology," and "Primary prevention"). References of the articles assessed for eligibility were also reviewed and included if considered relevant.

Inclusion and Exclusion Criteria

Studies that described the incidence and/or characteristics of ADR in pediatric oncohematology patients or in pediatric population with a differentiated oncohematology subgroup were included in this systematic review. Articles describing infectious outbreaks related to immunosuppression, data from national or international clinical databases of spontaneous pharmacovigilance reporting systems and pharmacovigilance studies including one single drug or specific ADR were excluded. No language or other search filters were applied.

Screening and Data Extraction

All articles were screened independently by two authors (KA-H, ID) to identify relevant studies based on titles and abstracts, and on full texts of potentially relevant papers if study relevance could not be determined from the titles and abstracts. For studies meeting inclusion criteria, data were extracted independently using a standardized data collection form defined and agreed previously. Data extracted included article identification, methodology characteristics (study design, setting, study aim, ADR definition and detection method, and causality, severity and preventability scales used), and relevant results (sample size, study duration, population characteristics, ADR frequency and description, severity and preventability). A third author (AA) participated in the review and in the data extraction in case of disagreement.

Data Analysis and Quality Assessment

\This review focuses on both the incidence of ADR in a high-risk population and on the methodological characteristics of the studies included. Quality assessment was performed independently by two authors (KA-H, ID), using a scale designed and previously published (Laatikainen et al., 2017), available in the **Supplementary Material**. The scale includes six questions related to study design, study population, ADR definition and identification, causality assessment and result description. Each question can be evaluated as 0 or 1, where 0 indicates the poor quality of the study regarding that item. A third author (AA) participated in the critical appraisal in case of disagreement.

RESULTS

Using the research strategies defined previously, 7,712 studies were retrieved from PubMed database. Forty articles were considered relevant for eligibility and finally, considering inclusion and exclusion criteria, 14 studies were included in the systematic review (Collins et al., 1974; Mitchell et al., 1988; Queuille et al., 2001; Le et al., 2006; Gallagher et al., 2012; Posthumus et al., 2012;

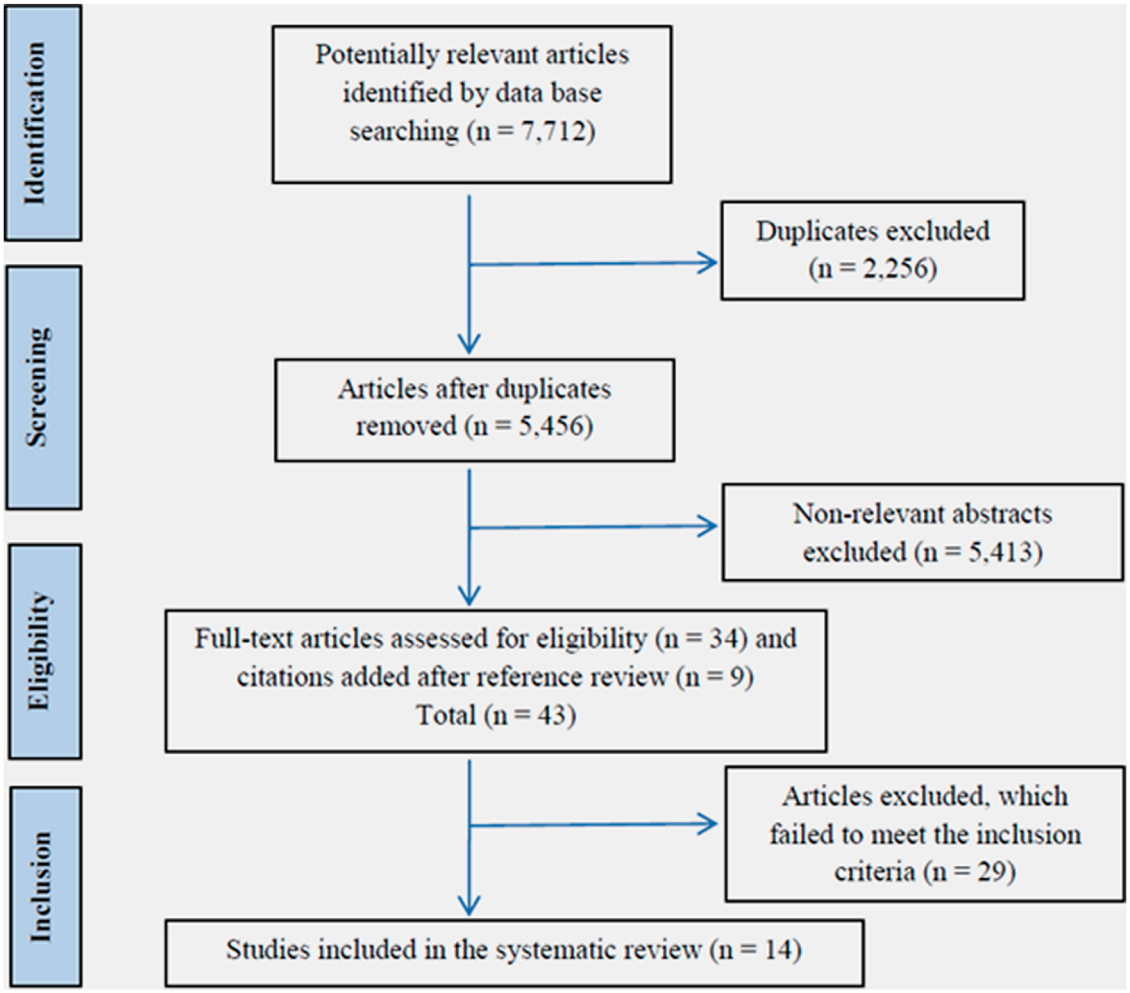

FIGURE 1 | Study flow chart.

Barrett et al., 2013; Call et al., 2014; Langerová et al., 2014; Makiwane et al., 2019; Dittrich et al., 2020; Joseph et al., 2020; Morales-Ríos et al., 2020; Workalemahu et al., 2020). Due to the characteristics of studies found, a meta-analysis was considered not feasible to be carried out. **Figure 1** shows the study flow chart.

Study characteristics and main results are summarized in **Tables 1–4**. Of the 14 studies included, six were carried out in pediatric oncology and hematology patients, and eight were carried out in general pediatrics and included a clear pediatric oncohematology subgroup. Four studies (Collins et al., 1974; Mitchell et al., 1988; Queuille et al., 2001; Le et al., 2006) were published before 2010, and 10 studies (Gallagher et al., 2012; Posthumus et al., 2012; Barrett et al., 2013; Call et al., 2014; Langerová et al., 2014; Makiwane et al., 2019; Dittrich et al., 2020; Joseph et al., 2020; Morales-Ríos et al., 2020; Workalemahu et al., 2020) were published later.

Methodological Results

Eight observational studies collected data prospectively, whereas six were performed retrospectively. Seven studies focused on hospitalized patients, four included admissions related to ADR and three analyzed both settings. No studies assessing outpatient setting were found. Twelve studies evaluated ADR, predominantly using WHO or Edward and Aronson definitions, and two studies used adverse drug events (ADE). Causality was estimated in nine studies, using mainly Naranjo and WHO-UMC scales. Severity was assessed in 11 studies, mostly using Hartwig et al. scale and NCI CTCAE criteria. Finally, preventability was only evaluated in five studies out of 14, using Shumock and Thorton in two of them. Ten studies used a single ADR detection method, and four studies used a combination of them: intensive monitoring chart review method was used in seven studies, chart review was used in four studies, and three studies based their results in triggers.

Critical appraisal is summarized in the supplementary material. Most of the studies defined adequately the study population and stated the causality assessment tool used (questions 2 [Q2] and 5 [Q5]). In contrast, results were considered not clearly described in half of the studies (Q6), as the information provided by the original articles on number of

TABLE 1 | Methodology characteristics in pediatric oncohematology studies.

References	Design	Setting	Main study aim	ADR definition	Detection method	Causality scale	Severity scale	Preventability scale
Barrett et al. (2013)	Retrospective	Inpatients	Assess the diversity of toxicities, the association with drug pairs and to compare the reported incidence of specific toxicities based on differences in dosing patterns	ADR (WHO)	Single centre pharmacovigilance database	NS	NCI CTCAE v4	Not evaluated
Call et al. (2014)	Retrospective	Inpatients	Investigate the effectiveness and efficiency of the use of a trigger tool for ADE detection	ADE	Triggers + chart review	NS	NCI CTCAE v4 NCC MERP MEI	NS
Collins et al. (1974)	Prospective	Inpatients Admission	Assess the incidence and characteristics of ADR	ADR (WHO)	Intensive monitoring chart review + medical round + direct observation	NS	NS	Not evaluated
Joseph et al. (2019)	Prospective	Inpatients	Determine the incidence and characteristics of ADR, drug interactions and drugs involved	ADR (Edwards and Aronson)	Intensive monitoring chart review	Naranjo et al	Hartwig et al	Shumock and Thornton
Queuille et al. (2001)	Prospective	Inpatients	Evaluate ADE frequency and characteristics	ADE	Intensive monitoring chart review	Bégaud et al	EORTC tool Hartwig et al	FAMC
Workalemahu et al. (2020)	Retrospective	Inpatients	Evaluate ADR associated to chemotherapy and related risk factors	ADR (WHO)	Chart review	WHO-UMC	Hartwig et al	Not evaluated

ADR, adverse drug reaction; ADE, adverse drug event; FAMC, factorial analysis of multiple correspondences; NCC MERP MEI, national coordinating council for medication error reporting and prevention medication error index; NCI CTCAE, National Cancer Institute common terminology criteria for adverse events; NS, not specified; WHO, World Health Organization; WHO-UMC, World Health Organization–Uppsala Monitoring Centre.

patients or ADR was missing. In addition, study design (Q1), ADR definition (Q3), and ADR detection method (Q4) were not clearly mentioned in three studies.

Clinical Results

Sample size varied from 52 to 10,297 patients, as well as study duration, which ranged from 30 days up to 12 years. Age was expressed in means in five studies, as median in four or with percentage of patients in an age range (2–12 years old) in three; age values can be found in **Tables 3**, **4**. Gender varied from 44.1 to 61.3% of males, and was not stated in four studies. Leukemia and solid tumors were the main cancer diagnosis, stated in seven studies. ADR incidence varied depending on study setting: it ranged from 14.4 to 67% in hospitalized patients, 19.6–68.1% in admissions caused by an ADR, and 2.12–71% in studies evaluating both settings. Chemotherapy toxicity described in the studies was related to hematological toxicity (anemia, febrile neutropenia), gastrointestinal toxicity (nausea, vomiting, transaminases increase), and skin (alopecia, rash). Both chemotherapy agents such as methotrexate, doxorubicin or vincristine, and antimicrobials were frequently related to ADR in oncohematology population. Severe ADR frequency described was 11–16.4%, and preventability also varied from 0 to 74.5%. Only four studies reported fatal cases, shown at the results tables.

Four studies included in this systematic review also assessed risk factors for an ADR. In general pediatric studies, Langerová et al. described oncology patients as an independent risk factor (OR = 9.8 [95% CI: 5.8–16.7]), as well as Makiwane et al. (OR = 7.3 [95% CI 3.0–18.9] and Gallagher et al., finding an even higher risk (OR = 29.7 [95% CI 17.4–50.9]). Workalemahu et al. described an increased risk for etoposide (OR = 1.99 [95% CI 0.93–4.27]), mercaptopurine (OR = 3.91 [95% CI 1.1–14.5), doxorubicin (OR = 2.32 [95% CI 1.3–4.2]) and >4 chemotherapy agents (OR = 2.7 [95% CI 1.5–4.7).

DISCUSSION

Even though ADR are an important cause of morbidity and mortality, are frequent in oncology and hematology, and chemotherapy is described as a risk factor, only 14 studies that assessed ADR were found. Incidence rates ranged from 14.4 to 61.3% in hospitalized patients and 19.6–68.1% in ADR leading to admission. A high heterogeneity in methodological aspects

TABLE 2 | Methodology characteristics in pediatric studies with oncohematology subpopulation.

References	Design	Setting	Main study aim	ADR definition	Detection method	Causality scale	Severity scale	Preventability scale
Dittrich et al. (2020)	Retrospective	Inpatients	Identify if ADR are adequately documented and reported to pharmacovigilance databases	ADR (WHO)	Chart review	WHO-UMC	NCI CTCAE v5.0	Not evaluated
Gallagher et al. (2012)	Prospective	Admission	Identify ADR causing admission in order to quantify and characterise them, and to determine their avoidability	ADR (Edwards and Aronson)	Intensive monitoring chart review	LCAT	Hartwig et al	Hallas et al
Langerová et al. (2014)	Prospective	Admission	Ascertain the incidence and characteristics of ADR related hospital admissions, and determine drug groups involved	ADR (Edwards and Aronson)	Intensive monitoring chart review	Naranjo et al LCAT Edwards and Aronson	Not evaluated	Not evaluated
Le et al. (2006)	Retrospective	Inpatients Admission	Evaluate the incidence and common types of ADR in hospitalized children	ADR (WHO)	Spontaneous notification + triggers + medical round + drug monitoring	NS	Hartwig et al	Not evaluated
Makiwane et al. (2019)	Prospective	Inpatients	Describe the prevalence of ADR in pediatric inpatients	ADR (WHO)	Intensive monitoring chart review	Naranjo et al	Hartwig et al	Not evaluated
Mitchell et al. (1988)	Prospective	Admission	Provide information regarding pediatric hospital admissions prompted by ADR	ADR (NS)	Intensive monitoring chart review	NS	Not evaluated	Not evaluated
Morales-Ríos et al. (2020)	Retrospective	Inpatients Admission	Estimate the frequency of ADR and their characteristics in hospitalized patients, as well as drugs related	ADR (WHO)	Single centre pharmacovigilance database	Naranjo et al	NOM-220-SSA1-2012	Not evaluated
Posthumus et al. (2012)	Prospective	Admission	Investigate the incidence and characteristics of hospital admissions related to ADR	ADR (Edwards and Aronson)	Triggers + chart review	Naranjo et al	Hartwig et al	Schumock and Thornton

Seriousness was evaluated in three studies using the following tools: ICH CIOMS definitions (Makiwane et al., 2019), ICH E2A guidelines (Dittrich et al., 2020) and NOM-220-SSA1-2012 guidelines (Morales-Ríos et al., 2020)

ADR, adverse drug reaction; ADE, adverse drug event; LCAT, Liverpool ADR, causality assessment tool; NCI CTCAE, National Cancer Institute common terminology criteria for adverse events; NS, not specified; WHO, World Health Organization; WHO-UMC, World Health Organization–Uppsala Monitoring Centre.

reviewed was also described and has likely influenced on the observed results. To our knowledge, this is the first systematic review on pharmacovigilance regarding pediatric oncology and hematology.

As mentioned previously, a high variability regarding methodology was found in almost every aspect of study design: sample size, study duration, study setting, population of interest, ADR detection method, the assessment of severity and preventability, and the different scales used. These findings could be explained by the different aim of each study, the effort to adapt the study to each local environment and available resources, research experience of the team and the moment in which they were designed and carried out, since methodology has evolved over time. These methodological differences have probably influenced on the clinical results found. A systematic review on ADR detection methods in hospitalized children was carried out (Ramos et al., 2021) and found that methods such as intensive monitoring chart review or trigger tools are more effective but time consuming, whereas spontaneous notification showed the lowest rate of detection. They concluded that most of the studies used a combination of methods, which might indicate a growing concern in ADR care in hospitalized children. This improvement in combined methods for ADR detection was previously suggested (Gonzalez-Gonzalez et al., 2013).

To our knowledge, there is no reference quality assessment tool for observational studies with other designs than cohort or case-control studies. A systematic review (Katrak et al., 2004) pointed out the variability in 121 published critical appraisal tools, regarding its intent, components and construction; this finding was later confirmed in another systematic review (Page et al., 2018), which concluded that there are several limitations of existing tools for assessing risk of reporting biases. STROBE statement (von Elm et al., 2007) or Johanna Briggs Institute (Munn et al., 2015) critical appraisal checklists are the most known tools, but their application was complex and troublesome. Therefore, the choice of the checklist used in this systematic review (Laatikainen et al., 2017) was agreed by the research team due to the lack of a standardized tool, its suitability to the type of studies included in the systematic review and to the aim of the critical appraisal analysis, and its easy

TABLE 3 | Clinical results in pediatric oncohematology studies.

References	Sample size	Duration	Population characteristics	ADR frequency	ADR description	Severity (%)	Preventability (%)
Barrett et al. (2013)	1,713p	6.5 y	Age: 64% (2–12 y) Gender: 53.1% male Dx: 52.1% leukemia, 28.5% neuroblastoma	Incidence per year: 14.4–23.5% 326p, ADR NS	Frequent ADR were neutropenia, increased ALT and febrile neutropenia (especially G3-4). The most toxic drug pair was methotrexate—vincristine. Twelve deaths were reported	NS	Not evaluated
Call et al. (2014)	390p	4 y	Mean age: 11 y Gender: 55% male Dx: 54% leukemia, 24% solid tumor	Incidence: NS Patients NS, 38 ADE	Sodium polystyrene sulfonate and naloxone were the triggers most frequently related to an ADE	21 ADE (G3-4)	63.6%
Collins et al. (1974)	63p	15 w	Mean age: 8.9 y Gender: 51% male Dx: 44.4% leukemia	Incidence: 71% (45p/63p) 154 ADR: 63 (admission) and 91 (during hospitalization)	CT and antimicrobials were the drugs most frequently related to ADR. Gastrointestinal and hematologic ADR were the most frequently described during hospitalization. ADR most frequent during admission were nausea and vomiting with cyclophosphamide (9), cytosine arabinoside (6) and/or vincristine (6)	11% severe during hospitalization	Not evaluated
Joseph et al. (2019)	176p	18 m	Age: 66.1% (2–12 y) Gender: 55.9% female Dx: 67.9% leukemia	Incidence: 67% (118p/176p) 131 ADR	The most frequent ADR was rash (19), itching (10), anemia and gastrointestinal complaints (8). The most frequent drugs were vincristine (19) and methotrexate (16). Rashes were related to co-trimoxazole, allopurinol, dexamethasone, methotrexate and vincristine. Cases of itching were related to dexamethasone	16.4% severe	74.5%
Queuille et al. (2001)	52p	50 d	NS	Prevalence: 65% (34p/52p) 155 ADE	Allergic reactions and medication errors were the most preventable ADE. CT was involved in >50% ADE	16% severe	53%
Workalemahu et al. (2020)	287p	25 m	Mean age: 7.1 y Gender: 61.3% male Dx: 23.3% leukemia, 22.6% Wilms tumor	Prevalence 41.5% (119p/287p) 147 ADR	Most frequent ADR were vomiting (16.3%), alopecia (15%) and febrile neutropenia (10.2%). Vincristine (85.4%), doxorubicin (61.7%) and cyclophosphamide (57.8%) were the most frequently prescribed drugs. Concomitant medication, etoposide, mercaptopurine, doxorubicin and >4 CT agents were identified as risk factors	74.1% moderate	Not evaluated

ADR, adverse drug reaction; ADE, adverse drug event; CT, chemotherapy; d, day; Dx, diagnosis; G3-4, grade 3–4; m, month; NS, not specified; p, patients; y, year.

application. The main area of improvement was the presentation of results, as results were insufficient or missing in half of the studies, and therefore it was considered to be the aspect most susceptible to introduce bias. Moreover, an adequate study design statement, ADR definition and identification clearly mentioned would likely reduce the risk of bias and improve study quality. Ten studies were published after the STROBE statement, which suggests a need to reinforce the use of these tools both during study design and manuscript drafting.

Incidence described in oncohematology pediatric patients was higher, in contrast with studies in pediatrics, which described an overall rate of ADR of 9.53 and 2.09% (hospitalized and admission, respectively) (Impicciatore et al., 2001). This finding is expectable and consistent with chemotherapy safety profile and ADR risk factors, such as cancer diagnosis or number of concomitant drugs. Moreover, it is likely that the use of different scales in causality and severity assessment has influenced on the results observed too.

Hematological, gastrointestinal and skin toxicities are the most frequently described ADR in the articles included, which are in tune with the expected safety profile of conventional chemotherapy. No studies with novel drugs such as monoclonal antibodies or tyrosine kinase inhibitors were found up to 2020. A recently published study (Amaro-Hosey et al., 2021) prospectively assessed drug safety with some specific therapies, including novel drugs and conventional chemotherapy. The most frequent ADR were hematological, infections and gastrointestinal. Incidence using days at risk was calculated

TABLE 4 | Clinical results in pediatric studies with oncohematology subpopulation.

References	Sample size	Duration	Population characteristics	ADR frequency	ADR description	Severity (%)	Preventability (%)
Dittrich et al. (2020)	T: 301p; POH: 31p	1 m	Median age (T): 5 y Gender (T): NS; Dx: NS	T: 26.9%; 81p; 132 ADR POH: % NS; p NS; 56 ADR	All patients suffering multiple ADRs received CT. Cytostatics was the drug group most frequently associated to ADR (28.8%). Leukopenia and febrile neutropenia were the most common ADR	T: 12.1% G3-4 POH: NS	Not evaluated
Gallagher et al. (2012)	T: 6,821p; POH: 74p	1 y	Median age (T): 3 y and 1 m (IQR: 9 m, 9 y) Gender (T): 58.1% male; Dx: NS	T: 2.9% (240adm/ 8,345adm); 178p; 249 ADR POH: NS; 41p, 120 ADR	The most common ADRs were oncology related neutropenia (89), thrombocytopenia (55) and anemia (38); and immunosuppression (74) occurring in both oncology and non-oncology patients. The most frequent drugs were dexamethasone (68), vincristine (51) and doxorubicin (38). Oncology patients were much likely to have an ADR	T: 6.8% (≥G4) POH: NS	T: 22% POH: 6.7%
Langerová et al. (2014)	T: 2.405p, 2,903adm POH: p NS, 143adm	9 m	Mean age (T): 7.1 ± 5.7 y; Gender (T): 57.1% male Dx: NS	T: 2.2% (64adm/ 2,903adm); p NS; ADR NS POH: 19.6% (28 adm/143 adm); p NS; ADR NS	The most frequent ADR were infections (16), febrile neutropenia (12) and mucositis (5). Cancer was described as a risk factor	Not evaluated	Not evaluated
Le et al. (2006)	T: NS POH: NS	9y	Mean age (T): 7.0 ± 6.2 y; Gender (T): 52% male Dx: 31.5% had hematologic malignancies or disorders, or solid tumors	T: 1.6%/10 y (per year: 0.4–2.3%); 1,009p; 1,087 ADR POH: % NS; 318p; ADR NS	ADR with antibiotics were usually mild; anticonvulsants and CT were associated more commonly with severe reactions. Asparaginase was associated with 3% of ADR. One death in an oncohematological patient was reported	T: 11% POH: NS	Not evaluated
Makiwane et al. (2019)	T: 282p POH: 23p	3 m	Median age (T): 1.4 y Gender (T): NS; Dx: NS	T: 18.4%; 52p; 61 ADR POH: 56.5%; 13p; ADR NS	ADRs were associated with CT (44.3%). ADR in POH included febrile neutropenia (6), anemia (4) and pancytopenia (3). Drugs related were doxorubicin, etoposide, vincristine, carboplatin and asparaginase. Oncology patients had an increased risk of an ADR	T: 11.5% POH: NS	Not evaluated
Mitchell et al. (1988)	T: 10,297p POH: 725p	12 y	NS	T: 2.85%; 294p; ADR NS POH: 22%; 157p; ADR NS	The most frequent ADR in POH were neutropenia (41%), fever (38%) and leukopenia (29%). CT was involved in 94% of POH admissions. Three deaths were reported in oncohematological patients	Not evaluated	Not evaluated
Morales-Ríos et al. (2020)	T: NS POH: NS	4 y	Age (T): 56.9% (2–11 y) Gender (T): 52% female; Dx (T): 72.2% neoplasms	T: 2.12–8.07% per year; 1,649p; 2,166 ADR POH: % NS; 1,190p; 1,494 ADR	91.9% ADR led to admission and 94.5% required treatment in POH. Serious ADR were frequently related to antineoplastic drugs (81.2%), being febrile neutropenia (52.4%) the most common serious ADR. Cancer patient deaths were drug-related in 1.4% cases (febrile neutropenia commonly	T: 14.4% POH: 14.2%	Not evaluated

(Continued on following page)

TABLE 4 | (*Continued*) Clinical results in pediatric studies with oncohematology subpopulation.

References	Sample size	Duration	Population characteristics	ADR frequency	ADR description	Severity (%)	Preventability (%)
					associated to death). Seventeen deaths were reported in oncohematological patients		
Posthumus et al. (2012)	T: 258p POH: 47p	5 m	Median age (T): 3 y and 6 m Gender (T): 56.6% male; Dx: NS	T: 18.2%; 47p; ADR NS POH: 68.1%; 32p; ADR NS	21 febrile neutropenia cases related to 20 different CT drugs (6 cases due to vincristine)	T: 0% POH: 0%	T: 13% POH: 0%

Adm, admissions; ADR, adverse drug reaction; CT, chemotherapy; d, day; Dx, diagnosis; G3-4, grade 3–4; m, month; NS, not specified; p, patients; POH, pediatric oncohematology subgroup; T, total population; y, year.

regarding novel therapies: 1.1 and 5.3 ADR/100 days at risk for blood disorders and 0.8 and four ADR/100 days at risk for infections, related to pegaspargase and thioguanine respectively; and 0.6 ADR/100 days at risk for infections attributed to rituximab. Only four out of 14 studies included in the systematic review reported fatal cases, and the global incidence of fatal cases could not determined because the total population was not specified in two studies (Le et al., 2006; Morales-Ríos et al., 2020). This finding has been previously described and could either suggest that fatal ADR are very rare in children or are frequently underreported or not suspected (Bouvy et al., 2015).

ADR preventability is a key aspect to analyze, in order to identify areas of improvement to reduce ADR occurrence and improve patients' life quality. A systematic review (Smyth et al., 2012) identified that preventability was only assessed in 19 out of 102 studies, and ranged from 7 to 98%. This finding is similar to result obtained in the current systematic review, which evidences that it's an aspect poorly evaluated in pharmacovigilance studies and therefore should be encouraged.

This systematic review tries to add some evidence on an important health problem insufficiently studied that affects a fragile population. Summarized data on characteristics and incidence of ADR in this population is provided, as well as a methodological description in order to find areas of improvement. Defined inclusion and exclusion criteria, the selection of studies in pediatrics with an oncohematology subgroup, the lack of non-standardized critical appraisal tool that fitted the study characteristics and the use of a selected/concrete critical appraisal tool may have introduced bias, but was agreed and considered appropriate to enrich the results and the discussion. Great heterogeneity makes it difficult to compare results, but can also be interpreted as a need to establish methodology standards or to reinforce their use during study design and manuscript drafting, such as STROBE statement. Ultimately, our aim should be to provide a high quality research and healthcare to our patients and to improve their quality of life, regarding drug efficacy and safety.

In conclusion, ADR in oncohematology pediatric patients are more frequent than in general pediatric population, as expected. A high variability in study design and results has been found, which indicates a need to reinforce the use of methodological standards both in study design and manuscript drafting, in order to allow comparability between studies and to identify areas of prevention and improvement. Preventability assessment should be strongly encouraged in order to provide a high quality healthcare and to improve patients' quality of life.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KA-H and ID contributed to the conception and design of the study, and contributed to the recording of the data. Substantial contribution to the analysis or interpretation of data for the work was made by KA-H, ID and AA. KA-H wrote the first draft of the manuscript. All authors substantially contributed to the manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.777498/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A Narrative Review on Efficacy and Safety of Proton Pump Inhibitors in Children

Valeria Dipasquale[1], *Giuseppe Cicala*[2], *Edoardo Spina*[2] *and Claudio Romano*[1]*

[1]*Pediatric Gastroenterology and Cystic Fibrosis Unit, Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy,* [2]*Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy*

Proton pump inhibitors (PPIs) are among the most prescribed drugs worldwide and include omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole. Their use in pediatrics is approved for children older than 1 year, for the short-term treatment of symptomatic gastroesophageal reflux disease (GERD), healing of erosive esophagitis, treatment of peptic ulcer disease, and eradication of *Helicobacter pylori*. PPIs are also considered the standard of care for pediatric eosinophilic esophagitis. Despite the strict range of indications, the use of this class of molecules has increased in all pediatric age ranges. The long-term gastric acid suppression in children has been linked to increased risks of gastrointestinal and lower respiratory tract infections, bone fractures, and allergy. This study aims to provide a comprehensive overview of the mechanism of actions, use (and misuse) in infants and children, and safety of PPIs.

Keywords: adverse reaction, indication, pediatrics, proton pump inhibitor, safety

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***Correspondence:**
Claudio Romano
romanoc@unime.it

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1 INTRODUCTION

Proton pump inhibitors (PPIs), such as omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole, are among the most often prescribed medications in the world. Despite the limited number of indications, the use of this class of compounds has risen across the pediatric age spectrum (Levy et al., 2020). From 2000 to 2015, a 16-year register-based analysis based on Danish nationwide healthcare registries showed 212,056 filled in PPI prescriptions dispensed to 78,489 children (0–17 years old), with total annual use of PPIs among children increasing eightfold (Aznar-Lou et al., 2019). A recent Irish retrospective drug utilization study using national prescription reimbursement data found a significant and statistically significant increase in PPI prescriptions in infants under the age of 1 year over 10 years (from 1011 in 2009–2763 in 2018; $p < .00001$) (O'Reilly et al., 2020). Similar trends have been found in other European countries and in the United States (Barron et al., 2007; De Bruyne et al., 2014; Illueca et al., 2014). Furthermore, several prescriptions were discovered to be off-label due to an inadequate indication of use (i.e., outside from the license for use for children) (Levy et al., 2020; O'Reilly et al., 2020). Indeed, PPIs are often empirically prescribed for infantile reflux, functional dyspepsia, chronic cough, and asthma without documented associated gastroesophageal reflux disease (GERD). The empiric use of PPIs is being scrutinized more closely as a body of evidence accumulates about their possible dangers (Chung and Yardley, 2013; Cohen et al., 2015; Stark and Nylund, 2016; De Bruyne and Ito, 2018; Pasman et al., 2020). In this study, we will provide a comprehensive overview of the mechanism of actions, use (and misuse) in infants and older children, and safety profile of PPIs. The main goal of the present review is to re-evaluate the evidence, limitations, efficacy, and safety of PPI use in the pediatric population.

2 METHODS

Relevant studies published over the last 20 years were identified via a PubMed/Medline search using different combinations of the following search terms: "proton pump inhibitors," "children" and "infants." Additional papers were identified by reviewing reference lists of relevant publications. Particular emphasis was placed on original studies investigating efficacy and/or safety issues. The summaries of product characteristics (SmPCs) were used for verification of approved indications. Non-English publications were excluded. A systematic approach to study selection was not implemented. Instead, data were extracted based on their relevance to the topic.

3 PHARMACOLOGICAL CHARACTERISTICS

Effective treatment with PPIs requires an understanding of their pharmacokinetics (Ward and Kearns, 2013). The H^+-K^+-adenosine triphosphatase (H^+-K^+-ATPase) is the enzyme responsible for acid secretion by the parietal cell in the stomach, after stimulation by the binding of different ligands, such as acetylcholine, histamine, or gastrin (Litalien et al., 2005; Roche, 2006; Shin et al., 2009). Ten of the H + -K + -ATPase's 28 cysteine (CYS) molecules are accessible to PPIs for binding. To bind to the CYS of the ATPase, PPIs must be activated, and the rate of this activation varies depending on their structures. (Litalien et al., 2005; Roche, 2006; Shin et al., 2009). PPIs are acid-sensitive weak bases that require an enteric coating to protect them from gastric acid destruction and allow absorption in the intestine. PPIs now on the market have a basic structure that connects a benzimidazole ring and a pyridine ring via a sulfinyl bond, with numerous substitutions on these rings affecting their chemistry (Roche, 2006). To chemically connect to the CYSs of the ATPase, the sulfinyl must obtain energy from the acidic environment within the parietal cell. Two protons are added to the nitrogens on either side of the sulfinyl group to activate the PPI. (Roche, 2006; Shin et al., 2009). Once active, the PPI can suppress the proton pump by forming disulfide bonds with CYS molecules on the ATPase. For the proton pump to be inhibited, the PPI must first reach the acidic site of action within the parietal cell, where it will receive the acidic activation described above (Litalien et al., 2005). The pharmacokinetics of the PPI determine the concentrations at the site of action, starting with inactive form absorption, distribution, processing by cytochrome P450 (CYP) 2C19 or CYP3A4, and clearance. Blockade of the proton pump necessitates pump activation before the PPI is eliminated from circulation, which influences the rate of absorption, latency to highest concentration, and rate of medication removal from circulation (Litalien et al., 2005). Because gastrin production after a meal is one of the most powerful H + -K + -ATPase inducers, the PPI should be taken long enough before a meal to be absorbed when the proton pump is most active. Acid secretion is stopped once the activated PPI attaches to the H + -K + -ATPase, either reversibly or permanently, long after the PPI has been removed from circulation. Because of variations in binding to the proton pump's CYSs, omeprazole blocks acid secretion for 24 h compared to 46 h for pantoprazole (Shin et al., 2009). Because not all proton pumps are blocked after the initial dosage, it takes around 3 days to reach steady state.

4 CLINICAL USES

4.1 Children and Adolescents

PPIs are licensed for use in children over the age of 1 year for the short-term treatment of symptomatic GERD, erosive esophagitis healing, peptic ulcer disease therapy, and *Helicobacter pylori* eradication. They are also considered the standard of care for pediatric eosinophilic esophagitis (EoE) (Levy et al., 2020). A systematic review including 12 studies on the use of PPIs (esomeprazole, lansoprazole, omeprazole, and pantoprazole) in children with GERD, has identified four trials in which PPIs were more effective for gastric acidity than placebo, alginic acids or ranitidine (van der Pol et al., 2011). In three of the studies, PPIs did not show significant difference from ranitidine or alginates in reducing histological alterations (van der Pol et al., 2011). A multicenter, double-blind, parallel-group study showed that rabeprazole is effective in 1- to 11-year-old children with endoscopically/histologically proven GERD randomized to receive a .5 or 1.0 mg/kg rabeprazole for 12 weeks, with further dose adjustment by weight (Haddad et al., 2013). The same authors have additionally determined the efficacy of maintenance treatment with rabeprazole in 1- to 11-year-old children suffering from GERD, showing healing maintenance in 90% of the children (Haddad et al., 2014). In children with typical GERD symptoms, current pediatric guidelines recommend a 4- to 8-week trial of PPIs (Rosen et al., 2018). Anyway, a recent study investigating the frequency of GERD in 85 toddlers with GERD symptoms found a very low incidence of GERD (3 children had abnormal reflux index at 24-hour esophageal pH monitoring, while 7 had reflux esophagitis at upper endoscopy), thus suggesting considering to be cautious with diagnostic PPI trials (Yang et al., 2019). In the pediatric age group, peptic acid disorders, such as erosive esophagitis and peptic ulcer disease, are rather uncommon conditions (Pasman et al., 2020; Ward and Kearns, 2013). The pharmacodynamics of PPIs for treatment of peptic acid disorders for children aged 1 year or older are comparable to that in adult patients (Ward and Kearns, 2013). In an international, multicenter, randomized, double-blind study conducted on children aged 1–11 years with endoscopically/histologically proven erosive esophagitis, PPI treatment (esomeprazole 0.2–1.0 mg/kg) led to healing of erosions in 89% of cases after 8 weeks (Tolia et al., 2015). In the case of *Helicobacter pylori* infection, eradication therapy should consist of a PPI-based triple regimen that includes *Helicobacter pylori*-susceptible antibiotics based on antimicrobial susceptibility testing of the bacterium (Jones et al., 2017). PPIs are also recommended for the second-line eradication therapy. The dosage of PPIs used for eradication therapy in children is shown in **Table 1**. EoE is an immune-mediated cause of esophageal inflammation with incidence and prevalence rates in children of 5.1 cases/100,000 persons/year and

TABLE 1 | Dosage of PPIs used for *Helicobacter pylori* eradication therapy (Tolia et al., 2015).

PPI	Dosage (mg/kg/day)	Maximum daily dose (mg/day)
	Twice daily	
Lansoprazole	1.5	60
Omeprazole	1.0	40
Rabeprazole	.5	20
Esomeprazole	≥4 years old Weight <30 kg, 20 mg/day Weight ≥30 kg, 40 mg/day	40

PPIs, proton pump inhibitors.

19.1 cases/100,000 persons, respectively (Arias et al., 2016). Initially, PPIs were used to differentiate EoE from PPI-responsive esophageal eosinophilia, which was thought to be related to GERD. According to the European Guidelines on EoE and the AGREE consensus statement, PPI therapy is no longer necessary for diagnosis of EoE and can be considered as part of the therapeutic management (Lucendo et al., 2017; Spergel et al., 2018). Several prospective randomized controlled trials support the efficacy of PPI therapy in inducing remission of EoE (Peterson et al., 2010; Dellon et al., 2013; Moawad et al., 2013; Vazquez-Elizondo et al., 2013), with clinic and histologic remission rates (defined as <15 eosinophils/high power field at biopsy) ranging from 33 to 57%, based on the study design and patient population. A systematic review and meta-analysis of 33 studies, including 619 EoE patients (431 adults and 188 children), reported histologic remission in about 50.5% of patients on PPI (95% CI 42.2–58.7%), with symptomatic improvement in 60.8% (95% CI 48.38–72.2%) (Lucendo et al., 2016). No significant differences were found in patients' age, study design, and type of PPI assessed (Lucendo et al., 2016). PPIs appear to have two main favorable effects on EoE: 1) lowering acid exposure, which reduces esophageal damage; and 2) lowering eotaxin-3 levels, a Th-2 cytokine involved in eosinophil-mediated inflammation (Lucendo et al., 2017; Spergel et al., 2018). There are currently no known predictors of PPI responsiveness in EoE. Esophageal miRNAs with different expression values between PPI responsive and PPI not responsive children have been recently proposed (Cañas et al., 2020). PPI therapy is currently considered a valid first-line treatment in patients with EoE, with recommended doses of omeprazole 1–2 mg/kg twice daily or equivalent in children (Lucendo et al., 2017). Such doses should be administered for 8 weeks to assess the response. To date, few observational studies in children and adults have investigated the long-term outcomes of patients with EoE who respond to PPI therapy (Molina-Infante et al., 2015; Gómez-Torrijos et al., 2016; Gutiérrez-Junquera et al., 2018). In a recent prospective multicenter study, 78.6% of pediatric patients maintained a clinic-pathologic remission at 1-year follow-up on maintenance treatment with standard doses of esomeprazole 1 mg/kg daily (Molina-Infante et al., 2015).

4.2 Infants

Contrary to the indications in older children, the indications for PPI use in infants are less clear. Current consensus guidelines for treatment of GERD in children under age 1 advocate for a trial of PPI to be considered after referral to a pediatric gastroenterologist, and only after failure of 1) first-line treatments, such as thickening of feeds and avoidance of overfeeding, and 2) second-line strategies, such as a trial of cow's milk elimination and allergy immunology consultation (due to the recognized link between cow's milk protein allergy and GER) (Rosen et al., 2018). Esomeprazole is the only PPI approved for use in patients 1 month to younger than 12 months of age. Recommendations are consistent with available evidence that PPIs are not effective for treating symptoms usually attributed to GERD in otherwise healthy infants, such as unexplained crying, irritability, or sleep disturbance. A double-blind placebo-controlled trial of omeprazole in irritable infants with a reflux index >5% found no difference in the duration of crying between the omeprazole-treated and placebo groups, despite highly effective acid suppression in the experimental arm (Moore et al., 2003). Another large double-blind trial of 162 infants randomized to receive 4 weeks of placebo or lansoprazole, revealed the same 54% response rate in both groups, using as an endpoint >50% reduction of measures of feeding-related symptoms (crying, irritability, arching and other parameters of the Infant Gastroesophageal Reflux Questionnaire) (Orenstein et al., 2009). Similar results have also been revealed in studies of the use of pantoprazole and esomeprazole in infants (Baker et al., 2010; Winter et al., 2012). Although an improvement of symptoms during the open-label run-in period was reported, no statistically significant difference between the PPI and placebo groups was found during the stopping phase (Baker et al., 2010; Winter et al., 2012). Springer et al. (2008) analyzed the effect of lansoprazole in infants and preterm infants with GERD symptoms and reported similar profiles of changes in pHmetry parameters and gastric pH in both the treated and placebo groups. Hussain et al. (2014) studied the efficacy and safety of rabeprazole in 1- to 11-month-old infants with symptomatic GERD that was resistant to conservative therapy and/or previous exposure to anti-acid drugs. A total of 344 patients were included in an open-label phase that lasted one to 3 weeks and received rabeprazole (10 mg/day). Patients were assigned to receive placebo, rabeprazole 5 mg, or rabeprazole 10 mg in the 5-week double-blind stopping phase after caregiver-rated clinical improvement during the open-label phase, with equal improvements in symptoms and weight in all three arms (Hussain et al., 2014). Infantile reflux is frequently physiologic, and it improves on its own between the ages of 6 and 12. Caregivers reported 4159 symptoms bouts of reflux in a large study involving 186 esophageal multichannel intraluminal impedance monitoring paired with pHmetry investigations in newborns, of which only 369 could be associated with an acidic reflux event. (Garza et al., 2011). Despite the above reported evidence, there is a huge increase of prescriptions of PPIs in infants (Blank and Parkin, 2017; Levy et al., 2020; O'Reilly et al., 2020). In a recent study including over 850,000 children, 8% were prescribed a PPI before age one (Malchodi et al., 2019).

4.3 Other Uses

A recent 20-year observational, retrospective study analyzing PPI-related ADR reports in a national spontaneous reporting system database found more than a half (55.7%) of prescriptions

being off-label. However, neither the severity nor the outcome of ADRs seemed to be linked to the label (Dipasquale et al., 2021). Chronic cough is a common off-label indication for PPI prescription in the pediatric age group, even before 1 year of age. The use of PPIs for chronic cough in children is not recommended unless there is evidence of GERD (Chang et al., 2019). In a recent retrospective study enrolling children under age 5, only 3.5% were found to have chronic cough due to GER (Chen et al., 2019). A trial evaluating 22 infants with chronic cough/wheezing found that the use of omeprazole alone did not reduce number of coughing episodes per day among those diagnosed with GER by pHmetry (Adamko et al., 2012). A larger, randomized placebo-controlled study of children with poorly controlled asthma showed no improvement in asthma control scores or pulmonary function tests when lansoprazole was added to their asthma therapy (Writing Committee for the American Lung Association Asthma Clinical Research Centers et al., 2012). Given the possibility of a link between persistent cough and GERD in children, referring them to an aerodigestive clinic might help them get better therapy.

PPIs are also widely used as stress ulcer prophylaxis, recommended in some situations to prevent upper gastrointestinal bleeding for patients admitted to the intensive care unit (Joret-Descout et al., 2017). Moreover, PPIs have showed to have benefits for reducing serum ferritin in patients with thalassemia major and intermedia (Eghbali et al., 2019).

5 SAFETY

The recent retrospective study from a national spontaneous reporting system database found 70 PPI-related adverse reaction reports in children (.01% of all database reports and 2% of all PPI adverse reaction reports, excluding literature cases), most of which were not serious or irreversible and presented with gastrointestinal (24%) and/or skin manifestations (21.3%). Notably, combination therapy (i.e., antibiotics) appeared to be positively linked with the severity of ADRs (Dipasquale et al., 2021). In terms of short-term side effects, 34% of children using PPIs experience headaches, nausea, diarrhea, or constipation (Cohen et al., 2015). In children, chronic PPI usage has been associated to an increased risk of gastrointestinal and lower respiratory tract infections, bone fractures, and allergies (De Bruyne and Ito, 2018). Although the toxicity profile of PPIs is unknown, particularly in children, pathogenetic pathways have been proposed, which are primarily connected to long-term gastric acid suppression (**Table 2**). Some of the most relevant literature studies on PPIs safety are summarized in **Table 3**.

5.1 Infections

A number of adult studies have found a possible link between PPIs and an increased risk of enteric infections, but only a few studies in children have looked into this (De Bruyne and Ito, 2018). Continuous PPI use was found to have a relative risk of 1.81 (95% CI 1.98–2.42) in a study of adults and children with acute gastroenteritis during the cold season (Vilcu et al., 2019). *Clostridium difficile* infection is the most commonly discussed infection linked to PPI use. A meta-analysis included 14 trials and 10,531,669 pediatric patients found that taking PPIs raised the probability of *C. difficile* infection by 1.33 (95% CI 1.07–1.64) (Anjewierden et al., 2019). In a more recent retrospective analysis of 124 children (1–18 years old) who had diarrhea and were positive for *C. difficile*, 49 had *C. difficile* infection and 75 had *C. difficile* colonization. Interestingly, age over 4 years (adjusted odds ratio 5.83; 95% CI 1.05–32.27) and PPI use (adjusted odds ratio 7.25; 95% CI 1.07–49.07) were the independent variables for serious illnesses among patients with the infection (Chang et al., 2020). Some studies even call for hospital regulations prohibiting the simultaneous administration of PPI and antibiotics, or for lower PPI doses, in order to reduce the risk of *C. difficile* infection (Thachil, 2008). PPIs in neonatal intensive care units have been identified as a risk factor for necrotizing enterocolitis (NEC) and sepsis, but evidence is conflicting. A prospective randomized trial showed an increased incidence of NEC in preterm infants treated with PPIs compared to the control group (Patil et al., 2017). However, a retrospective population-based study did not find any increase in NEC stage 2 and above or late onset sepsis, or mortality (Singh et al., 2016). However, most studies investigated

TABLE 2 | Main pathogenic mechanisms hypothesized for PPI-related adverse reactions in children.

System	Adverse reaction	Mechanism	
		Cause	Effect
Bone	Bone fractures	Hypochlorhydria	-Reduction of calcium ionization and subsequent calcium intestinal absorption -Inhibition of osteoclasts function and subsequent reduced bone resorption and remodelling
Digestive	Gastroenteritis	Hypochlorhydria	-Alterations in the gastrointestinal microbiome -Reduction of gastric mucus viscosity and leucocyte activity and subsequent enhanced bacterial invasion
Respiratory	Respiratory tract infections	Hypochlorhydria	-Invasion of microorganisms from the gastrointestinal tract into the upper and lower respiratory tract
Immune	Allergy	Hypochlorhydria	-Impairment of gastric and pancreatic protease activation and subsequent diminished protein processing and development of food-specific IgE -increase in mucosal permeability -Changes in the gastrointestinal microbiome

PPI, proton pump inhibitor.

TABLE 3 | Some relevant studies on PPIs safety in children.

Concern	Details	Evidence
Infections	Increased risk of enteric infections commonly *C. difficile* driven has been observed in relation to PPI treatment in children	-A meta-analysis included 10,531 669 pediatric patients and found that taking PPIs raised the probability of *C. difficile* infection (Anjewierden et al., 2019) -Age over 4 years and PPI use were the independent variables for serious *C. Difficile* disease among patients with the infection in a retrospective analysis (Chang et al., 2020)
Bone fractures	Increased risk of osteoporosis and bone fractures has been observed with PPIs administration	-In a retrospective study of 851,631 children, of whom 97,286 (11%) were had received acid suppression <1 year of age, the use of PPI was associated with an increased risk of fracture (Malchodi et al., 2019) -Acid suppression treatment before the age of one was correlated to an earlier median age at first fracture (Wang et al., 2020)
Allergic diseases	Concerns have recently emerged regarding the use of PPIs and the development of allergy disorders	-A retrospective cohort study involving 792 130 children, highlighted that the risk of developing an allergic disease such as food allergies, medication allergies, anaphylaxis, allergic rhinitis, and asthma was increased in those who had received anti-acids during the first 6 months of life (Mitre et al., 2018)

PPIs, proton pump inhibitors.

the effect of probiotics (*Lactobacilli, Bifidobacteria* or *Saccharides*) and bovine lactoferrin to prevent NEC and sepsis. The combination of both was shown to be more effective in decreasing NEC and sepsis (Meyer and Alexander, 2017; Hagen and Skelley, 2019).

The evidence on the link between PPI usage and an increased risk of community-acquired pneumonia is mixed. In a prospective trial of PPI and ranitidine-associated infections in newborns, researchers discovered that both PPI and ranitidine usage over an 8-week period increased the risk of pneumonia (odds ratio 6.39, 95% CI 1.38–29.70) in the 4 months after enrollment (Canani et al., 2006). More recently, in New Zealand, a case-control study found no link between PPI usage and community-acquired pneumonia (n = 65) or lower respiratory tract infections (n = 566) in infants (Velasco-Benítez, 2019). The risk of respiratory tract infections in children related with PPI medications has yet to be determined.

5.2 Bone Fractures

Data on the risk for osteoporosis and bone fractures mainly comes from adults. In a population-based study including 124,799 cases between ages 4–29 years, a higher risk of fractures in young adults (18–29 years) on PPIs was found, but not in children (adjusted odds ratio of 1.39 vs. 1.13, respectively) (Freedberg et al., 2015). When compared to controls, children who were taken acid suppression treatment before the age of one had an earlier median age at first fracture (3.8 vs. 4.5 years) in a retrospective cohort of patients followed for 2 years. Acid suppression before the age of one and therapy for a longer period were linked to a higher risk of fracture (Wang et al., 2020). In a retrospective study of 851,631 children, 97,286 (11%) were administered acid suppression before the age of one. The use of PPI in single therapy resulted in a hazard ratio of 1.23 (95% CI 1.15–1.32) for fracture, whereas the use of a combination PPI and H2-receptor antagonist resulted in a hazard ratio of 1.32 (95% CI 1.26–1.38) for fracture (Malchodi et al., 2019).

5.3 Allergic Diseases

Concerns have recently been aroused about the use of PPIs and the development of allergy disorders in children, although further research is needed. Food allergies (adjusted hazard ratio 2.59, 95% CI 2.25–3.00), medication allergies (adjusted hazard ratio 1.84, 95% CI 1.56–2.17), anaphylaxis (adjusted hazard ratio 1.45, 95% CI 1.22–1.73), allergic rhinitis (adjusted hazard ratio 1.44, 95% CI 1.36–1.52), and asthma (adjusted hazard ratio 1.41, 95% CI 1.31–1.52) (Mitre et al., 2018). There appears to be a connection between prenatal PPI usage and the development of allergy (Lai et al., 2018). A meta-analysis of eight population-based studies examining anti-acids usage during pregnancy and the risk of childhood asthma symptoms indicated that mothers who used prenatal PPIs had a higher risk of childhood asthma (relative risk 1.34, 95% CI 1.18–1.52, $p < .001$) (Lai et al., 2018).

6 CONCLUSION

PPIs should only be used in children who have GERD or gastrointestinal bleeding, which should be differentiated from non-pathological GER, especially in children under the age of 1 year. In many circumstances, the risk of adverse events is minimal. However, the safety profiles of PPI usage, particularly chronic PPI use, have yet to be thoroughly defined. Current research suggests that long-term use of PPIs is linked to a variety of side effects, the most common of which are gastrointestinal events (i.e., gastrointestinal infections). Clinicians should assess if a real indication exists before prescribing PPIs, as well as the impact of PPI use on clinics and the potential harmful effects on a child's future health.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Biosimilars in Pediatric Inflammatory Bowel Diseases: A Systematic Review and Real Life-Based Evidence

Valeria Dipasquale[1], *Giuseppe Cicala*[2], *Edoardo Spina*[2] *and Claudio Romano*[1]*

[1]Pediatric Gastroenterology and Cystic Fibrosis Unit, Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy, [2]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

Background: Many pediatric inflammatory bowel disease (IBD) patients are now using biosimilars of anti-tumor necrosis factor-α (TNF-α), with increasing trends in recent years. This study reviewed all available data regarding the use of biosimilars in children with IBD.

Methods: PubMed, Google Scholar, Scopus, and CENTRAL databases were searched through keywords; inflammatory bowel diseases, Crohn's disease, ulcerative colitis, biosimilar and child were combined using "AND" and "OR." Original research articles involving pediatric patients receiving one of the biosimilar medications based on the anti-TNF-α biologic drugs approved for pediatric IBD treatment, independently from efficacy and drug response, were included.

Results: Nine studies were included in the evidence synthesis. CT-P13 was the biosimilar used in all studies. Four studies assessed the induction effectiveness of CT-P13. Clinical response and remission rates of biosimilar treatment were 86–90% and 67–68%, respectively, and they were not significantly different to the originator group. Five prospective studies on patients elected to switch from originator IFX to CT-P13 yielded similar results. Adverse events related to CT-P13 were mostly mild. The most frequently reported were upper respiratory tract infections. The switch from the originator had no significant impact on immunogenicity.

Conclusion: The current review showed reported CT-P13 effectiveness as measured by clinical response and/or remission rates after induction or during maintenance and suggest that there is no significant difference with that of the originator IFX. Further studies are warranted, including clinical, and pharmacovigilance studies.

Keywords: biosimilar, crohn's disease, CT-P13, inflammatory bowel disease, pediatrics, safety, ulcerative colitis, anti-TNF-α

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Daniela Pugliese, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Italy

***Correspondence:**
Claudio Romano
romanoc@unime.it

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INTRODUCTION

Biologics were first introduced roughly 20 years ago, and have radically modified the treatment and prognosis of pediatric inflammatory bowel disease (IBD). Tumor necrosis factor-α (TNF-α), an inflammatory cytokine released by immune cells, was the target of the first biologics used to treat IBD patients (Laharie et al., 2005; Hyams et al., 2007; Hyams et al., 2012). The anti-TNF-α originator drugs available to treat IBD children are infliximab (IFX; Remicade©, Janssen) and adalimumab (Humira©, AbbVie). Their remarkable efficacy and safety profile has led to earlier ("top-down

therapy") and/or longer treatment duration, particularly in patients with a more severe course, and/or poor prognosis (Ruemmele et al., 2014; Turner et al., 2018). The patent on IFX expired in 2013, allowing the companies to launch its biosimilars. According to the World Health Organization, a biosimilar is defined as a "biotherapeutic product, which is similar in terms of quality, safety, and efficacy to an already licensed reference biotherapeutic product" (World Health Organization, 2009). CT-P13 was the first biosimilar IFX to be approved by the regulatory agencies, in 2013 by the European Medicine Agency (EMA) and in 2016 by the Food and Drug Administration (FDA) (Organization site European Medicines Agency, 2013; Organization site World Health Organization, 2017). IFX biosimilars are commercialized under different brand names, including Remsima© (Celltrion) and Inflectra© (Hospira) for CT-P13, or Flixabi© (Biogen) and Renflexis® (Merck) for SB2. ABP501 (Amgevita©, Amgen) was the first approved biosimilar to adalimumab. Based upon extrapolation of thorough *in vivo* experiments and two randomized controlled clinical studies in adult patients with rheumatologic diseases, biosimilars were authorized for the same indications as the original drug, including adult, and pediatric IBD (Park et al., 2013; Yoo et al., 2013; Alten and Cronstein, 2015). Extrapolation is the process of licensing a biosimilar for all the originator drug's approved indications, even though the biosimilar has not been formally investigated in all the originator drug's indications or populations (Weise et al., 2014; Alten and Cronstein, 2015; Vande Casteele and Sandborn, 2015). Extrapolation of compounds in the same class with the same mechanism of action from adult to pediatric or across indications is prevalent in clinical practice when there is insufficient evidence or clinical studies are underway (Weise et al., 2014; Vande Casteele and Sandborn, 2015). Adult patients have been the focus of studies evaluating the efficacy and safety of biosimilars in IBD (Farkas et al., 2015; Jahnsen et al., 2015; Gecse et al., 2016; Ye et al., 2019). According to the European Crohn's and Colitis Organization's (ECCO) guidelines, CT-P13's effectiveness and safety are equivalent to those of its originator product drug in patients who are naïve to anti-TNF-α therapy or who have switched to CT-P13 (Danese et al., 2017). Many pediatric IBD patients are now using biosimilars, with growing trends in recent years. Data on the effectiveness and safety of biosimilars in pediatric IBD are steadily increasing (Dipasquale and Romano, 2020). CT-P13 can be regarded as a good alternative to the originator for induction and maintenance of remission in children with IBD, according to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Pediatric IBD Porto Group (de Ridder et al., 2015; de Ridder et al., 2019). A recent nationwide web survey conducted in Italy showed that most pediatric IBD experts have good knowledge about biosimilars, with awareness of similar efficacy and safety in comparison to the originator (Dipasquale et al., 2021).

The aim of this review was to analyze all the literature data, published after biosimilar use approval in 2013, regarding the use of biosimilars of anti-TNF-α in pediatric IBD patients, and to assess effectiveness, immunogenicity, and safety profiles, as well as cost concerns.

METHODS

Search Strategy

Studies identification, screening, and extraction of relevant data were conducted according to the 2020 version of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Literature searches and screening of titles, abstracts and full text articles were conducted by two authors (VD; GC) independently. The research was conducted using the PubMed, Google Scholar, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) database—the latter also includes data from the clinicaltrials.gov and the World Health Organization International Clinical Trials Registry Platforms. Records provided by the academic search engine Google Scholar were also scanned. The considered timeframe for all scanned databases and searches was from 2013 to December 2021. For PubMed, Google Scholar and Scopus research, a query structure based on Boolean combinations of the terms "inflammatory bowel diseases," "Crohn's disease," "ulcerative colitis," "biosimilar" and "child," with terms variations, was used. For Google scholar the search filter "only scientific articles" was also applied. For the complete query structure and the full list of filters and refinement used see **Supplementary Box S1, S2**. As for the CENTRAL database search, a multiple query strategy was used: a general query for IBDs, with Boolean combinations of the same terms used for other databases; two other queries of analogous structure to account for specific trials regarding Crohn's disease and ulcerative colitis. For all the CENTRAL queries, the option of "search for word variations" was selected (full details are available in **Supplementary Box S1**). The references of all collected publications were also checked to find any missing relevant studies.

Inclusion and Exclusion Criteria

Papers that fulfilled the following criteria were included: original research articles involving pediatric patients of any gender and ethnicity receiving one of the biosimilar medications based on the anti-TNF-α biologic drugs approved for pediatric IBD treatment, independently from efficacy and drug response. Studies were excluded if *1*) the originator drug only was used; *2*) biosimilars were used to treat diseases other than IBDs; *3*) articles were written in a language other than English.

Data Extraction and Management

Data of relevance were extracted by a single author (V.D.) by the means of a data extraction sheet. Data regarding *1*) type of IBD treated, *2*) number of patients, *3*) type of biosimilar used, *4*) study duration, *5*) clinical evaluations, *6*) direct costs of treatment, were extracted. Missing data entries were marked with N/A (not available). Clinical response and/or remission as measured by the Pediatric Crohn's Disease Activity Index (PCDAI) for CD or the Pediatric Ulcerative Colitis Activity Index (PUCAI) for UC were the primary outcomes. In most studies, clinical response was defined by a PCDAI drop of >15 and a PUCAI score of >20, while remission was defined by a PCDAI or a PUCAI score of 10 or less. No statistical analyses were performed due to the limited number

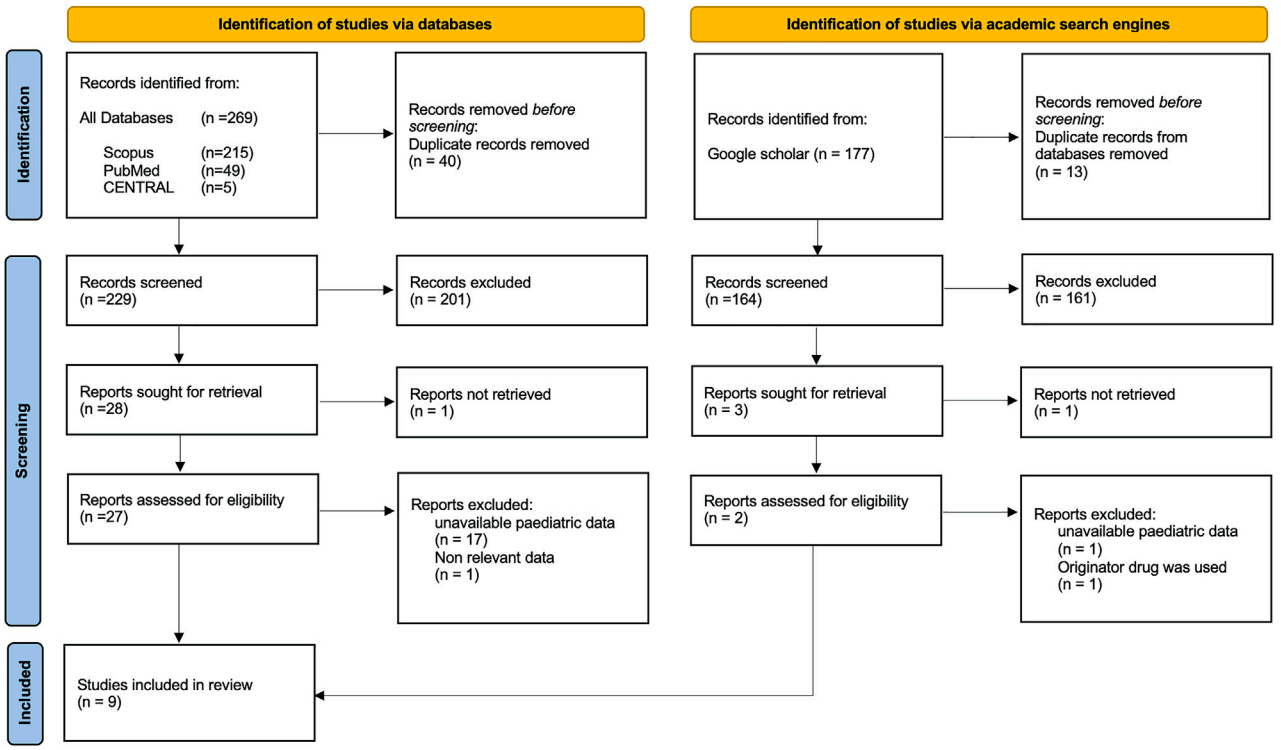

FIGURE 1 | Flow chart for study retrieval and selection.

of available studies and the heterogeneity in the reported data. Thus, the findings are presented in a descriptive manner.

RESULTS

In total, 384 records were retrieved, 9 of which met the inclusion criteria (**Figure 1**) (Sieczkowska et al., 2016; Sieczkowska-Golub et al., 2017; Chanchlani et al., 2018; Gervais et al., 2018; Kang et al., 2018; Richmond et al., 2018; van Hoeve et al., 2019; Nikkonen and Kolho, 2020; Cheon et al., 2021). A total of 394 pediatric IBD patients (316 CD, 61 UC, and 17 IBD-U) was comprised. CT-P13 was the biosimilar used in all studies. No studies on other biosimilars of IFX (PF-06438179/GP1111, SB2) or adalimumab biosimilars in pediatric IBD have been performed so far.

Clinical Endpoints

Each of the studies considered is summarized in **Table 1**. Some of them compared the outcomes with historical or reference cohorts. In most studies, patients received induction doses at 5 mg/kg at weeks 0, 2, and 6 (Sieczkowska-Golub et al., 2017; Kang et al., 2018; Richmond et al., 2018). In one study it was reported that 57% (16/28) of patients on CT-P13 received induction dose at > 5 mg/kg (Nikkonen and Kolho, 2020). The median age of included patients on CT-P13 was similar, ranging from approximately 11 to 14 years.

Biosimilars as Primary Indication for anti-TNF-α

In a prospective Polish study, 36 pediatric CD patients were recruited from three institutions where the originator IFX was no longer accessible (Sieczkowska-Golub et al., 2017). CT-P13 treatment was indicated in the case of severe luminal CD and/or perianal disease that was resistant to standard treatment. Clinical response (a reduction of 12.5 points on the PCDAI) and remission (a PCDAI score of 10) were obtained in 86% and 67% of patients, respectively, at the end of the induction (week 14). No significant difference in remission rates between naïve and non-naive patients was found. The findings of this study were compared to those of the REACH study (Hyams et al., 2007), which established the efficacy and safety of the originator IFX, and identical clinical improvement and remission after three doses of biosimilar were shown. Other studies have shown similar remission rates (Chanchlani et al., 2018; Richmond et al., 2018). A prospective analysis of 278 IBD children from 27 UK sites found no differences in clinical response or remission rates after induction between the originator IFX (n = 82) and biosimilar IFX (n = 21) groups (Chanchlani et al., 2018). No significant difference in remission rates between the two groups was found. They were also compared new anti-TNF-α therapy patients to historical data from 398 patients who started on originator IFX in a prior United Kingdom IBD biologics audit (2011–2015) and they were found no significant differences in clinical response and remission rates at the same timepoint (Lynch et al., 2013; Russell et al., 2013). A retrospective

TABLE 1 | Efficacy of biosimilars for pediatric inflammatory bowel disease.

References	Study design	Patients	Age, years[a]	Disease duration[b]	Controls	Time of assessment	Main outcomes
Sieczkowska-Golub J et al. (Sieczkowska-Golub et al., 2017)	Prospective	36 CD children, 27 anti-TNF naïve	11.79 ± 4.07	14 months (0.5–164)	No	Before the first and the fourth infusion (week 14)	86% (31/36) clinical response rate and 67% (24/36) remission rate
Richmond L et al. (Richmond et al., 2018)	Prospective	40 IBD children (29 CD, 11 UC)	12.7	12 months	No	At initiation and at week 12	67% (14/21) remission rate for CD patients
Chanchlani N et al. (Chanchlani et al., 2018)	Prospective	82 IBD children (63 CD, 14 UC, 5 IBD-U)	N/A	11.3 months (4.8–25.16)	175 (148 CD, 33 UC, 15 IBD-U) children on originator IFX	At initiation and at week 12	79% (19/24) and 68% (25/37) remission rates for biosimilar and originator IFX groups, respectively
Nikkonen A et al. (Nikkonen and Kolho, 2020)	Retrospective	28 IBD children (16 CD, 3 UC, 9 IBD-U)	12	13.2 months (0–87.6)	23 IBD children (17 CD, 2 UC, 4 IBD-U) on originator IFX	At initiation, at the third infusion, and at 1 year	90% clinical responses during induction with no difference between the two groups 65 vs. 61% on maintenance treatment at 1 year ($p > 0.05$), respectively
Sieczkowska J et al. (Sieczkowska et al., 2016)	Prospective	39 IBD children (32 CD, 7 UC) elected to switch	11.1 ± 3.3 (CD) and 12.3 ± 2.3 (UC)	N/A	No	At switching (shortly before the first infusion), after the first and the second doses of biosimilar, and at the last follow-up assessment (mean 8 ± 2.6 months)	Statistically significant ($p < 0.05$) switching-related change in PCDAI 88% (28/32) and 57% (4/7) clinical remission rate at the last follow-up assessment for CD and UC patients, respectively
Kang B et al. (Kang et al., 2018)	Prospective	38 IBD children (32 CD, 6 UC) elected to switch	14	N/A	36 IBD children (28 CD, 8 UC) on originator IFX	At switching (anytime during maintenance phase) and at 1 year	77.8% (28/36) and 78.9% (30/38) clinical remission rate for biosimilar and originator IFX groups, respectively
Gervais L et al. (Gervais et al., 2018)	Prospective	33 IBD children (26 CD, 4 UC, 3 IBD-U) elected to switch	11.8	N/A	No	Before the first dose of biosimilar, 6 and 12 months after switching	87% (25/31) and 83% (24/29) remission rates at 6 and 12 months, respectively No significant difference in remission rates within 12 months after switch
van Hoeve K et al. (van Hoeve et al., 2019)	Prospective	42 IBD children (26 CD, 16 UC) elected to switch	11.8	N/A	No	6 months before switching (baseline), at the last infusion before switching and 6 months after switching	83.3% (35/42) clinical remission rate 6 months after switching No significant difference in remission rates in comparison to baseline or at the last infusion before switch
Cheon JH et al. (Cheon et al., 2021)	Prospective	56 CD children (15 after switch)	N/A	N/A	No	At baseline, and at 6, 12, 24, 36, 42, and 48 months	Reduced PCDAI score at month 6 compared with baseline, remaining relatively consistent at most time points Lower proportion of PCDAI responders in the switch group

TNF, tumor necrosis factor; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; IBD-U, inflammatory bowel disease unclassified; N/A not available; IFX, infliximab; PCDAI, Pediatric Crohn Disease Activity Index.

[a]*At diagnosis.*

[b]*Before CT-P13 initiation.*

Finnish study found that the originator IFX and biosimilar IFX therapies had similar first-year therapy outcomes, such as treatment intensification during follow-up (83 vs. 82%); treatment discontinuation during induction (8.7 vs. 3.6%) or follow-up (because of loss of response or adverse reaction; 39 vs. 36%); and treatment discontinuation due to anti drug antibodies (ADA) (17 vs. 3.4%) (Nikkonen and Kolho, 2020).

Biosimilars in Patients Switching From Originator anti-TNF-α

A total of 152 children (116 CD, 33 UC, and 3 IBD-U) were examined in five studies after switching from the originator IFX to CT-P13 (Sieczkowska et al., 2016; Gervais et al., 2018; Kang et al., 2018; van Hoeve et al., 2019; Cheon et al., 2021). In a prospective study, 39 IBD children were switched after ($n = 37$) or during ($n = 2$) induction (Sieczkowska et al., 2016). The effectiveness at the last biosimilar doses was assessed, and clinical remission rates for CD and UC patients were found to be 88% and 57%, respectively. Eighty percent of CD patients and all 4 UC patients who continued biosimilars at the last assessment visit (i.e., 11 months after the first patient had been switched, after a mean follow-up of 8 ± 2.6 months) were in remission (Sieczkowska et al., 2016). Later studies found similar results, with no clinically important changes in disease activity after switching. A prospective single-center study conducted in South Korea compared 38 IBD patients after the switch to CT-P13 with 36 patients remained on the originator IFX (Kang et al., 2018). Maintenance treatment of 1-year duration was continued by 86.1% of the patients on originator IFX, and 92.1% of those on biosimilar IFX. Eight patients did not complete the year of follow-up, because of complete remission ($n = 3$), loss of response and change to adalimumab ($n = 3$), and loss at follow-up ($n = 2$). Similar rates (77.8 vs. 78.9%) of sustained remission (i.e., 1 year of corticosteroid-free clinical remission with no further dose intensification) were observed in the two groups (Kang et al., 2018).

Biomarkers Changes

Seven out of nine studies evaluated inflammatory biomarker changes (**Supplementary Box**). The Polish study evaluated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelets, and as well as hemoglobin (Hb) levels (Sieczkowska-Golub et al., 2017). CRP, ESR, and platelets had a significant reduction in all children who achieved a clinical response (Sieczkowska-Golub et al., 2017). More than half (59%) of individuals with elevated CRP levels at baseline had their CRP levels totally restored by week 14. In addition, one of the three children with anemia at week 0 had normalized Hb levels at week 14 (Sieczkowska-Golub et al., 2017). Similarly, Richmond et al. (Richmond et al., 2018) showed a significant decrease in CRP, ESR, and albumin serum levels at the end of induction with CT-P13. Studies investigating the switching from originator IFX to CT-P13 found no significant changes of inflammatory markers after switching (Sieczkowska et al., 2016; Gervais et al., 2018; Kang et al., 2018; van Hoeve et al., 2019). Fecal calprotectin was included in the analysis in four studies (Gervais et al., 2018; Kang et al., 2018; Richmond et al., 2018; Nikkonen and Kolho, 2020). Decreases were found to be not significant neither between baseline and follow-up visits, nor after switching from the originator IFX.

Through Concentration

Five studies evaluated trough levels (TL) of IFX biosimilar (**Table 2**) (Gervais et al., 2018; Kang et al., 2018; Richmond et al., 2018; van Hoeve et al., 2019; Nikkonen and Kolho, 2020). Therapeutic trough values, when reported, were assessed to be in the range of 3–7 mg/L post-induction. When comparing CT-P13 patients to those on originator IFX, there were no significant differences in TL. Likewise, there was no substantial difference in TL changes after switching from originator IFX to CT-P13. Dose escalation or treatment intensification were used to optimize treatment for patients with subtherapeutic levels at baseline (Nikkonen and Kolho, 2020). Switching on immunogenicity has been examined in five pediatric studies (Gervais et al., 2018; Kang et al., 2018; Richmond et al., 2018; van Hoeve et al., 2019; Nikkonen and Kolho, 2020). After switching to the biosimilar CT-P13, it was not found any substantial increase in immunogenicity. When available, mean ADA levels did not differ substantially.

Safety and Immunogenicity

Current available literature data reported only mildly to moderately severe adverse events (AEs) related to the IFX biosimilar. AEs related to IFX biosimilars in pediatric IBD patients were investigated in eight studies (**Table 3**) (Sieczkowska et al., 2016; Sieczkowska-Golub et al., 2017; Chanchlani et al., 2018; Gervais et al., 2018; Kang et al., 2018; Richmond et al., 2018; van Hoeve et al., 2019; Nikkonen and Kolho, 2020). In comparison to patients on originator IFX, CT-P13 patients had no significant differences in AE rates. Similarly, there was no significant difference when switching from originator IFX to CT-P13. Mild infections, predominantly upper respiratory tract infections, were the most commonly reported AEs. Three cases of Herpes zoster reactivation have been documented, one of which occurred after the first infusion of biosimilar IFX and necessitated therapy withdrawal (Sieczkowska et al., 2016). In seven cases, acute infusion reactions (AIRs) were observed, and in three of these, therapy was stopped (Sieczkowska et al., 2016; Sieczkowska-Golub et al., 2017; van Hoeve et al., 2019). During biosimilar treatment, one patient developed an ovarian teratoma (Sieczkowska et al., 2016). There was no information provided on demographics or disease progression. The patient had a total surgical ovary excision between consecutive biosimilar infusions. There was no need to adjust the dose. Cheon et al. (2021) found no additional safety findings in IBD patients treated with CT-P13 for up to 5 years, whether they were treated with or switched to CT-P13. In any case, there was no age-based subgroup analysis.

Costs

Three out of nine studies reported comparison of costs between originator IFX and CT-P13 (**Table 4**) (Chanchlani et al., 2018; Gervais et al., 2018; Richmond et al., 2018). All available data

TABLE 2 | Studies investigating trough levels.

References	Therapeutic range	Method	Time of assessment	Findings
Richmond L et al. (Richmond et al., 2018)	3–7 mg/L	N/A	Post-induction	Median TL 3.85 mg/L in 20/40 patients; level outside therapeutic range in 10/20
Nikkonen A et al. (Nikkonen and Kolho, 2020)	N/A	N/A	Third infusion (a) At any point (b)	Median TL 8.9 mg/L (originator group) and 14 mg/L (biosimilar group) (a) TL < 2 mg/L in 61% of patients (originator group) and in 36% of patients (biosimilar group) (b) No significant difference between the two groups
Kang B et al. (Kang et al., 2018)	≥3 μg/ml	ELISA	1 year	Therapeutic TL in 90.3 and 88.6% of patients in originator and switch group, respectively No significant difference in therapeutic TL between the two groups No significant difference in therapeutic TL or median TL between baseline and 1-year follow up in the switch group
Gervais L et al. (Gervais et al., 2018)	3–7 mg/L	N/A	N/A	No significant changes in TL post-switch
van Hoeve K et al. (van Hoeve et al., 2019)	Lower limit 0.3 mcg/mL, upper limit 12 mcg/mL	ELISA	6 months before (baseline, a) and 6 months after switching (b)	Median TL 5.7 mcg/mL versus 6.5 mcg/mL (no significant difference) No significant difference between the proportion of patients with subtherapeutic levels at baseline or at the last infusion before switching and 6 months after

TL, trough level, ELISA enzyme-linked immunosorbent assay, N/A not available.

TABLE 3 | Reported adverse events.

References	Premedication	AE	Discontinuation	ADA
Sieczkowska-Golub J et al. (Sieczkowska-Golub et al., 2017)	Yes	Upper respiratory tract infection (*n* = 6), AIR (*n* = 2), immediate raised blood pressure (*n* = 1), arthralgia (*n* = 1), Herpes simplex (*n* = 1), Herpes zoster (*n* = 1), pancreatitis (*n* = 1), suspected latent tuberculosis (*n* = 1)	*n* = 1 (AIR)	N/A
Richmond L et al. (Richmond et al., 2018)	N/A	AIR (*n* = 1)	Yes	Positive *n* = 2 at the end of the induction
Chanchlani N et al. (Chanchlani et al., 2018)	N/A	*n* = 2	N/A	N/A
Nikkonen A et al. (Nikkonen and Kolho, 2020)	N/A	Recurrent abscesses (n = 1)	Yes	Positive *n* = 2 at the end of the induction
Sieczkowska J et al. (Sieczkowska et al., 2016)	N/A	AIR (*n* = 3), upper respiratory tract infection (*n* = 7), viral diarrhea (*n* = 2), nausea, headache (*n* = 2), seborrhea (*n* = 1), epistaxis (*n* = 1), conjunctivitis (*n* = 1), pneumonia (*n* = 1), Herpes zoster (*n* = 1)	*n* = 2 (AIR, Herpes zoster)	N/A
Kang B et al. (Kang et al., 2018)	N/A	Upper respiratory tract infection (*n* = 10), acne (*n* = 4), hair loss (*n* = 3), aggravation of perianal fistula (*n* = 3), rash (*n* = 2), arthralgia (*n* = 1), leukopenia (*n* = 2), liver enzyme elevation (*n* = 1), headache (*n* = 1), Herpes zoster (*n* = 1), Norovirus infection (*n* = 1), viral conjunctivitis (*n* = 1)	No	Positive *n* = 2 at baseline, *n* = 2 at 12 months post-switch
Gervais L et al. (Gervais et al., 2018)	N/A	No significant AE reported; no AIR	No	Positive *n* = 16 at baseline, *n* = 8 at 6 months, *n* = 6 at 12 months post-switch
van Hoeve K et al. (van Hoeve et al., 2019)	N/A	AIR (*n* = 1), upper respiratory tract infections (*n* = 25), arthralgia (*n* = 5), gastroenteritis (*n* = 4), headache (*n* = 3), pharyngitis (*n* = 2), otitis media (*n* = 2), sinusitis (*n* = 1), conjunctivitis (*n* = 1), rash (*n* = 1)	*N* = 1 (AIR)	Positive *n* = 1 post-switch (not related to the AIR)

AE, adverse event; ADA, antidrug antibodies; AIR, acute infusion reaction.

TABLE 4 | Cost saving in comparison to treatment with originator.

References	Drugs	Estimated saving	Time period
Richmond L et al. (Richmond et al., 2018)	Remsima© vs. Remicade©	38% average per phial £47,800 (€57,000) for the total number of infusions	12 weeks
Chanchlani N et al. (Chanchlani et al., 2018)	Remsima© or Inflectra© vs. Remicade©	£875,000 (€998,526) for the total number of infusions	1 year
Gervais L et al. (Gervais et al., 2018)	Remsima© vs. Remicade©	£66,000 (€75,900) £1,500 per patient per year	1 year

reported considerable cost reductions from using biosimilar IFX based on estimated and averaged local procurement rates.

DISCUSSION

Overall, the findings of this systematic review article establish CT-P13 effectiveness as measured by clinical response and/or remission rates after induction or during maintenance and suggest that it does not significantly differ from that of the originator IFX.

Because the originator IFX is often no longer available, most IBD units have had to switch to or to start with its biosimilars (de Ridder et al., 2019; Dipasquale and Romano, 2020). Access to the originator IFX is also often limited due to the originator's relatively expensive cost (de Ridder et al., 2019; Hughes et al., 2021). A growing number of children with IBD who have used biologics are being elected to switch to biosimilars (de Ridder et al., 2019; Dipasquale and Romano, 2020). Children with IBD were effectively transitioned from the originator IFX to CTP13 in available studies, without affecting the effectiveness, pharmacokinetics, immunogenicity, and safety (Sieczkowska et al., 2016; Gervais et al., 2018; Kang et al., 2018; van Hoeve et al., 2019). Switching occurred primarily during the maintenance phase and did not appear to be associated with a loss of efficacy over time, even in patients with mild-to-moderate disease activity. Single switches have been used in all studies. Following at least three induction infusions, ESPGHAN guidelines recommend transitioning to CT-P13 in IBD children in clinical remission (de Ridder et al., 2019). Because evidence on interchangeability is still sparse, multiple switches (>1 switch) between different biosimilars or between biosimilars and the originator are not currently advised (de Ridder et al., 2019). Switching to biosimilar IFX might raise the risk of immunogenicity, which is one of the key concerns about biosimilar usage in the pediatric IBD group. Loss of response, AEs, and delayed hypersensitivity responses are all linked to immunogenicity, as well as the formation of IFX ADA (Allez et al., 2010). All biologics have varying degrees of immunogenicity, and even modest variations in the formulation, purity, or packaging of a biological medication might impact its immunogenicity pattern (Gabbani et al., 2017). The findings of this systematic review suggest that biosimilars appear to be safe in pediatric IBD patients. Additionally, switching from the originator drug does not appear to raise immunogenicity considerably.

Biosimilars offer a more advantageous costing and reimbursement strategy, with price cuts ranging from 25 to 70% in Europe when compared to originator products (Brodszky et al., 2016). More than 90% of respondents in the previously cited Italian survey believed biosimilars to be cost-effective, with cost savings being the most important benefit of using biosimilars (Dipasquale et al., 2021). If the cost savings from the use of biosimilars were used to fund more biological treatments, several more IBD patients could be treated.

The extensive and systematic literature search is one of the strengths of this systematic review. The limitations largely reflect the shortcomings of the studies reviewed. First, they are observational studies reporting real-life data. Second, some of the included studies were limited with respect to sampling and generalizability. Moreover, the efficacy of biosimilars in the induction of mucosal healing was not investigated. Pediatric clinical trials and eventually more research into post-marketing surveillance data on effectiveness, safety, and immunogenicity are highly needed. Data on the efficacy and safety of adalimumab biosimilars in children with IBD are also warranted.

CONCLUSION

More experiences regarding the effectiveness, immunogenicity, and interchangeability of biosimilars in pediatric IBD have been reported over the last few years. Their utilization has almost completely substituted that of the originator IFX due to greater availability and lower costs. There are no differences in efficacy and safety between originator IFX and CT-P13, according to current evidence. Nonetheless, regulatory legislation needs to be standardized, and more data on the interchangeability, pharmacokinetics, as well as pediatric specificities, are still desirable.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.846151/full#supplementary-material

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Effectiveness of Sodium Bicarbonate Infusion on Mortality in Critically Ill Children With Metabolic Acidosis

Huabin Wang[1,2†], Rui Liang[3†], Tianqi Liang[1,2], Songyao Chen[4], Yulong Zhang[5], Lidan Zhang[1,2] and Chun Chen[1,2*]*

[1]Division of Hematology/Oncology, Department of Pediatrics, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China, [2]Department of Pediatric Intensive Care Unit, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China, [3]Department of Colorectal Surgery, The Third Affiliated Hospital of Kunming Medical University, Kunming, China, [4]Center of Digestive Disease, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China, [5]Scientific Research Center, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China

Objective: Metabolic acidosis often occurs in the paediatric intensive care unit (PICU). Although sodium bicarbonate (SB) has been widely used in paediatrics, data on the effect of SB on children with metabolic acidosis in the PICU are scarce.

Methods: Patients with metabolic acidosis who were treated with SB within 48 h of PICU admission were screened. Multivariate logistic regression, subgroup analysis, and propensity score matching (PSM) were used to investigate the relationships between SB infusion and clinical outcomes.

Results: A total of 1,595 patients with metabolic acidosis were enrolled in this study. In the multivariate logistic regression model, SB infusion was not correlated with in-hospital mortality (odds ratio (OR) 0.87, 95% confidence interval (CI) 0.47–1.63, $p = 0.668$), but was significantly correlated with hypernatraemia (OR 1.98, 95% CI 1.14–3.46, $p = 0.016$), hypokalaemia (OR 2.01, 95% CI 1.36–2.96, $p < 0.001$), and hypocalcaemia (OR 4.29, 95% CI 2.92–6.31, $p < 0.001$). In the pH value, lactate level, acute kidney injury level, age grouping, and anion gap level subgroups, the ORs for SB and in-hospital mortality were not statistically significant. After PSM, the results remained unchanged.

Conclusion: SB infusion does not reduce the in-hospital mortality of severely ill children with metabolic acidosis and increases the risk of hypernatraemia, hypokalaemia, and hypocalcaemia. More effort should be focused on eliminating the causes of metabolic acidosis rather than SB infusion.

Keywords: sodium bicarbonate, pediatric intensive care unit, metabolic acidosis, prognostic value, cohort study

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****Correspondence:***
Lidan Zhang
xinxian1116@163.com
Chun Chen
chenchun69@126.com

†These authors share first authorship

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INTRODUCTION

Metabolic acidosis is a disorder of the body's acid-base balance characterized by a primary decrease in plasma bicarbonate concentration (BC) caused by an increase in hydrogen ions or a loss of bicarbonate in the extracellular fluid (Fujii et al., 2019). Metabolic acidosis can be divided into lactic acidosis, ketoacidosis, hyperchloremic acidosis and so on and has a high incidence in the paediatric intensive care unit (PICU) (Kraut, 2018). Diseases such as sepsis, severe hypoxemia, and cardiogenic shock are the main causes of metabolic acidosis (Velissaris et al., 2015; Haas et al., 2016). Metabolic

acidosis can cause haemodynamic instability, decrease myocardial contractility and arterial vasodilation, decrease the cellular oxygen supply and mitochondrial oxygen consumption, impair responsiveness to catecholamines, and induce insulin resistance, leading to increased mortality (Schotola et al., 2012; Kraut and Madias, 2014).

At present, the most basic treatment for metabolic acidosis is to eliminate the underlying causes, such as treating infections and increasing the oxygen supply. However, some institutions have used sodium bicarbonate (SB) or other alkaline drugs after ineffective treatments. Theoretically, SB infusion can neutralize excess acid, thereby restoring the blood pH (Loomba et al., 2020). However, several studies have shown that SB infusions in critically ill patients with metabolic acidosis do not reduce mortality and increases the risk of adverse reactions. It is highly controversial whether SB should be infused in critically ill patients with metabolic acidosis. Existing studies mostly focus on adults, and there have been few reports on severely ill children; therefore, PICU physicians have no clear guidance regarding the use of SB. As such, the purpose of this study was to evaluate whether SB infusion could improve the prognosis of critically ill children with metabolic acidosis.

MATERIALS AND METHODS

Database Introduction

The Paediatric Intensive Care (PIC) database is a completely open, single-centre, bilingual Chinese-English database developed by the Children's Hospital of Zhejiang University School of Medicine (Zeng et al., 2020). It is a database specifically for PICU patients and includes diagnostic, testing, and monitoring data for non-adult patients aged 0–18 years from multiple intensive care units (ICUs). This database contains information regarding 13,499 hospital stays for 12,881 paediatric patients. The PIC database is based on the widely used Medical Information Mart for Intensive Care (MIMIC) database and can be downloaded and used after registration, application, and certification. The data used in this study were collected in adherence to Health Insurance Portability and Accountability Act (HIPAA) standards. This project was approved by the Institutional Review Committee of the Children's Hospital of Zhejiang University School of Medicine and exempt from obtaining patient informed consent.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) patients between 1 month and 18 years old; 2) if a patient was hospitalized multiple times, only information from the first hospitalization was selected; and 3) patients with metabolic acidosis in the first 48 h of ICU admission (pH < 7.35, BC < 22 mmol/L).

The exclusion criteria were as follows: 1) patients in the neonatal intensive care unit; 2) lack of chart event data in the database; 3) patients undergoing cardiac surgery; and 4) patients with combined respiratory acidosis (partial pressure of carbon dioxide ($PaCO_2$) > 50 mmHg).

If pH, BC, and $PaCO_2$ were measured multiple times within the first 48 h of ICU entry, the minimum pH and BC values and the maximum $PaCO_2$ value during this period were used.

Data Extraction and Definition of the Primary and Secondary Outcomes

The extracted demographic information included age, sex, and type of ICU admission; experimental variables included white blood cell (WBC) count, platelet (PLT) count, activated partial thromboplastin time (APTT), anion gap, and lactic acid concentration, and the initial values at ICU admission were used for the above variables. Comorbidities included anaemia, hypertension, acute kidney injury (AKI), liver dysfunction, and ketoacidosis. All comorbidities were diagnosed within 48 h of ICU admission. We used the pROCK criterion, which defines AKI as an increase in creatinine levels of ≥20 μmol/L and ≥30% within 7 days (Xu et al., 2018). The pROCK classifies AKI stages 2 and 3 as creatinine increases of ≥40 μmol/L and ≥60% and ≥80 μmol/L and ≥120%, respectively. The diagnostic criteria of other comorbidities are provided in **Supplementary Table S1**. The percentage of missing values of each variable was less than 5%; therefore, the mean or median was used to replace the missing value.

The primary outcome measure was in-hospital mortality, defined as death during hospitalization. The secondary outcome measures included hypernatraemia, hypokalaemia, hypocalcaemia, hospitalization length of stay (LOS) and 30-days mortality. Only hypernatraemia (serum sodium >150 mmol/L), hypokalaemia (serum potassium <3.5 mmol/L), and hypocalcaemia (free calcium <1.2 mmol/L) that occurred after the use of SB were counted. The maximum sodium value and minimum potassium and calcium values were selected.

Statistical Analysis

Continuous variables with a normal distribution are presented as the mean ± standard deviation, and continuous variables with a nonnormal distribution are presented as the median (quartile). Student's t test, the Wilcoxon rank sum test, or the chi-squared test was used to compare differences between two groups. A backward stepwise method was used to screen the covariates for multivariate logistic regression. Considering that pH, lactate, and AKI are important factors that affect the use of SB in clinical practice and that different physiopathological situations may arise at different ages and anion gaps, the above factors were used for subgroup analysis. To verify the interaction between SB and these variables, multiplicative interaction terms were incorporated in the regression model.

Propensity score matching (PSM) was used to minimize the influence of confounding factors that may cause bias in the results. PSM scores were assigned based on the probability of patients receiving SB treatment and were estimated using a multivariate logistic regression model. The 1:1 nearest neighbour matching algorithm was used for matching, and the caliper value was set to 0.05. Based on **Table 1**, the following variables were selected to generate the PSM score: age, ICU type, APTT, anion gap, anaemia, hypertension, and AKI.

TABLE 1 | Comparisons of the baseline characteristics between patients with or without sodium bicarbonate use.

Variable	All patients (n = 1,595)	Non-SB group (n = 1,045)	SB group (n = 550)	*P*
Age, months	17 (5–50)	17 (5–52)	16 (5–43)	0.593
Age, n (%)	—	—	—	0.077
<12 months	692 (43.4)	452 (43.3)	240 (43.6)	—
≥12 months and <60 months	598 (35.6)	365 (34.9)	203 (36.9)	—
≥60 months and <120 months	207 (13.0)	131 (12.5)	76 (13.8)	—
≥120 months	128 (8.0)	97 (9.3)	31 (5.6)	—
Male, n (%)	904 (56.7)	598 (57.2)	306 (55.6)	0.543
ICU type, n (%)	—	—	—	0.033
PICU	417 (26.1)	291 (27.8)	126 (22.9)	—
SICU	1,178 (73.9)	754 (72.2)	424 (77.1)	—
Laboratory data	—	—	—	—
WBC (<4 or >12, 10^9/L)	679 (42.6)	435 (41.6)	244 (44.4)	0.293
Platelet (<100, 10^9/L)	100 (6.3)	66 (6.3)	34 (6.2)	0.916
Lactate (≥2.0, mmol/L)	625 (39.2)	412 (39.4)	213 (38.7)	0.786
APTT (>45, s)	246 (15.4)	186 (17.8)	60 (10.9)	<0.001
PH (<7.2)	81 (5.1)	56 (5.4)	25 (4.5)	0.482
BC (<14, mmol/L)	173 (10.8)	113 (10.8)	60 (10.9)	0.953
Anion gap (8–16, mmol/L)	510 (32.0)	404 (38.7)	106 (19.3)	<0.001
Comorbidities, n (%)				
Anemia	1,093 (68.5)	693 (66.3)	400 (72.7)	0.009
Hypertension	349 (21.9)	183 (17.5)	166 (30.2)	<0.001
AKI	181 (11.3)	133 (12.7)	48 (8.7)	0.017
AKI stage ≥2	48 (3.0)	16 (1.5)	32 (5.8)	0.865
Liver dysfunction	309 (19.4)	198 (18.9)	111 (20.2)	0.553
Diabetic ketoacidosis	117 (7.3)	76 (7.3)	41 (7.5)	0.895
Clinical outcome				
Hypernatremia	59 (3.7)	31 (3.0)	28 (5.1)	0.033
Hypokalaemia	121 (7.6)	64 (6.1)	57 (10.4)	0.002
Hypocalcemia	135 (8.5)	51 (4.9)	84 (15.3)	<0.001
Hospital LOS (day)	11 (7–18)	11 (7–18)	12 (6–19)	0.667
30 days mortality, n (%)	58 (3.6)	40 (3.8)	18 (3.3)	0.574
Hospital mortality, n (%)	61 (3.8)	44 (4.2)	17 (3.1)	0.268

AKI, acute kidney injury; APTT, activated partial thromboplastin time; BC, bicarbonate concentration; LOS, length of stay; PICU, pediatric intensive care unit; SB, sodium bicarbonate; SICU, surgery intensive care unit; WBC, white blood cell.

A p value <0.05 was considered statistically significant. Statistical analysis was performed using STATA (V.16), SPSS (V.24), and R (V.3.6.3).

RESULTS

Baseline Characteristics

A total of 1,595 patients were enrolled; the study flowchart is shown in **Figure 1**. **Table 1** provides the baseline characteristics data. The overall median age was 17 months. A total of 56.7% of patients were male, and the in-hospital mortality was 3.8%. Comparisons between the SB and non-SB groups indicated that there were significant differences in ICU type, APTT, anion gap, anaemia, hypertension, and AKI. The occurrence of hypernatraemia (5.1 vs. 3.0%, $p = 0.033$), hypokalaemia (10.4 vs. 6.1%, $p = 0.002$), and hypocalcaemia (15.3 vs. 4.9%, $p < 0.001$) in the SB group was higher than that in the non-SB group. However, there were no significant differences between the two groups in LOS, 30-days mortality, or in-hospital mortality.

Primary Outcome

The results of univariate and multivariate logistic regressions with in-hospital mortality as the outcome variable are shown in **Table 2**. Regardless of univariate analysis or multivariate analysis, there was no significant correlation between SB use and in-hospital mortality. In the multivariate analysis, ICU type, lactate level, APTT, and AKI were independent risk factors for in-hospital death. Subgroup analysis was performed based on pH, lactate level, AKI, age and anion gap (**Figure 2**). When constructing a logistic regression model for the subgroup with age ≥120 months, the small number of deaths in this subgroup precluded statistical analysis. Therefore, we set age ≥60 months as a subgroup. The odds ratios (ORs) for SB and in-hospital death in the different subgroups were not statistically significant. In addition, the *p-interaction* between SB and pH, lactate, AKI, age, or anion gap was greater than 0.05.

Secondary Outcomes

Multivariate logistic regression was performed using hypernatraemia, hypokalaemia, hypocalcaemia, LOS, and 30-day mortality as the outcome variables, as shown in **Table 3**. LOS was converted to a

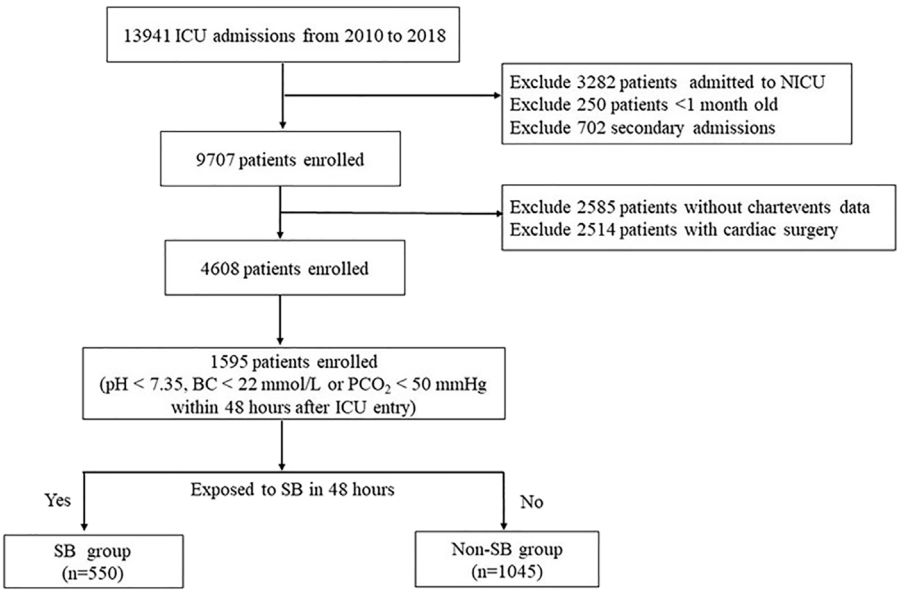

FIGURE 1 | Flow diagram of patient recruitment. NICU, neonatal intensive care unit; SB, sodium bicarbonate.

dichotomous variable based on the median value (11.7 days). The use of SB was associated with an increased risk for hypernatraemia (OR 1.98, 95% CI 1.14–3.46, $p = 0.016$), hypokalaemia (OR 2.01, 95% CI 1.36–2.96, $p < 0.001$) and hypocalcaemia (OR 4.29, 95% CI 2.92–6.31, $p < 0.001$). The OR values for other covariates are provided in **Supplementary Table S2**.

PSM Results

Utilizing the 1:1 matching algorithm, there were 518 matched pairs (**Table 4**). The overall quality of the matched samples was assessed by examining the propensity scores between groups (**Supplementary Figure S1**). All covariates were balanced between the two groups. There were no significant differences in in-hospital mortality, 30-days mortality, or LOS between the two groups; however, in the SB group, there were increased incidences of hypernatraemia (5.4 vs. 2.7%, $p = 0.027$), hypokalaemia (10.6 vs. 6.0%, $p = 0.007$), and hypocalcaemia (15.8 vs. 6.2%, $p < 0.001$).

Sensitivity Analysis

To test the reliability of the results after PSM, different covariates were included for the sensitivity analysis. As seen in **Table 2**, the relationships between the use of SB and clinical outcomes were

TABLE 2 | Logistic regressions of sodium bicarbonate use for in-hospital mortality.

	Crude OR	95% CI	*P*	Adjusted OR	95% CI	P
Age						
<12 months		Ref.-			Ref	
≥12 months and <60 months	1.18	0.67–2.08	0.576	1.26	0.67–2.37	0.472
≥60 months and <120 months	1.07	0.48–2.42	0.866	1.12	0.46–2.69	0.805
≥120 months	0.86	0.29–2.52	0.784	0.58	0.18–1.83	0.351
Gender (female)	0.84	0.50–1.42	0.523	0.67	0.39–1.17	0.160
ICU type (PICU)	6.31	3.65–10.91	<0.001	3.54	1.96–6.42	<0.001
WBC (<4 or >12, 10^9/L)	2.00	1.19–3.36	0.009	1.54	0.88–2.67	0.127
Platelet (<100, 10^9/L)	3.57	1.80–7.10	<0.001	1.45	0.66–3.18	0.358
Lactate (≥2.0, mmol/L)	6.12	3.29–11.40	<0.001	4.39	2.32–8.31	<0.001
APTT (>45, s)	3.83	2.25–6.53	<0.001	2.30	1.30–4.06	0.004
Anion gap (8–16, mmol/L)	1.61	0.96–2.71	0.071	1.04	0.58–1.84	0.903
Anemia	0.94	0.54–1.62	0.822	0.87	0.48–1.57	0.635
Hypertension	0.45	0.20–1.00	0.051	0.54	0.23–1.23	0.142
Acute kidney injury	4.88	2.82–8.44	<0.001	2.26	1.24–4.11	0.007
Liver dysfunction	3.53	2.09–5.94	<0.001	1.50	0.83–2.70	0.180
Diabetic ketoacidosis	2.29	1.10–4.76	0.027	1.19	0.53–2.66	0.673
Sodium bicarbonate use	0.73	0.41–1.28	0.270	0.87	0.47–1.63	0.668

APTT, activated partial thromboplastin time; CI, confidence interval; OR, odds ratio; PICU, pediatric intensive care unit; WBC, white blood cell.

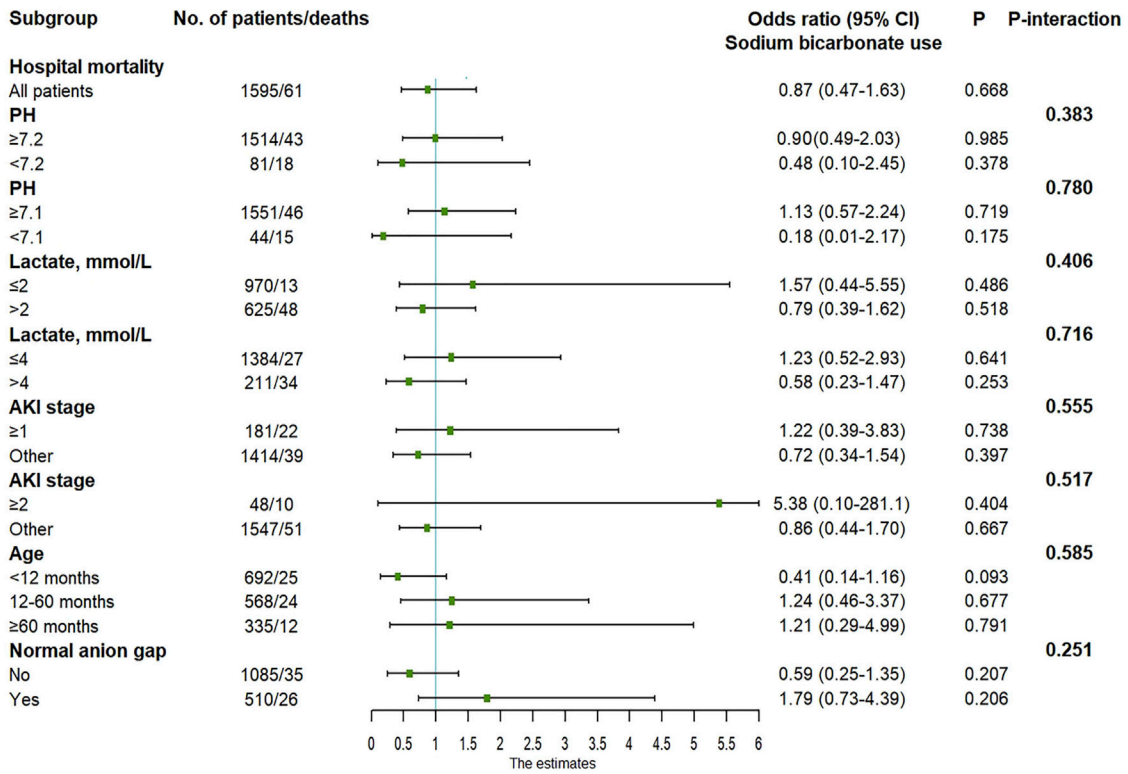

FIGURE 2 | Adjusted odds ratio in the subgroup analysis. AKI, acute kidney injury; CI, confidence interval.

TABLE 3 | Multivariate logistic regression of sodium bicarbonate infusion for secondary outcome.

Secondary outcome	OR	95% CI	*P*
Hypernatremia	1.98	1.14–3.46	0.016
Hypokalaemia	2.01	1.36–2.96	<0.001
Hypocalcemia	4.29	2.92–6.31	<0.001
Longer hospital LOS (>11.3 days)	0.96	0.77–1.20	0.726
30 days mortality	1.03	0.55–1.90	0.937

CI, confidence interval; LOS, length of stay; OR, odds ratio.

confounded to some extent by some experimental variables, such as WBC count, PLT count, and lactate level. Therefore, these three variables were added for PSM; there were no significant differences in the covariates after PSM (**Supplementary Table S3**, **Supplementary Figure S2**), and the results remained stable.

DISCUSSION

The main purpose of this study was to evaluate the effect of SB infusion on the prognosis of PICU patients with metabolic acidosis. To the best of our knowledge, this is the largest study on this subject ever conducted in a PICU. The results indicated that SB infusion was not correlated with changes in mortality in critically ill children with metabolic acidosis, even in subgroups with pH < 7.1, AKI and hyperlactacidaemia. Additionally, SB infusion was associated with an increased risk of hypernatraemia, hypokalaemia, and hypocalcaemia.

Currently, whether SB infusion improves survival in patients with metabolic acidosis is debated. Several studies have shown that SB treatment does not improve patient prognosis but increases the incidence of adverse reactions and even mortality. Loomb et al. conducted a systematic review and meta-analysis, which included 341 children from six studies, to determine whether SB treatment can improve haemodynamics, gas exchange, and blood oxygen saturation in infants with metabolic acidosis (Loomba et al., 2020). The results suggested that the use of SB did not improve oxygen saturation as measured by pulse oximetry, heart rate, blood pressure (BP), pH, or oxygen partial pressure. Kim et al. retrospectively analysed 103 patients with lactic acidosis and found that the use rate of SB in the deceased group was higher than that in the survival group (p = 0.006); multivariate logistic regression suggested that the administration of SB was an independent risk factor for in-hospital death (OR 6.27, 95% CI 1.1–35.78) (Kim et al., 2013). In terms of basic research, there are two experiments with dogs with lactic acidosis. Compared with those in dogs in the control group, the blood pH and mortality did not improve in dogs that received SB treatment; however, BP and cardiac output decreased (Arieff et al., 1982; Graf et al., 1985).

TABLE 4 | Comparisons of the covariates after propensity score matching.

Variable	All patients (n = 1,036)	Non-SB group (n = 518)	SB group (n = 518)	*P*
Age, months	17 (5–44)	17 (5–43)	17 (5–47)	0.525
ICU type, n (%)	—	—	—	1.000
PICU	240 (23.2)	120 (23.2)	120 (23.2)	—
SICU	796 (76.8)	398 (76.8)	398 (76.8)	—
APTT (> 45, s)	115 (11.1)	55 (10.6)	60 (11.6)	0.621
Anion gap (8–16, mmol/L)	207 (20.0)	101 (19.5)	106 (20.5)	0.698
Comorbidities, n (%)				
Anemia	735 (71.0)	367 (70.9)	368 (71.0)	0.945
Hypertension	261 (25.2)	127 (24.5)	134 (25.9)	0.616
Acute kidney injury	92 (8.9)	44 (8.5)	48 (9.3)	0.662
Clinical outcome				
Hypernatremia	42 (4.1)	14 (2.7)	28 (5.4)	0.027
Hypokalaemia	86 (8.3)	31 (6.0)	55 (10.6)	0.007
Hypocalcemia	114 (11.0)	32 (6.2)	82 (15.8)	<0.001
Hospital LOS (day)	12 (7–19)	12 (7–18)	12 (6–19)	0.767
30 days mortality, n (%)	39 (3.8)	22 (4.3)	17 (3.3)	0.414
Hospital mortality, n (%)	40 (3.9)	24 (4.6)	16 (3.1)	0.197

APTT, activated partial thromboplastin time; LOS, length of stay; PICU, pediatric intensive care unit; SB, sodium bicarbonate; SICU, surgery intensive care unit.

It seems intuitive to add an alkaline agent to acidic blood to increase the pH; however, the actual treatment approach is much more complicated (Quade et al., 2021). The inability of SB to improve the prognosis of children with metabolic acidosis can be explained as follows. First, although SB infusion can increase blood pH in a short period of time (Mintzer et al., 2015), it can also increase CO_2 production and aggravate intracellular acidosis in children with severe circulatory failure (Kim et al., 2013; Collins and Sahni, 2017). Second, SB treatment can lead to fluid overload and electrolyte disorders, including hypocalcaemia, which in turn affects the function of the heart and vascular smooth muscle (Kimmoun et al., 2015; Drumheller and Sabolick, 2021), resulting in cerebral and cardiovascular haemodynamic fluctuations and functional abnormalities (Aschner and Poland, 2008; Berg et al., 2010; Katheria et al., 2017). Studies have reported that SB infusion can increase the risk of cerebral oedema in paediatric patients with diabetic ketoacidosis (Glaser et al., 2001). Third, SB infusion may change the oxyhaemoglobin saturation relationship and increase the production of lactic acid in anaerobic glycolysis (Forsythe and Schmidt, 2000). The results of this study are consistent with the above view that SB cannot reduce the mortality of children with metabolic acidosis and that SB treatment causes hypernatraemia, hypokalaemia, and hypocalcaemia.

As described above, many studies have shown that patients with metabolic acidosis do not benefit from SB infusion. However, some patients with specific diseases or severe conditions may benefit from SB infusion. In a multicentre, phase III randomized controlled trial (RCT) that included 389 patients with severe metabolic acidaemia (pH ≤ 7.20), there was no significant difference in the survival rate at day 28 between the control group and the SB group. However, for patients with an AKI network score of two to three points, the survival rate in the SB group was higher than that in the control group on day 28 (63 vs. 46%, $p = 0.028$) (Jaber et al., 2018). Zhang et al. retrospectively analysed 1718 sepsis patients with metabolic acidosis in the MIMIC database (500 patients in the SB group and 1,218 patients in the non-SB group) (Zhang et al., 2018). The results indicated that there was no significant difference in mortality between the two groups overall (hazard ratio (HR) 1.04, 95% CI 0.86–1.26, $p = 0.670$) but that SB use benefitted the population with grade 2 or three AKI and pH < 7.2 (HR 0.74, 95% CI 0.51–0.86, $p = 0.021$). However, in this study, for PICU patients, SB infusion did not benefit patients in the severe acidosis (pH < 7.2 or pH < 7.1) and AKI subgroups. Notably, the causes of metabolic acidosis or AKI in children are different from those in adults, and different aetiologies may cause different patient responses to SB treatment (Joannes-Boyau and Forni, 2018).

Our research presents some advantages. Thus far, this is the largest study on the efficacy of SB in the PICU; therefore, enough confounding variables were included, and subgroup analysis was performed. However, this study also has limitations. First, this is a retrospective study. Although PSM and sensitivity scores were used to balance the important confounding factors, there may still be some unknown confounding factors that affected the results. Second, multiple subgroup analyses were conducted, but the small number of cases in the pH < 7.1 subgroup may lead to false negative results. Third, due to limitations with respect to the database, data for some treatments were not available, such as balanced salt solution and haemodialysis, which would affect the acid-base balance of patients. Fourth, because of database limitations, we could not precisely classify

the type of metabolic acidosis in each patient. For example, organic acidemias are mostly diagnosed by some sophisticated laboratory tests, such as tandem mass spectrometry and gas chromatography–mass spectrometry, which are not available in databases. Therefore, we could not clarify whether the patient had organic acidemia.

CONCLUSION

Acidaemia in critically ill children is a common concern of paediatricians. Based on the conclusions of this study, SB infusion may not be beneficial. More effort should be focused on eliminating the causes of metabolic acidosis rather than SB infusion. Notably, RCTs targeting specific disease populations and different types of acidosis are needed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Committee of the Children's Hospital of Zhejiang University School of Medicine. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LZ and CC conceptualized and designed the study and reviewed and revised the manuscript. HW coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript. RL carried out the analyses, interpreted the results and drafted the initial manuscript. TL, SC, and YZ contributed to data collection and reviewed and revised the manuscript. All authors contributed to manuscript revision and read and approved the submitted version. HW and RL contributed equally to this work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.759247/full#supplementary-material.

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SYSTEMATIC REVIEW
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Efficacy of Systemic Biologic Drugs in Pediatric Psoriasis: Evidence From Five Selected Randomized Clinical Trials

*Vito Di Lernia[1], Laura Macca[2], Lucia Peterle[2], Ylenia Ingrasciotta[3], Gianluca Trifirò[4] and Claudio Guarneri[5]**

[1]Dermatology Unit, Arcispedale S. Maria Nuova, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy, [2]Department of Clinical and Experimental Medicine, Section of Dermatology, University of Messina, Messina, Italy, [3]Department of Biomedical and Dental Sciences and Morpho-functional Imaging, Section of Pharmacology, University of Messina, Messina, Italy, [4]Department of Diagnostics and Public Health, Section of Pharmacology, University of Verona, Verona, Italy, [5]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Section of Dermatology, University of Messina, Messina, Italy

Background: Psoriasis is a chronic, immune-mediated skin disease that may occur at any age. Prevalence in children ranges between 0.5 and 1.0% across Europe. Approximately 10–20% of paediatric psoriasis patients are moderate-to-severe in severity and may require the use of systemic therapy.

Objective: Recently, newer targeted, systemic therapies have been licensed for treatment of moderate-to-severe paediatric psoriasis. The objective of this study was to evaluate the short-term efficacy of available antipsoriatic systemic drugs in children with a narrative synthesis of key efficacy from randomized clinical trials.

Methods: A systematic review of literature was performed on Medline and embase databases and the Cochrane Central Register of Controlled Trials. Randomized clinical trials investigating the efficacy of treatments licensed by the US Food and Drug Administration and/or the European Medicines Agency for paediatric and adolescent psoriatic population were retrieved and analyzed. Data from this literature review was assessed in line with GRADE (grading of recommendations, assessment, development and evaluations). The short-term (12-16 weeks) clinical efficacy from baseline was evaluated according to the Psoriasis Area and Severity Index (PASI) 75 and 90 compared to baseline. Illustrative comparative risks, relative risk (RR) and the number needed to treat (NNT) for response on PASI 75 and PASI 90 were extracted.

Results: A total of five relevant studies were identified on two TNF-alpha blockers (etanercept and adalimumab), the IL12/23 inhibitor ustekinumab and two IL-17 inhibitors (ixekizumab, secukinumab). Comparators were placebo (3 studies), placebo and etanercept (1 study) methotrexate (1 study). All examined drugs resulted efficacious. The probability to achieve PASI 75 and PASI 90 was higher for the IL-12/23 and IL-17 inhibitors. Overall, the anti-IL17s and the anti-IL12/23 antibodies showed a more favourable NNT for PASI 75, whereas IL-17 inhibitors for PASI 90.

Conclusion: The approved biological therapies may be beneficial for the treatment of moderate to severe plaque psoriasis in children and adolescents. Since psoriasis is a

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****Correspondence:***
Claudio Guarneri
cguarneri@unime.it

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chronic and often challenging condition with no definitive solution, systematic evaluations of long-term efficacy, drug survival and adverse effects may help careful, individualized, patient-centered clinical decision making.

Keywords: psoriasis, paediatric, childhood, treatment, therapy

INTRODUCTION

Psoriasis is a chronic inflammatory cutaneous disorder affecting 2-4% of the world's population (Parisi et al., 2013; Ingrasciotta et al., 2021). Onset of psoriasis in infancy and adolescence is relatively common. Prevalence among children ranges between 0.5 and 1% across Europe and the median in individuals younger than 18 years is about 0.7% (Augustin et al., 2010). Psoriasis can appear at any age and all age groups may be affected (Augustin et al., 2010; Tollefson et al., 2010). The prevalence rates of the disease in childhood and adolescence are expected to increase in an approximately linear manner from the age of 1 year to the age of 18 years. The median prevalence in individual younger than 18 years is about 0.7% (Augustin et al., 2010). Therefore, onset of psoriasis in infancy and adolescence is relatively common (Raychaudhuri and Gross, 2000).

Although psoriasis severity in children is mild in the majority of patients, moderate to severe psoriasis can and do occur in children as well. Disease severity assessment is similar to adults. The Psoriasis Area and Severity Index (PASI), and the Body Surface Area (BSA) are the currently available tools for measurement of psoriasis severity in childhood as well (Spuls et al., 2010; Cannavò et al., 2017; Lavaud and Mahé, 2019). Skin symptoms are associated with significant impact on quality of life (QOL) for the patients and their parents and carers (Kim and Fischer, 2021). The Children Dermatology Life Quality Index (CDLQI) is considered a validated tool for measuring the impact of psoriasis in children. The majority of cases of psoriasis is mild and easily managed with topical treatments. Children with extensive lesions require systemic treatment and/or phototherapy. Validated definition of "severe disease" include the presence of at least one of the following criteria: PASI >10, BSA >10, CDLQI >10, according to the so-called "rule of tens" (Finlay, 2005). In addition, the involvement of sensitive and visible skin areas with high impact on QOL, as face, hands and feet, nails, intertriginous areas must be taken into account when evaluating the clinical severity of the disease (Fortina et al., 2017; Gisondi et al., 2021).

Severe psoriasis requires continuous systemic treatment with an effective and safe long-term therapy in consideration of the long-lasting natural history of the disease (Marcianò et al., 2020; Talamonti et al., 2020). However, the treatment of severe psoriasis in children may be challenging. Despite the lack of evidence for the non-biologic therapeutic options for paediatric psoriasis, these therapies have been featured prominently in the armamentarium of clinicians and a number of case series have been published (van Geel et al., 2015; Di Lernia et al., 2016a; Di Lernia et al., 2016b; Charbit et al., 2016; Di Lernia et al., 2017). This management may be driven by the extrapolation of evidence in adult psoriasis, the perceived risk-benefit profile of these therapies and the lack of the availability of medicines with an appropriate label for paediatric use. Indeed, methotrexate, cyclosporin, acitretin have not been evaluated formally among children and adolescents and are not licensed for paediatric psoriasis. Two TNF-alpha blockers, namely etanercept and adalimumab, were approved by European Medicine Agency (EMA) for the treatment of severe psoriasis in children (>6 years for etanercept, > 4 years for adalimumab), while only one of them, etanercept, was approved by the United States (US) Food and Drug Administration (FDA) in children >4 years. Ustekinumab, an anti-IL12/23 antagonist, was firstly been approved by the EMA and FDA for treatment of moderate to severe plaque psoriasis for children ≥12 years old. After the evaluation of the safety, efficacy, and pharmacokinetics in an open-label, single-arm, study in younger patients, (Philipp et al., 2020), ustekinumab use was expanded by the EMA and FDA also in patients from 6 to 11 year-old of age (Cvenkel and Starbek Zorko, 2021). More recently two biologic drugs targeting the IL-17A pathway, secukinumab and ixekizumab, were approved by the EMA and FDA as new treatment options for the treatment of moderate to severe psoriasis in patients aged 6–17 years.

The aim of this systematic review is to summarise the evidence on the efficacy of systemic treatments for moderate-to-severe psoriasis in children and adolescent patients based on randomized controlled trials (RCTs). This revision of studies does not focus on safety issues. Invaluable data about will be generated by long term observations, post marketing reviews and registries, since information on really relevant data cannot be drawn by RCTs.

MATERIALS AND METHODS

We conducted a systematic literature review of RCTs for the evaluation of treatments for moderate-to-severe psoriasis in children and adolescents. This study was organized according to the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement (Moher et al., 2015).

Literature Search

A literature search was implemented to identify pertinent articles published in PubMed, embase, and the Cochrane Central Register of Controlled Trials. The main search terms were "psoriasis" and "paediatric". The literature search was limited to English and French language and human studies. In addition, the references of these articles were also examined for additional related articles.

Study Selection

The inclusion and exclusion criteria were defined before the search. Eligible trials were required to 1) be randomized clinical trials (RCT) on treatments for moderate-to-severe

chronic plaque psoriasis; 2) have study participants younger than 18 years of age; 3) evaluate pharmacologic interventions limited to systemic drugs licensed by the EMA or US FDA; and 4) examine the efficacy and safety of antipsoriatic drugs for plaque psoriasis as measured by changes from baseline in the PASI and indicate the proportions of patients who achieved at least a 75% reduction in PASI by the end of the primary response period (short-term: 12–16 weeks from baseline). As comparators, we accepted any other type of management for psoriasis, including placebo or active surveillance.

We accepted studies published in English and French without date restrictions. Non-randomized trials were excluded. Studies were not included if they were only available as abstracts from conference proceedings or if published in a language other than English and French, or if they were long-term extensions or analysis of already selected studies. As this is a review of previously published results of clinical trial data, no institutional board review was required. This article is structured on previously published studies and does not include any new studies with human participants performed by any of the authors.

Types of Outcome Measures

Investigator-assessed improvement: proportion of participants achieving PASI 75 and PASI 90.

Selection of Studies

Two review authors (VD and CG) independently checked abstracted data using a predefined data extraction form for all included studies. The full text of potentially relevant studies for assessment was retrieved. Both review authors independently judged if, from reading the full text, each study met the predefined selection criteria.

Data Extraction and Analysis

Two review authors (VD and CG) performed data extraction. The following information was extracted from each study: author, year of publication, study type, study time frame, type of population (minimal age); sample size; the specific active treatment and comparator treatment employed as well as the dosing and regimens; primary endpoints. Measures of treatment effect were considered the proportion of participants obtaining a PASI 75 and a PASI 90. For dichotomous variables we expressed the results as risk ratios (RRs) and 95% confidence interval (CI). In addition, we calculated the number needed to treat (NNT) which represents the number of participants needed to be treated to achieve one additional positive outcome relative to the control group. We expressed the NNT for each treatment relative to placebo by the end of the primary assessment period. We summarized the included reports through descriptive analyses to provide an overview of studies' characteristics, quality, effectiveness of the treatment investigated.

RESULTS

Results of the Search

We identified 3,099 articles matching the search criteria (**Figure 1**). After removing duplicates, 490 articles remained and were screened by title and abstract. Of the 79 articles that underwent full text screening, we retained five articles (Paller et al., 2008; Landells et al., 2015; Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021). Of these, all examined the efficacy of five biological drugs in the treatment of moderate-to severe psoriasis of children and/or adolescents. Basically, each of these five drugs has been evaluated in one study. The process of selecting articles for inclusion in this review is shown graphically in the flow diagram in **Figure 1**. The strengths of recommendations ratings, and the respective symbols used are summarised in **Table 1**.

Baseline characteristics of the included studies are summarized in **Table 2**. Participants had stable moderate to severe plaque psoriasis at screening, defined as a PASI score equal to or greater than 12 in two studies (etanercept and ustekinumab trials), equal to or greater than 20 in the remaining three (adalimumab, secukinumab, ixekizumab trials); stable disease; PGA of at least three in two studies (etanercept and ustekinumab trials) or of at least four in the remaining three trials (adalimumab, secukinumab, ixekizumab trials); BSA involvement of at least 10% (etanercept, ustekinumab, secukinumab trials) or of at least 20% (adalimumab trial); a history of psoriasis in the last 6 months. The presence of psoriatic arthritis in enrolled patients was mentioned in three studies (Paller et al., 2008; Papp et al., 2017; Bodemer et al., 2021), but was not formally evaluated. A 75% improvement in the baseline PASI score, also known as a PASI 75 was the most frequently used primary endpoint in four out of five clinical trials (Paller et al., 2008; Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021). Only one study selected PGA 0/1 as primary endpoint and PASI 75 as secondary endpoint (Landells et al., 2015). PGA 0/1 (or alternatively IGA 0/1) was generally chosen as a co-primary endpoint to assess treatment efficacy (Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021). PASI 90 was a secondary endpoint in all five examined studies (Paller et al., 2008; Landells et al., 2015; Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021). PASI 100 was a secondary endpoint in three of out five studies (Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021). The primary end points were assessed at week 12 in four studies (Paller et al., 2008; Landells et al., 2015; Paller et al., 2020; Bodemer et al., 2021) and at 16 weeks in one study (Papp et al., 2017).

Anti-TNF-Alpha Agents

Etanercept (1 Trial)

In a placebo-controlled study on 211 patients, 4–17 years of age, Paller et al. (Paller et al., 2008) reported that etanercept 0.8 mg per kilogram of body weight (to a maximum of 50 mg) resulted in a greater percentage reduction in PASI 75 score versus placebo (57 vs. 11%, P=<0.001) at week 12. Similar results were observed for the secondary outcomes, with a higher proportion of reduction of PASI 50 (75 vs. 23%), PASI 90 (27 vs. 7%), and physician's global assessment (PGA) of clear or almost clear (53 vs. 13%) in etanercept group vs. placebo ($p < 0.001$).

Adalimumab (1 Trial)

Papp et al. (Papp et al., 2017) compared adalimumab 0.8 mg/kg or 0.4 mg/kg subcutaneously with oral methotrexate once weekly

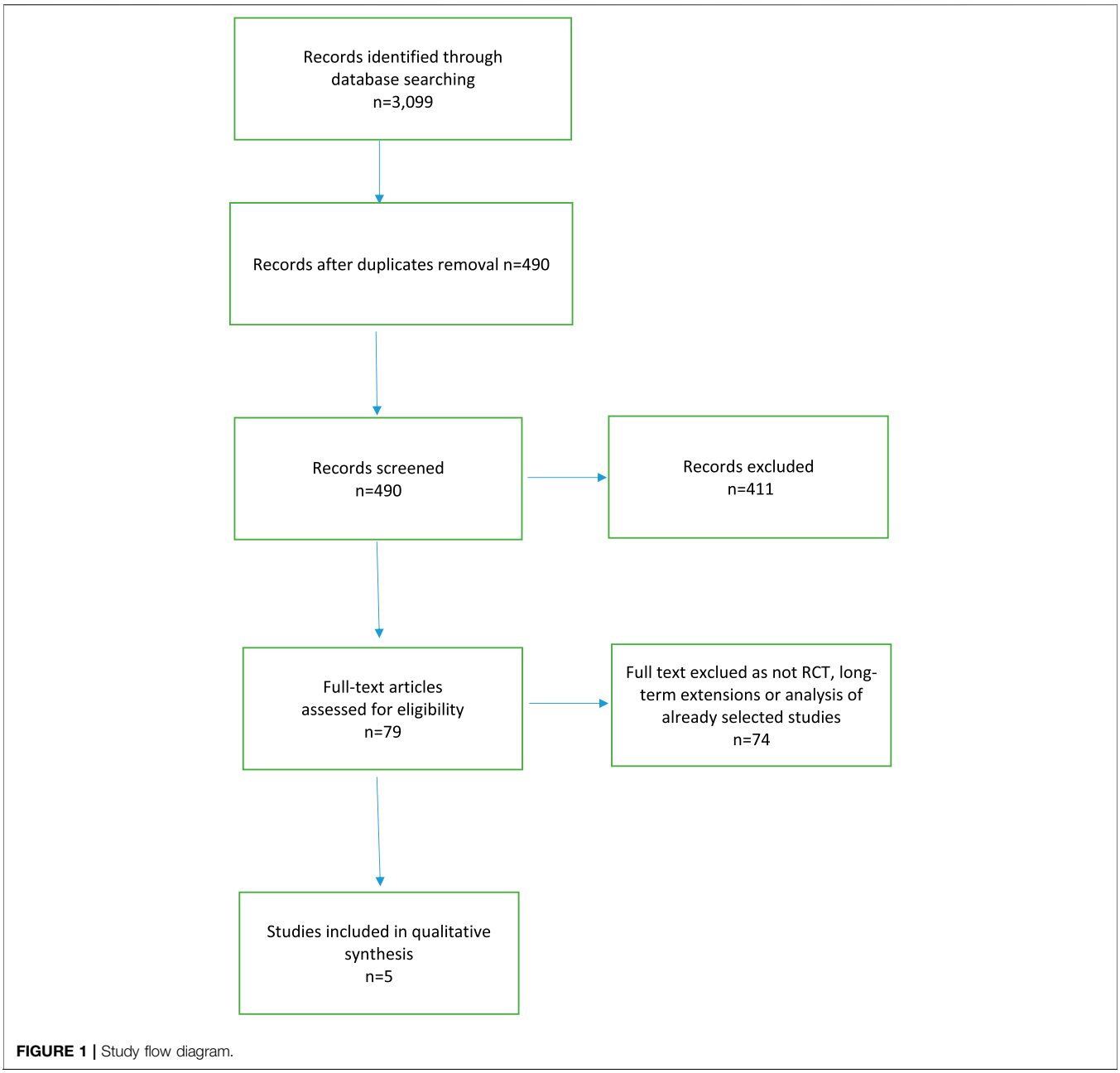

FIGURE 1 | Study flow diagram.

TABLE 1 | Strength of recommendation ratings, symbols used in subsequent statements and their corresponding definitions.

Strength of recommendation	Symbol	Definition
High	⊕⊕	High quality body of evidence from robust, large, well conducted trials, where benefits of treatment outweigh risks and adverse effects
Low	*	Low quality body of evidence from smaller studies, risks of bias where benefits of treatment, risks and adverse effects are closely matched
No Recommendation	X	Insufficient evidence
Against	-	Sufficient body of evidence where risks of treatment outweigh benefits

TABLE 2 | Summary of main characteristics of the selected clinical trials.

Author/ Study Acronim	Study design	Patient Eligibility	Dose	Number of Patients	Duration	Primary Endpoints	Statistical Analysis	Quality of Evidence (GRADE)
Paller (Paller et al., 2008)	Multicentre, double-blind	PASI≥12	-Etanercept 0.8 mg/kg (max 50 mg)	106	12 weeks	PASI 75	Intention to treat	⊕⊕ high
		PGA≥3 BSA≥10%	-Placebo	105				
Papp (Papp et al., 2017)	Multicentre, double-blind, multiperiod, phase 3 trial	PASI≥20 or ≥10 and at least one of active psoriatic arthritis unresponsive to non-steroidal anti-inflammatory drugs	-Adalimumab 0.8 mg/kg (max 40 mg)	38	16 weeks	PASI 75	Intention to treat	⊕⊕ high
		PGA≥4	-Adalimumab 0.4 mg/kg (max 20 mg)	39		PGA 0/1		
		BSA>20% or ≥10% with very thick lesions	-Methotrexate 0.1–0.4 mg/kg (max 25 mg per week total dose)	37				
		CDLQI≥10 or clinically relevant facial, genital, or hand or foot involvement						
Landells/ CADMUS (Landells et al., 2015)	Multicentre, double-blind, phase 3 trial	PASI≥12	-Ustekinumab Standard Dose 0.75 mg/kg	36	12 weeks	PGA 0/1	Non responder imputation	⊕⊕ high
		PGA≥3	-Ustekinumab Half-Standard Dose 0.375 mg/kg	37				
		BSA≥10%	-Placebo	37				
Bodemer (Bodemer et al., 2021)	Multicentre, double-blind, placebo- and active controlled	PASI≥20	-Secukinumab low dose (LD) 150 mg	40	12 weeks	PASI 75	Non responder imputation	⊕⊕ high
		IGA≥4	-Secukinumab high dose (HD) 300 mg	40		IGA 0/1		
		BSA≥10	-Etanercept	41				
			-Placebo	41				
Paller/IXORA Ped (Paller et al., 2020)	Multicentre, double-blind, phase 3 trial	PASI ≥20 sPGA ≥4	-Ixekizumab[1]	115	12 weeks	PASI 75 sPGA 0/1	Non responder imputation	⊕⊕ high
			-Placebo	56				

According to body weight, dosing was as follows: subjects >50 kg received a starting dose of 160 mg, then 80 mg every 4 weeks (Q4W) thereafter; subjects 25–50 kg received a starting dose of 80 mg, then 40 mg Q4W thereafter; subjects <25 kg received a starting dose of 40 mg, then 20 mg Q4W thereafter).

(0·1–0.4 mg/kg) for 16 weeks in 114 patients (aged ≥4 to <18 years). Adalimumab 0.8 mg/kg induced greater improvement in the PASI 75 score than methotrexate (58 vs. 32%, $p = 0.027$) and the clear or minimal PGA score (61 vs. 41%, $p = 0.083$) with respect to oral methotrexate. Adalimumab 0.8 mg/kg was also superior to oral methotrexate in the secondary efficacy end point of a PASI 90 response at week 16 (29 vs. 22%, $p = 0.466$), without statistical significance (Di Lernia, 2017; Papp et al., 2017).

Anti-IL12/23 Agents

Ustekinumab (1 Trial)

Landells et al. (Landells et al., 2015) compared in the CADMUS trial ustekinumab standard dosing (0.75 mg/kg for patients ≤60 kg, 45 mg for patients 60-≤100 kg, and 90 mg for patients >100 kg) or half-standard dosing (0.375 mg/kg for patients ≤60 kg, 22.5 mg for patients 60-100 kg, and 45 mg for patients >100 kg) versus placebo during 12 weeks of treatment in 110 patients aged 12–17 years. Treatment with ustekinumab standard and half standard dosing respectively resulted in significantly better percentage improvement in the primary endpoint PGA score 0/1 than the placebo group (69.4 and 67.6% vs. 5.4%, $p < 0.001$). Similarly, using ustekinumab standard and half standard dosing respectively resulted in significant improvement also for major secondary endpoints compared with placebo, in particular for PASI 75 (80.6 and 78.4% vs. 10.8%, $p < 0.001$), PASI 90 (61.1 and 54.1% vs. 5.4%, $p < 0.001$) and CDLQI (-6.7 and -5.6 vs. -1.5, $p < 0.01$).

Anti-IL17 Agents

Secukinumab (1 Trial)

Bodemer et al. (Bodemer et al., 2021) compared secukinumab (low dose, 150 mg, and high dose, 300 mg) with placebo and etanercept in 162 patients aged six to <18 years during 12 weeks.

TABLE 3 | Efficacy outcome: PASI 75. Active treatment (intervention) compared to placebo or active comparator (methotrexate, etanercept).

Publication	Intervention	Illustrative Comparative Risks*		Relative risk (95% CI)	No of Participants**	Number needed to treat (NNT)
		Assumed risk	Corresponding risk			
Paller (Paller et al., 2008)	Etanercept	**Placebo** 114 per1000	**Etanercept** 566 per1000	RR 4.95 (2.83–8.65)	211	2.2
Papp (Papp et al., 2017)	Adalimumab	**Methotrexate** 324 per1000	**Adalimumab 0.8 mg/kg** 578 per1000	RR 1.79 (1.04–3.06)	75	3.8
Landells (Landells et al., 2015)	Ustekinumab	**Placebo** 108 per1000	**Ustekinumab standard dose** 805 per1000	RR 7.45 (2.91–19.06)	73	1.4
Bodemer (Bodemer et al., 2021)	Secukinumab	**Placebo** 146 per1000	**Secukinumab Low dose** 800 per1000	RR 5.47 (2.57–11.64)	81	1.5
		Etanercept 634 per1000	**Secukinumab Low dose** 800 per1000	RR 3.17 (2.20–4.57)	81	6
Paller (Paller et al., 2020)	Ixekizumab	**Placebo** 250 per1000	**Ixekizumab** 887 per1000	RR 3.55 (2.24–5.61)	171	1.6

**Illustrative comparative risk is presented in the form of a number of people experiencing the chosen event (PASI, 75) per 1,000 persons. The assumed risk is calculated in a group of people (placebo or active comparator group) who did not receive the intervention (drug under investigation). The corresponding risk is calculated in the group who received the intervention.*
***Number of participants involved in the efficacy outcome may differ from total number of trial participants due to the presence of more than two groups of patients treated with different drug dosages in some of the selected studies (24–25).*

The co-primary objectives of the study were met with both secukinumab doses. Treatment with low and high dose secukinumab respectively compared with placebo resulted in greater improvement in the PASI 75 score (80% and 77,5 vs. 14.6%, $p < 0.0001$), IGA 0/1 (70 and 60% vs. 4.9%, $p < 0.0001$). In addition, both secukinumab dose groups (low and high dose) respectively achieved significantly higher ($p < 0.05$) response versus etanercept with respect to IGA 0/1 (70.0 and 60% versus 34.1%) and PASI 90 (72.5 and 67.5% versus 29.3%). Treatment with low and high dose secukinumab compared with placebo resulted in significant improvements in other secondary endpoints as well, as PASI 100 (30.0 and 27.5% vs. 0%) and CDLQI 0/1 (44.7 and 50% vs. 15%, P 0.05 and 0.001).

Ixekizumab (1 Trial)

In a placebo-controlled study (IXORA-PEDs) with 171 patients aged six to <18 years, Paller et al. (Paller et al., 2020) highlighted that ixekizumab resulted in significantly better percentage improvement in the primary endpoints PASI 75 and sPGA 0/1 respectively than the placebo group (PASI 75 89% vs. placebo 25%, $p < 0.0001$) (sPGA 81 versus 11%). Ixekizumab was also superior for all secondary endpoints, including PASI 90 (78% versus placebo 5%) PASI 75 and sPGA (0,1) at week 4, improvement in itch, and complete skin clearance.

Efficacy outcomes relative to PASI 75 and PASI 90 are presented respectively in **Table 3**; **Table 4**. A two-sided asymptotic 95% CI was calculated for the relative risk (RR) of a PASI 75 and PASI 90 response at week 12 or at week 16 for adalimumab/methotrexate. RR of PASI 75 response was 7.45 for ustekinumab, 5.47 for secukinumab, 4.95 for etanercept, 3.55 for ixekizumab, 1.79 for adalimumab (**Table 3**). RR of PASI 90 response was 29.73 for secukinumab, 11.22 for ixekizumab, 9.47 for ustekinumab, 3.28 for etanercept, 1.34 for adalimumab (**Table 4**).

In addition, the NNT for the outcome PASI 75 and PASI 90 in the exposed groups compared to those receiving placebo (Paller et al., 2008; Landells et al., 2015; Paller et al., 2020; Bodemer et al., 2021) or methotrexate and etanercept (Landells et al., 2015; Papp et al., 2017) have been exploited. NNT for PASI 75 response was 1.4 for ustekinumab, 1.5 for secukinumab, 1.6 for ixexizumab, 2.2 for etanercept, 3.8 for adalimumab (**Table 3**). NNT for PASI 90 response was 1.4 for secukinumab and ixekizumab, 1.8 for ustekinumab, five for etanercept, 14.3 for adalimumab (**Table 4**)

DISCUSSION

The exact role of biologics in the treatment of paediatric psoriasis is evolving (Napolitano et al., 2016). This systematic review summarizes the up-to-date evidence on the short-term efficacy of licensed biologic therapies in the treatment of paediatric psoriasis.

Five studies referring to five biologic agents for moderate-to-severe psoriasis were included.

We selected PASI 75 as our primary outcome due its relatively wide use in current psoriasis trials, and PASI 90. The measure of improvement in a patient-oriented score, such as CDLQI, has not been investigated.

Heterogeneity of patient age and psoriasis severity across the selected five studies was found. Younger enrolled patients were children aged four in one study (Papp et al., 2017), six in three studies (Paller et al., 2008; Paller et al., 2020; Bodemer et al., 2021) and 12 in one study (Landells et al., 2015). PASI baseline was ≥20 in three studies (Papp et al., 2017; Paller et al., 2020; Bodemer et al., 2021) and ≥12 in the remaining two (Paller et al., 2008; Landells et al., 2015).

Effect size estimates suggested that etanercept, ustekinumab, secukinumab and ixekizumab reduced overall psoriasis symptoms more than placebo; secukinumab more than etanercept; adalimumab more than methotrexate. The latter result was not statistically significant probably due to the limited sample size and power of the study (Di Lernia, 2017).

TABLE 4 | Efficacy outcome: PASI 90. Active treatment (intervention) compared to placebo or active comparator (methotrexate, etanercept).

Publication	Intervention	Illustrative Comparative Risks*		Relative Risk (95% CI)	No of Participants **	Number Needed to Treat (NNT)
		Assumed risk	Corresponding risk			
Paller (Paller et al., 2008)	Etanercept	**Placebo** 67 per1000	**Etanercept** 273 per1000	RR 3.28 (1.51–7.12)	211	5
Papp (Papp et al., 2017)	Adalimumab	**Methotrexate** 216 per1000	**Adalimumab 0.8 mg/kg** 289 per1000	RR 1.34 (0.61–2.95)	75	14.3
Landells (Landells et al., 2015)	Ustekinumab	**Placebo** 54 per1000	**Ustekinumab standard dose** 611 per1000	RR 9.47 (2.42–37.11)	73	1.8
Bodemer (Bodemer et al., 2021)	Secukinumab	**Placebo** 24 per1000	**Secukinumab low dose** 725 per1000	RR 29.73 (4.25–207.90)	81	1.4
		Etanercept 293 per1000	**Secukinumab low dose** 725 per1000	RR 2.48 (1.48–4.14)	81	2.3
Paller (Paller et al., 2020)	Ixekizumab	**Placebo** 53 per1000	**Ixekizumab** 782 per1000	RR 11.22 (3.75–33.55)	171	1.4

** Illustrative comparative risk is presented in the form of a number of people experiencing the chosen event (PASI, 90) per 1,000 persons. The assumed risk is calculated in a group of people (placebo o active comparator group) who did not receive the intervention (drug under investigation). The corresponding risk is calculated in the group who received the intervention. **Number of participants involved in the efficacy outcome may differ from total number of trial participants due to the presence of more than two groups of patients treated with different drug dosages in some of the selected studies (Landells et al., 2015; Papp et al., 2017).*

According to RR, when compared with placebo the decreasing rank order for PASI 75 endpoint was ustekinumab, secukinumab, etanercept, ixekizumab, for PASI 90 endpoint secukinumab, ixekizumab, ustekinumab, etanercept. Noteworthy, PASI 75 response rate of ixekizumab was higher than etanercept, but the high placebo PASI 75 response rate (Paller et al., 2020) had influence on the assessment.

NNT can help to quantify efficacy outcomes and give support to place various therapeutic options into clinical perspective. NNT for additional benefit on the PASI 75 outcome showed as the most effective options ustekinumab, secukinumab and ixekizumab with very low difference among them. NNT for additional benefit on PASI 90 showed as the most effective option secukinumab and ixekizumab.

CONCLUSION

In this study, we highlighted that the available biologic therapies for psoriasis are efficacious for paediatric psoriasis. Our study showed that there was a trend to better response to certain biological classes and in particular that anti-IL-17 agents seem to be superior to anti-TNF-alpha therapies in the treatment of paediatric psoriasis, consistent with their corresponding efficacy in adults (Fahrbach et al., 2021; Sbidian et al., 2021). However, all the drugs were compared in a blinded, randomised comparison in the short term.

Finally, long term observations and large registries are necessary to enhance our knowledge about efficacy and safety of these drugs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

VD, GT and CG: conceptualization. VD: writing. GT and CG: supervision. LM, LP and YI: software and methodology, data curation. VD and CG: investigation and formal analysis. All authors contributed to the article and approved the submitted version.

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Safety of Off-Label Pharmacological Treatment in Pediatric Neuropsychiatric Disorders: A Global Perspective From an Observational Study at an Italian Third Level Children's Hospital

*Maria Sole Giurin†, Marta Paulina Trojniak *†, Anna Arbo, Marco Carrozzi, Giuseppe Abbracciavento, Lorenzo Monasta and Caterina Zanus*

Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy

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Serafina Perrone, University of Parma, Italy

Reviewed by:
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***Correspondence:**
Marta Paulina Trojniak
marta.trojniak@burlo.trieste.it

†These authors have contributed equally to this work

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Background: The acquisition of proper and relevant pediatric clinical data is essential to ensure tolerable and effective pediatric drug therapies. In the field of pharmacological treatment of neuropsychiatric disorders, the lack of sufficient high quality scientific evidence for pediatric age results in the frequent need to prescribe off-label drugs. With the aim of improving knowledge about safety profile of off-label drug prescription in children and adolescent with neurological and/or psychiatric disorders, we realized a multidisciplinary pharmacovigilance study.

Materials and methods: An observational retrospective study was conducted to assess the safety of off-label pharmacological therapies in patients aged 0–18 years, admitted to the Neuropsychiatry Unit of the Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" between January 2016 and December 2018. Prescription patterns and adverse drug reactions were evaluated by a multidisciplinary team.

Results: Overall, 230 patients were enrolled, 48% boys (N = 111), 52% girls (N = 119), average age of 10 years, and a total of 534 prescriptions was analyzed. 54.5% (N = 125) of patients had epilepsy, 37.5% (N = 86) suffered from psychiatric disorders, 8% (N = 19) had other neurological disorders. The prevalence of off-label prescriptions was 32% and 50% of the study population received at least one off-label drug. A total of 106 ADRs was detected: 57% of ADRs were due to drug-drug interactions, 30% were due to off-label prescriptions, 10% were due to overdose and 3% were due to improper use. No significant association between emerged ADRs and off label prescriptions was found (Fisher's exact two-tailed test, $p = 1.000$). There was significant association between increasing number of administrated drugs and risk of ADRs (OR 1.99; IC95% 1.58–2.5; $p = 0.000$). Psychiatric disorders were associated with at least three times higher risk to be treated with an off-label drug (OR 3.30; IC95% 2.26–4.83; $p = 0.000$).

Conclusions: This study shows that off-label prescribing in neuropsychiatric disorders does not pose a greater risk of ADRs than on-label prescribing and highlights unmet clinical

needs in pediatric neuropsychopharmacology. The multidisciplinary approach can provide important contributions to improve therapeutic path of these already complex pathologies by careful monitoring of therapeutic appropriateness and drug interactions.

Keywords: off-label, antipsychotics, antiepileptics, pediatric, pharmacovigilance, clinical pharmacist, safety, neuropsychiatric disorders

INTRODUCTION

Children are not little adults, especially when they take drugs, not only do they differ from adults in size, but also as far as drugs' absorption, metabolism and excretion are concerned (Wagner and Abdel-Rahman 2013).

Due to ethical and commercial problems and to the lack of pharmaceutical research on pediatric neurology and psychiatry, some medications are unlicensed, holding no marketing authorization, or are used outside the indication or age range for which they are licensed. This makes selecting an appropriate drug even more complex. Pediatric neurology and psychiatry are therapeutic areas frequently requiring off-label approaches and this practice is challenging for prescribing physicians. Beside authorized indications, some off-label uses with good clinical evidence are already authorized and reimbursed in Italy based on Law 648/1996. **Tables 1** and **2** show authorized and reimbursed indications of antiepileptic and psychotropic drugs for children and adolescents in Italy. However, the limited indications of these drugs lead to widespread use of off-label prescriptions.

The Italian legislation governing the use of off-label drugs refers to Law 94/1998 (Law N 94/1998, 1998). The term "off-label" refers to an authorized pharmaceutical product used outside the terms of its marketing authorization and consequently not in line with the information contained within the summary of its characteristics.

The lack of clinical trials in pediatric population involves uncertainties in terms of efficacy and safety, this means that evidence derived from real world is crucial to define the benefit-risk profile of drugs in the pediatric population.

It is known that children are more subject to adverse drug reactions (ADRs) than adults, so the use of off-label drugs may expose them at a higher risk of toxicity or ineffective treatment (Rosli et al., 2017). There is little evidence about the use and the risk of ADRs associated with off-label drugs in pediatric neuropsychopharmacology, and scientific communities recognize the urgent need to carry out epidemiological studies not only on the frequency of off-label prescriptions,

TABLE 1 | Authorized and reimbursed indications of antiepileptic drugs for Children and Adolescents in Italy.

Drug name	Pediatric approved indications	Off label use authorized by law 648/96
ACTH	Infantile Epileptic, Encephalopathy with hypsarrhythmia	Add-on: ESES, Lennox-Gastaut syndrome, Severe Epileptic Encephalopathy
Ethosuximide	Absence Epilepsy	Add-on: ESES, Epileptic Negative Myoclonus
Lamotrigine	2–12 years: - Monotherapy for typical absences - in add-on in focal seizures, generalized TC and Lennox-Gastaut syndrome	Monotherapy for the treatment of Janz syndrome in >12 years old
Levetiracetam	Focal seizures with or without secondary generalization: - monotherapy: > 16 years old - Add on: > 1 month - 12 years Janz syndrome	Monotherapy >12 years for the treatment of Jan syndrome, ESES, Add-on: Typical absences
Rufinamide	Add-on: Lennox-Gastaut syndrome >4 years	Add-on for severe Encephalopathy >4 years
Topiramate	Focal and generalized seizures: - monotherapy: > 6 years Lennox-Gastaut syndrome - Add-on> 2 years	Drug-resistant Typical absence seizures
Zonisamide	No pediatric indications	Severe epileptic encephalopathies >4 years in add-on Typical pharmacoresistant absences
Clobazam	No indications in epilepsy	Severe drug resistant epilepsies over 3 years old
Dexamethasone	No indications in epilepsy	Not present in L. 648/96
Nitrazepam	No indications in epilepsy	Not present in L. 648/96
Perampanel	In add-on in patients aged> 12 years for the treatment of focal seizures, generalized TC	Not present in L. 648/96

TABLE 2 | Authorized and reimbursed indications of antipsychotic drugs in Children and Adolescents in Italy.

Drug name	Pediatric approved indications	Off label use authorized by law 648/96
Risperidone	Indicated for persistent aggressiveness associated to conduct disorder in children (>5 years old) and adolescents with intellectual disabilities or limit intellectual functioning, diagnosed according to DSM-V criteria	- Short-term treatment of moderate or severe behavioral problems such as irritability and aggression in individuals (≥5 years) with autism spectrum disorders - Tourette syndrome with moderate to severe functional impairment (≥7 years) - Add-on to methylphenidate in subjects (≥7 years old) with ADHD and oppositional defiant disorder, or aggressive behavior who have not responded effectively to methylphenidate treatment alone
Olanzapine	No pediatric authorization	>7 years schizophrenia and bipolar disorder
Quetiapine	No pediatric authorization	>12 years schizophrenia and bipolar disorder
Aripiprazole	>15 years schizophrenia >13 years bipolar I disorder	>13 years schizophrenia >10 years type1 bipolar disorder >6 years treatment of irritability in subjects with autism spectrum disorders >6 years Tourette's syndrome
Clozapine	>16 years schizophrenia and psychosis	Acute and chronic psychosis in adolescents and children >7 years of age
Delorazepam	No pediatric authorization	Not present in L. 648/96
Clothiapine	No pediatric authorization	Not present in L. 648/96
Promazine	Patients older than 12 years - Treatment of psychomotor agitation or aggressive behavior - Schizophrenia and other psychotic disorders	Not present in L. 648/96
Lithium	Prophylaxis and treatment of - states of excitement in forms of mania and hypomania - states of depression or chronic depressive psychosis manic-depressive psychosis	Not present in L. 648/96
Fluoxetine	>8 years major depression	Not present in L. 648/96
Sertraline	6 years obsessive compulsive disorder	Not present in L. 648/96

but also on the indications for their use and the monitoring of these therapies (Sharma et al., 2016).

In spite of this uncertainty, off-label prescribing is a tool of early access, particularly widespread in pediatrics, which allows physicians to treat young patients.

Pharmacists and pharmacologists play a significant role in the drug monitoring, consisting in appropriateness evaluation through a systematic review of the scientific literature, with the aim of identifying and summarizing evidence on the effectiveness and safety of the pharmacologic interventions. Pharmacotherapy is pivotal in treating patients with psychiatric and neurologic disorders; however, its success is often limited by adverse effects, inefficacy, inadequate therapy monitoring and follow up, and poor adherence (Persico et al., 2015). In many cases, adverse effects go unrecognized. The non-identification of ADRs can have dire consequences, leading to the so-called "prescription cascade". A lot can be done by the multidisciplinary team to prevent side effects, mainly: monitoring of therapies, patient education (advise the patient on how to best take his or her medications to maximize benefits while minimizing side effects), follow-up, critical appraisal of the literature, polypharmacy review and drug-drug interactions, develop a treatment plan to resolve any medication-related problems (e.g. change administration times; propose therapeutic drug monitoring and change dose if the therapy reveals to be toxic or ineffective, prepare information documents to inform the patient about the therapy and side effects so that he/she can recognize them early) (Werremeyer et al., 2020).

The lack of sufficient high quality scientific evidence and the frequent need to prescribe off-label drugs prompted us to start a pharmacovigilance study, with the aim of detecting ADRs and assessing safety profiles of off-label drug prescriptions as compared to on-label drugs.

With a multidisciplinary approach, the study was conceived through the collaboration of pharmacists, pharmacologists and clinicians who, at our Institute, routinely cooperate in all the prescription process.

MATERIALS AND METHODS

Objectives

This study aimed to assess if off-label use in third level children's hospitals may be associated with an increased risk of adverse drug reactions and to verify the real-world safety of these drugs.

Primary endpoint of the study:

- Estimate the incidence of adverse reactions in patients undergoing off-label drugs compared to the on-label in the inpatient setting of Pediatric Neuropsychiatry Department.

Secondary endpoints were:

- Analysis of the Prescriptions: estimate the prevalence of off-label drugs compared to on-label drugs, the frequency of different drugs used for off-label prescriptions and the indication for their use; analyze the quantity, quality, and consistency of evidence of off-label prescriptions; quantify the drug-drug interactions; evaluate the difference of off-label use between neurologic and psychiatric disorders.
- Analysis of the ADRs: estimate the prevalence of ADRs, characterization of ADRs and detection of ADR's risk factors.

The study was performed with the approval of the regional ethics committee.

Patients

To be eligible for participation in this study, patients had to be younger than 18 years; have confirmed evidence of psychiatric or neurological diagnosis; have been exposed to at least one medication and being hospitalized in the Pediatric Neuropsychiatry Department of the Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" in Trieste from 2016 to 2018. In the Italian organization of medical services, Neuropsychiatry deals with all the neurological and psychiatric disorders of children and adolescents, different from other European countries where pediatric neurological and psychiatric domains are separated.

Study Design

This is a retrospective, single-center, observational study.

The review of the cases was conducted through a multidisciplinary collaboration between physicians, pharmacists and pharmacologists with the aim of evaluating the appropriateness of the patients' pharmacological treatment. For every single patient enrolled in the study the pharmacist analyzed and collected: socio-demographic information (age, gender, weight, height); diagnosis; drug prescriptions during hospitalization found in the medical records (duration of treatment, indication, dosage, time of administration, a route), exposure to off-label/on label drug, drug-drug interactions and safety analysis. All prescriptions during patients' hospitalization were considered in the analysis.

Information regarding therapies and the clinical data (ADRs and biochemical parameters) of the study population was obtained from prescriptions in the electronic health record (clinical diary, drug prescription sheet), Hospital Mission Sheets (SDO), hospital discharge report and laboratory reports.

The collected clinical data was anonymized, and inserted into a certified database REDCap. The diagnoses were classified using the International Classification of Diseases, ninth revision (ICD - ICD-9-CM - International Classification of Diseases, 2021).

Classification of Medicines

The prescribed medicines were divided into: on-label (authorized and reimbursed indications including drugs form the list referred to the Law 648/1996) and off-label drugs (based on the Law 94/1998).

The evaluation was made by the pharmacist comparing the information from the prescriptions (patient's age, diagnosis, etc.) with the information in SmPC (Summary of Product Characteristics) reported by AIFA (Italian Drug Agency) and EMA (European Medicines Agency). For each off-label prescription, scientific evidence available for the given off-label drug use was evaluated, mainly phase I, phase II, phase III studies, observational studies, case series and case reports.

Evaluation of Off-Label Prescriptions

The Scottish Intercollegiate Guidelines Network (SIGN) has been used to evaluate the quality of the evidence (Baird and Lawrence 2014). SIGN method leads to guidelines that are essentially the direct product critical appraisal of the systematic review.

The level of evidence depends on:

- Quantity, quality, and consistency of evidence;
- External validity (generalizability) of studies;
- Direct applicability of the guidelines to the target population.

Classification of Drug-Drug Interactions

Drug interactions were investigated by the pharmacist using three different data sources Lexicomp® and Terap® and SmPC.

Interactions were classified as:

- Avoid combination (X): when data demonstrate that the specified agent may interact with the other in a clinically significant manner. The risks associated with concomitant use of these agents usually outweigh the benefits. These agents are generally considered contraindicated;
- Consider therapy modification (D): when data demonstrate that the two medications may interact with each other in a clinically significant manner. A patient-specific assessment must be conducted to determine whether the benefits of concomitant therapy outweigh the risks. Specific actions must be taken in order to maximize the benefit and/or minimize the toxicity resulting from concomitant use of agent. These actions may include aggressive monitoring, empiric dosage changes, choosing alternative agents;
- Monitor Therapy (C): when data demonstrate that the specified agents may interact with each other in a clinically significant manner. The benefits of concomitant use of these medications usually outweigh the risks. An appropriate monitoring plan should be implemented to identify potential negative effects. Dosage adjustments of one or both agents may be needed in a minority of patients.
- No Action Needed (B): when data demonstrated that the specified agents may interact with each other, but there is little to no evidence of clinical concern resulting from their concomitant use.
- No known Interaction (A): data has not demonstrated either pharmacodynamic or pharmacokinetic interactions between the specified agents.

Classification of ADRs

The ADRs were coded using the Medical Dictionary for Regulatory Activities (MedDRA). ADRs were detected by pharmacists from electronic health records (clinical diary, drug prescription sheet), Hospital Mission Sheets (SDO), hospital discharge report and laboratory reports, and then confirm by physicians. For each ADR, the Summary of Product Characteristics (SmPC) was investigated by pharmacist to understand if the ADR was already reported and to understand its relevance. A "serious" ADR was defined as "any untoward medical occurrence that at any dose results in death, requires hospital admission or prolongation of existing hospital stay, results in persistent or significant disability/incapacity or is life threatening or other clinically relevant condition" (Gautron et al., 2018). To avoid correlation biases between the ADR and the drug, the Naranjo algorithm was calculated.

Furthermore, the causal link between ADRs and drug-drug interactions was assessed by pharmacist. The Drug Interaction Probability Scale (DIPS) algorithm was run to assess the causality between ADRs and drug-drug interaction.

Based on the result of the Naranjo algorithm and the DIPS algorithm, the ADRs related to the drug or interaction between drugs can be: doubtful (≤0); possible (1–4); probable (5–8); certain (≥9) (Horn et al., 2007).

Statistical Analysis

For descriptive analyses, categorical variables were presented as frequencies and percentages, while continuous variables were reported as means and standard deviations (SDs) or as medians and interquartile ranges (IQR), as appropriate after verification of their distribution. The normality of the distribution of quantitative variables was tested by the Shapiro-Wilk test.

The main study outcome was the presence of ADRs, and the main independent variable was the prescription of off-labels versus on-labels. Associations were mainly studied using non-parametric tests. The two-tailed Fisher exact test was carried out to study the association between two dichotomous variables, while the Mann-Whitney rank-sum test was used to study the difference in the distribution of a continuous variables in two different groups.

Univariate and multivariate logistic regression have been used to study the association between type of drugs (off-label vs. on-label and drugs included in the Italian law 648/96) and health outcomes (ADRs, clinical outcomes), considering other relevant covariates that emerged in the study. Odds Ratios (ORs) and 95%CI were calculated for each variable included in the models.

The hypothesis of the study was to find a difference in the incidence of ADRs in the population of neuropsychiatric children undergoing off-label compared to on-label drugs of at least 20% (Bellis et al., 2014), based on our estimates of an ADR frequency of 10% in on-label and 30% in off-label uses. The total sample size needed was 200 subjects.

For all analyses, the significance level was set at $p< 0.05$. All analyses were performed using StataBE 17.0 (StataCorp, College Station, United States).

RESULTS

A total of 230 patients were enrolled: 48% boys (N = 111), 52% girls (N = 119), with an average age of 10 years. 54.5% (N = 125) of patients had epileptic diagnosis, 37.5% (N = 86) suffered from psychiatric pathologies, 8% (N = 19) had other neurological disorders.

Primary Endpoint

There was no association between ADRs and the prescription of an off-label, among 71 prescriptions with at least one ADR detected, 49 of them (69%) were due to on-label prescriptions and 22 (31%) were related to off-label drugs (**Table 3**).

Considering the number of off-labels as a categorical variable, the Odds Ratio increases as the number of off-label administered increases. One off-label had an OR = 1.72 (95%CI 0.81–3.66; p = 0.1569), not significant. Two off-labels had an OR = 6.19 (95%CI 2,43–15.72; p = 0.000), while three off-labels had an OR = 15.47 (95%CI 2.76–86.62; p = 0.002). This analysis was not possible for the prescription of more than three off-label due to data scarcity.

Secondary Endpoint

Analysis of the Prescriptions

A total of 534 prescriptions was analyzed. Off-label prescriptions represented 32% of the total (N = 169). 105 prescriptions referred to the psychiatric field, 52 to epileptic and 12 to other neurological problems.

In general, each patient received more than two therapies during hospitalization (**Table 4**). 50% of the patients (N = 115 out of 230) received at least one off-label drug with on average almost one and a half off-label prescriptions per person.

On the total of 534 prescriptions, 38 (7.2%) concerned medications included in the list based on Italian law 648/96.

Some 35.6% of off-label prescriptions were off-label with respect to the age of the patients, 44.4% with respect to the indication, 18.4% were both for age and indication in relation to the dose and 1.6% in relation to the therapeutic line.

The most common off-label drugs were: delorazepam, quetiapine, risperidone (off-labels with respect to age) and dexamethasone, nitrazepam, trihexyphenidyl (off-label with respect to indication).

The outcome related to the level of evidence and the grade of recommendation for off-label prescriptions result in 59.5% of grade 1A, 24.8% of grade 3D, 13.1% of grade 2C, 0.6% of grade 4D and 0.6% of grade 1B.

Sixty-three patients (27%) enrolled in the study experienced a drug-drug interactions (**Table 4**). A total of 113 drug-drug interactions were detected, with an average of 1.8 interactions per patient experiencing a drug-drug interaction. Of all drug-drug interactions, 59 were pharmacodynamic (52%), 45 were pharmacokinetic (40%), while nine were both (8%). Furthermore, 83% of drug-drug interactions were risk type C, requiring monitoring therapy and 17% were risk D, needing therapy modification. Valproic Acid, Carbamazepine, Lithium and Phenobarbital were the drugs causing most drug-drug interactions.

80% of patients with a psychiatric disorder have been exposed to more than one off-label drug. In the psychiatric field, off-label

TABLE 3 | Two-way relative frequency table between ADRs and off-label prescriptions.

		Off-label		Total	*p*-value of Fisher's exact test
		No	Yes		
Adverse Drug Reactions	**No**	316	147	463	1.000
		68.3%	31.7%	100%	
		86.6%	87.0%	86.7%	
	Yes	49	22	71	
		69.0%	31.0%	100%	
		13.4%	13.0%	13.3%	
Total		365	169	534	
		68.4%	31.6%	100%	
		100%	100%	100%	

TABLE 4 | Description of prescribing patterns.

Age groups	Freq	Off-label prescription	At least one off-label prescription	Average number of off-label prescriptions x person	Average number of on- and off-label prescription x person	Average number of drug-drug interaction per person
<1	17	10 (59%)	7 (41%)	1.57	2.29	1.00 (n = 3)
1–4	34	21 (62%)	13 (38%)	1.31	2.64	1.67 (n = 9)
5–11	60	36 (60%)	24 (40%)	1.42	2.35	1.68 (n = 19)
12–17	119	48 (40%)	71 (60%)	1.51	2.33	1.97 (n = 32)
Tot	230	115 (50%)	115 (50%)	1.47	2.32	1.79 (n = 63)

TABLE 5 | Two-way relative frequency table between off-label prescriptions and drug-resistant epilepsy.

		Drug-resistant epilepsy		Total	*p*-value of Fisher's exact two-tailed test
		No	Yes		
Off-label	**No**	72	16	88	0.000
		82%	18%	100%	
		82%	42%	70%	
	Yes	16	22	38	
		42%	58%	100%	
		18%	58%	30%	
Total		88	38	126	
		70%	30%	100%	
		100%	100%	100%	

prescriptions are much more frequent: the analysis showed that psychiatric pathology is associated with a higher risk to be treated with an off-label drug (OR = 3.30, 95%CI 2.26–4.83; $p = 0.000$) if compared to other diagnoses.

There is also a significant association between drug-resistant epilepsy and prescription of off-label drugs (**Table 5**). 58% of the prescribed drugs to drug-resistant patients are off-label, while the percentage of prescribed off-label drugs to other patients is only 18%.

Multinomial logistic regression has shown that the age group 12–17 years is the one with the highest risk of off-label prescriptions (RR 2.38, 95%CI 1.09–5.22; $p = 0.029$). The reference group was 1–4 years of age, the group with the

TABLE 6 | Distribution of Adverse Drug Reactions reported by MedDRA System Organ Classes (SOCs).

Adverse drug reactions	Frequency	Percentage
SNC disorders	47	44
Mental and behavioral disorders	19	18
Gastrointestinal disorders	13	12
Immune system disorders	7	7
Cardiovascular disorders	6	6
Eyes disorders	3	3
Endocrine disorder	3	3
Urinary and renal disorders	3	3
Others disorders (reproductive, metabolism etc.)	5	5
Total Adverse Drug Reactions	106	100

lowest risk if compared to each of the others (RR 1.13, 95%CI 0.3–3.7; $p = 0.8$).

Analysis of the ADRs

During 71 drug administrations, at least one ADR was detected and the total of ADRs was 106, for an average of 1.5 per subject who had at least one.

Table 6 shows that 44% of all ADRs were neurological reactions, in particular extrapyramidal symptoms and dystonia (due to the use of first- and second-generation antipsychotics) and dizziness (caused by antiepileptic drugs). 18% were mental and behavioral disorders like mood alteration, insomnia and irritability (**Table 6**).

Thirty-four medications were involved in adverse drug reactions: most ADRs were due to Risperidone (N = 17), Haloperidol (N = 9), Phenytoin (n = 8), Carbamazepine (N = 7) and Quetiapine (N = 6).

According to the Naranjo algorithm, 54% of all ADRs were probable, 35% possible, 7% certain and 4% doubtful. According to the DIPS algorithm, the causal link between ADRs and drug-drug interactions was 59% doubtful, 30% probable and 10% possible.

Thirty-four (32%) of a total of 106 ADRs were serious, one of them led to hospitalization or prolongation of hospitalization, the rest caused other clinically relevant conditions. Drugs that have caused serious adverse reactions were: Quetiapine, Valproic Acid, Risperidone, Phenytoin, Haloperidol, Vigabatrin, Dexamethasone, Oxcarbazepine, Levetiracetam, Clobazam. 65% of the ADRs caused serious CNS disturbances (drug-induced extrapyramidal adverse effect, convulsive seizure, hypotonia, dystonia and drowsiness), 3% caused CPK increase, 6% were related to reproductive and breast disease (gynecomastia and galactorrhea), 6% to endocrine disease (hyperprolactinemia, hypothyroidism), 3% gastrointestinal disease (mainly hypersalivation), 9% to cardiovascular disease (syncope hypotensive, tachycardia, retention water) and 9% to immune system disorders (tongue edema, rash and redness of face).

For some drugs, such as Haloperidol, Phenytoin, and Primidone, an ADR was observed in 50% of the administrations.

Overall, 57% of ADRs were due to drug-drug interactions, 30% to off-label prescriptions, 10% due to overdose and 3% due to improper use.

ADR's Risk Factors

From the logistic regression analysis, it emerged that there were no significant associations between occurrence of adverse events and age group (OR 1.1; 95%CI 0.3–3.83; $p = 0.17$ for age range 12–17; the reference group was 1–4 years) and sex (OR 0.71; 95% CI 0.39–1.31; $p = 0.28$, for females). While there was a statistically significant association between increasing number of drugs taken by the patient and the onset of ADRs (OR 1.99; 95%CI 1.58–2.50; $p = 0.000$).

As regards to clinical risk management, 15 therapeutic errors and 14 near miss events were detected (3% and 3% of total prescriptions, respectively): 28 were due to medication errors (82% for drug-drug interactions of contraindicated drugs combinations and 29% for higher dose) and one due to administration errors (manipulation and administration error). It was calculated that the risk of an ADR is over six times higher if a prescription error occurs (OR 6.69, 95%CI 3.33–14.77; $p = 0.000$).

DISCUSSION

The primary endpoint of this study was to assess the risk of ADR associated with off-label prescribing in the field of pediatric neuropsychiatry. In our study, 30% of the ADRs involved an off-label prescription and ADR occurrence was not significantly related to off-label prescribing. Findings are consistent with several studies (Neubert et al., 2004; Mason et al., 2012; Palmaro et al., 2015) that found no association between ADR and off label prescribing. Other studies (Horen et al., 2002; Neubert et al., 2004; Aagaard and Hansen 2011; Bellis et al., 2014; Pratico et al., 2018) instead found significant association between ADR and off-label prescription. In these studies, ADRs were related mainly to anti-infective and anti-asthmatic drugs and vaccines, which were not prescribed in our study. Bellis et al. found an increased risk related to off label drugs used in oncological practice and the result became statistically insignificant when oncology patients were excluded (Bellis et al., 2014). Thus, it is possible that the greater risk of ADRs observed by the other studies resulted from a different pattern of off-label drug use (Rusz et al., 2021).

The fact that off-label prescribing in our pediatric sample with neurodevelopmental disorders shown not to be associated with ADRs leads us to focus more on finding evidence from the literature in order to provide the access to the pharmacological treatments offering the best possible care, regardless of marketing authorization.

This study highlights the unmet clinical needs of pediatric population with neurodevelopmental disorders: the frequent use of off-label prescriptions underlines the strong need for studies especially for psychiatric pathologies (OR = 3.30 for off-label prescription). The treatment of psychiatric disorders represents an important therapeutic challenge, determined by the complexity of the disorders and the difficulty of producing clinical pharmacological recommendations in the absence of consolidated evidence. Psychiatric patients are associated with a high risk of suicide, high access to mental health services, severe impairment of psychosocial functioning and high social and economic costs with the need for emergency hospitalization therefore the implementation of research in this field is very important (Leichsenring et al., 2011).

Considering the indications approved for neuroleptics drugs, it is important to point out that the real word frequency of these authorized indications is very low: only 6.17 and 1.23% of the enrolled patients in our study suffered from bipolar disorder and ADHD respectively, and none was diagnosed with schizophrenia in our study. A possible interpretation of this finding could be that generally for adolescents with bipolar disorder or ADHD an inpatient care is needed only when psychiatric emergency presents, like severe mood disorder of psychosis, and in this case the main diagnosis reported are these.

The fact that mood disorders and eating disorders do not have a specific treatment in adolescence is confirmed by the high

frequency of off label prescriptions, in our series, among subjects with these disorders.

Another consideration must be done, concerning the specificity of the adolescent age and psychiatric diagnosis: this age represents a particular phase for neurodevelopmental processes (Pablo et al., 2019) when many symptoms can appear, even intense or severe, without this meaning a definite psychiatric diagnosis. It can be difficult to diagnose a psychotic/bipolar/schizophrenic disorder at this age, when many maturational processes are still in progress. Moreover, clinical pictures cannot be clear at the first hospitalization taking place during a psychiatric emergency. The burden of the potentially stigmatizing power of a psychiatric diagnosis can make it difficult to formalize it, mainly in the stormy context of the emergency hospitalization. This all factors can contribute to the evidence of a relatively low frequency of diagnosis of psychosis and to the use of antipsychotics out of indication, resulting in an off-label prescription.

Concerning the field of neurological disorders, off-label prescription is mainly needed in drug-resistant epilepsies, so when there is no therapeutic alternative (other therapeutic options have been tried).

Most of the identified ADRs affect the nervous system: it is critical to consider the dynamic effect of antipsychotic and antiepileptic drugs on the immature brain, which demonstrates plasticity in its ability to adapt to the external milieu and preventative interventions. In this study half of the extrapyramidal effects were determined by first generation antipsychotics and the other half by second generation antipsychotics, although the latter should be administered less frequently (Stahl 2017). Clinicians prescribing these medications should familiarize themselves with the most common adverse events, and work with pharmacists and pharmacologists to recognize them early through monitoring (neurological examination but also electrocardiograms, absolute neutrophil counts, blood glucose, blood LDL and weight).

In our study the overall incidence of therapeutic inappropriateness was 5.8%, consistent with the literature data (Lewis et al., 2009; Alshehri et al., 2017); 82% of them were associated with drug-drug interactions (mainly due to contraindicated drug combinations) and 29% of them led to overdose. 18% of the identified ADRs was preventable and correlated to therapeutic inappropriateness, a percentage which is consistent with the results from 20 studies which we reviewed in a systematic manner. From our results, therapeutic inappropriateness discovered to be a very high-risk factor for having an ADR (OR 6.69; IC95% 3.03–14.77; $p = 0.000$).

Furthermore, 73% of therapeutic inappropriateness were linked to psychotropic drugs prescription (Clothiapine, Clozapine, Haloperidol, Lithium, Olanzapine, Quetiapine Risperidone) and 27% were linked to antiepileptic prescriptions (Phenytoin, Primidone, Valproic Acid). The important issue to focus on is therapeutic inappropriateness consisting in drug-drug interactions and improper uses. A total of 113 drug-drug interactions were found and 27% of patients in our study experienced a drug-drug interactions. Valproic Acid caused 23 (20%) drug-drug interactions and appeared to be a broad-spectrum enzyme inhibitor as it inhibits the activity of UGT enzyme (UGT1A4 and UGT2B7) as well as CYP2C9 and, weakly, CYP2E1. Valproic Acid increases the serum concentration of Phenobarbital, Lamotrigine and Ethosuximide, resulting in possible enhancement of the adverse/toxic effect of its substrate. Valproate Products may decrease the protein binding of Fosphenytoin-Phenytoin: this appears to lead to an initial increase in the percentage of unbound (free) phenytoin (Perucca 2006; Zaccara and Perucca 2014).

Also, Carbamazepine and Phenobarbital caused drug-drug interactions (N = 26, 23%) both inducing CYP450 and glucuronyl transferase enzyme and so reducing serum concentration of substrate of the same enzyme. In our study we detected carbamazepine interactions with phenytoin, rufinamide, valproic acid, phenobarbital, levetiracetam, desmopressin, clobazam and levothyroxine.

We observed a poorly described particular drug–drug interaction that caused significant extrapyramidal adverse reaction: the concomitant use of lithium with first/second generation of antipsychotic. Considering that the extrapyramidal side effects are due an imbalance between dopaminergic and cholinergic systems, and considering the fact that lithium and psychotropic drugs are known to decrease the amount of dopamine, it is plausible to consider it as a pharmacodynamic drug-drug interaction (Baastrup et al., 1976; Addonizio 1985; Sachdev 1986; Tuglu et al., 2005).

This is the first detailed analysis of off-label prescription patterns in Child Neuropsychiatry in Italy. The context of Italian Child Neuropsychiatry, which unlike other European realities deals with both neurological and psychiatric pathology in the pediatric age, offers the possibility of a unique perspective that allows to extend the analysis of the problem of off-label prescribing to a wide spectrum of clinical areas, both very relevant: epilepsy, that is among the most frequent neurological pathologies in children, often due to rare diseases (Amann et al., 2013) and psychiatric disorders; both have very little evidence for drug use.

The study has several limitations. The first limitation is the retrospective nature of the study and its structure that do not allow the detection of variables with a potential confounding role. In addition, the low number of events with which some outcome occurred in our study did not allow the execution of appropriate subgroup analyzes in order to explore the validity of the relationships between different variables. Nevertheless, retrospective studies are an important tool to study rare diseases and findings of this study can form the basis on which prospective studies are planned.

CONCLUSION

This study assessed the prescriptive and safety profiles of pharmacological treatment for the inpatient care of children and adolescents with neuropsychiatric disorders. The findings of a high prevalence of off-label prescription highlights unmet needs in pediatric neuropsychopharmacology, mainly for what in psychiatry is concerned. This study has not detected greater

risks associated with off-label prescriptions supporting the relevance of research in this field. As members of a multidisciplinary team, pharmacists and pharmacologists may collaborate with clinician providing them their expertise by medication review of pharmacological therapies, leading to increased therapeutic appropriateness.

These first results give us several insights into areas for improvement, mainly preventing prescribing inappropriateness and polypharmacy. This study has shown that to increase therapeutic safety of neuropsychiatric therapies in pediatrics, monitoring of drug interactions and therapeutic appropriateness are needed both in on- and off-label use of drugs. Multidisciplinary team should take any effort to optimize the appropriateness and the safety of therapies as well as the resources of the National and Regional Health Systems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent to participate in research study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MPT and CZ study design; MSG, MPT, and CZ collect data, analyze data, write manuscript; GA support in data collection; AA, MC, and LM critical review; LM and MPT statistics.

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Population Pharmacokinetics of Intravenous Acyclovir in Oncologic Pediatric Patients

Natalia Maximova[1], Daniela Nisticò[2], Giacomo Luci[3], Roberto Simeone[4], Elisa Piscianz[5], Ludovica Segat[5], Egidio Barbi[1,2] and Antonello Di Paolo[3]*

[1]Department of Pediatrics, Institute for Maternal and Child Health—IRCCS Burlo Garofolo, Trieste, Italy, [2]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy, [3]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [4]Transfusion Medicine Department, Azienda Sanitaria Universitaria "Giuliano Isontina", Trieste, Italy, [5]Laboratory for Hygiene and Public Health, University Hospital of Trieste, Trieste, Italy

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****Correspondence:***
Natalia Maximova
natalia.maximova@burlo.trieste.it

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Background: Acyclovir represents the first-line prophylaxis and therapy for herpes virus infections. However, its pharmacokinetics in children exposes them to the risk of ineffective or toxic concentrations. The study was aimed at investigating the population pharmacokinetics (POP/PK) of intravenous (IV) acyclovir in oncologic children.

Methods: Patients (age, 8.6 ± 5.0 years, 73 males and 47 females) received IV acyclovir for prophylaxis (n = 94) and therapy (n = 26) under a therapeutic drug monitoring (i.e., minimum and maximal plasma concentrations, >0.5 and <25 mg/L, respectively). Plasma concentrations were fitted by nonlinear mixed effect modeling and a simulation of dosing regimens was performed. Findings were stratified according to an estimated glomerular filtration rate (eGFR) threshold of 250 ml/min/1.73 m^2.

Results: The final 1-compartment POP/PK model showed that eGFR had a significant effect on drug clearance, while allometric body weight influenced both clearance and volume of distribution. The population clearance (14.0 ± 5.5 L/h) was consistent across occasions. Simulation of standard 1-h IV infusion showed that a 10-mg/kg dose every 6 h achieved target concentrations in children with normal eGFR (i.e., ≤250 ml/min/1.73 m^2). Increased eGFR values required higher doses that led to an augmented risk of toxic peak concentrations. On the contrary, simulated prolonged (i.e., 2 and 3-h) or continuous IV infusions at lower doses increased the probability of target attainment while reducing the risk of toxicities.

Conclusion: Due to the variable pharmacokinetics of acyclovir, standard dosing regimens may not be effective in some patients. Prospective trials should confirm the therapeutic advantage of prolonged and continuous IV infusions

Keywords: acyclovir, pediatric patients, hematopoietic stem cell transplantation, pharmacokinetics, non-linear mixed effect modeling, prolonged infusion acyclovir, children, prolonged infusion

INTRODUCTION

Herpesvirus infections in immunocompromised patients, particularly in hematopoietic stem cell transplant (HSCT) recipients, lead to severe disease with high dissemination rates, complications, and mortality (Beyar-Katz et al., 2020). Herpes simplex virus (HSV) is a ubiquitous virus that results in lifelong infections due to its ability to alternate between lytic replication and latency (Ly et al., 2021). The worldwide prevalence of HSV-1 increases consistently with age, reaching 40% by age 15 and increasing to 60%–90% in older adults (Chayavichitsilp et al., 2009). Up to 80% of adult leukemia patients are HSV seropositive, as well as up to 80% of HSV-seropositive allogeneic HSCT recipients had post-transplant HSV reactivation (Styczynski et al., 2009; Flowers et al., 2013). In the first post-transplant year, symptomatic varicella-zoster virus (VZV) reactivation in adult recipients is described with rates of 13%–55% (Beyar-Katz et al., 2020). Similarly, 30%–33% of pediatric HSCT recipients had VZV reactivation, and 11% of these were disseminated (Fisher et al., 2008). In the current era of antiviral prophylaxis in seropositive HSCT recipients, the infection rate has decreased significantly, besides a significant reduction in mortality (Dadwal, 2019).

In pediatric patients undergoing allogeneic HSCT, surveillance algorithms, antiviral prophylaxis, or pre-emptive treatment are well established for many viruses due to the high incidence of severe systemic complications in this population (Czyzewski et al., 2019; Jaing et al., 2019). In contrast to HSCT recipients, data on systemic viral infections in children receiving chemotherapy for hematological malignancies are very limited (Buus-Gehrig et al., 2020). However, few reports confirm that children with malignant diseases who experience prolonged periods of myelosuppression due to cytotoxic chemotherapy are highly susceptible to invasive viral infections (Feldman and Lott, 1987).

Most viral reactivation in adult cancer patients during neutropenia after myelotoxic chemotherapy is due to HSV (Saral et al., 1984). However, despite the high infection rate, there is not enough evidence from randomized trials on acyclovir prophylaxis in patients with acute leukemia to establish a strong recommendation in adult and pediatric patients undergoing intensive chemotherapy (Styczynski et al., 2009).

Acyclovir (ACV) effectively prevents and treats HSV and VZV infections but demonstrates high interindividual variability in its treatment response. Indeed, ACV prophylaxis is recommended for all HSV-seropositive HSCT recipients from conditioning until engraftment or until mucositis resolves to prevent HSV reactivation during the early post-transplant period. For VZV-seropositive HSCT recipients antiviral prophylaxis is recommended for at least one year, while for VZV-seronegative HSCT recipients passive immunization is preferred (Tomblyn et al., 2009; Carreras et al., 2018). For instance, the prophylactic regimen of ACV for the European Blood and Marrow Transplant Group consists in 250 mg/m^2 (or 5 mg/kg) i.v., every 12 h, while the treatment of infections needs an intensified regimen (250 mg/m^2 or 5 mg/kg i.v., every 8 h for 7–10 days) (Carreras et al., 2018). Due to the time-dependent killing of ACV, plasma concentrations should be higher than 1 mg/L for at least 50% of time interval between two consecutive doses (Saiag et al., 1999), so that a minimum plasma concentrations >0.5 mg/L could be considered an appropriate target. Moreover, high peak concentrations in plasma (i.e., >50 mg/L) are associated with an increased rick of neurotoxicity (Wade and Monk, 2015), even if the correlation between high plasma concentrations and the risk of nephrotoxicity and bone marrow adverse events remains to be fully investigated.

ACV is eliminated by the kidney's glomerular filtration and tubular secretion, has low oral bioavailability, approximately 10% in children (Carcao et al., 1998), and a short half-life, hence it requires high and repeated doses to exceed the value of the inhibitory concentration (Abdalla et al., 2020). Furthermore, ACV has high interindividual variability (Blum et al., 1982; O'Brien and Campoli-Richards, 1989), which is particularly evident in the younger patients, and related to changes in renal function during the first months after the birth and body weight across the ages (De Miranda and Blum, 1983; Zeng et al., 2009; Abdalla et al., 2020). In addition to this, the genetic status of the patient may be considered a further cause of variability of pharmacokinetics and clinical outcome, as recently demonstrated for the NUDT15 polymorphism (Nishii et al., 2021).

Therefore, information regarding ACV optimal use in children with malignancies is restricted because pharmacokinetic data are limited in this population (Zeng et al., 2009; Abdalla et al., 2020). This study aims to characterize the pharmacokinetics of ACV following intravenous (IV) administration and to evaluate the adequacy of current dose regimens for children with malignancy undergoing myelosuppressive chemotherapy or HSCT. Additionally, the study aims to explore alternative dosing regimens that could be more effective and tolerable.

MATERIALS AND METHODS

Study Design and Population

This prospective, single-center, observational study was carried out at the pediatric Onco-Hematology Department and pediatric Bone Marrow Transplant Center of the Institute for Maternal and Child Health—IRCCS "Burlo Garofolo," Trieste, Italy, from 2011 to 2020. The Institutional Review Board of the IRCCS Burlo Garofolo (reference no. IRB RC 10/20) approved the protocol, and the study was conducted following the Declaration of Helsinki (Clinicaltrials.gov code: NCT05198570). The patients' parents gave their written consent to collect and use personal data for research purposes. From January 2011 to December 2020, consecutive patients aged 0–18 years, affected by hematological malignancies, undergoing ACV prophylaxis or treatment for HSV-VZV infection during allogeneic HSCT or ACV treatment during high-intensity chemotherapy were included in this study. All patients underwent ACV therapeutic drug monitoring (TDM), and consequent dose adjustment was applied to maintain minimum (C_{min}) and maximum (C_{max}) plasma

concentrations >0.5 mg/L and <25 mg/L, respectively, as per laboratory practice. Data collection included patient demographic characteristics as gender, age, weight, height, body mass index, body surface area, serum creatinine in addition to primary diagnosis, treatment for primary diagnosis, ACV dose, administration interval, concomitant medications, duration of ACV treatment, and cause of treatment interruption. The Schwartz formula determined the estimated glomerular filtration rate (eGFR) for each patient (Schwartz et al., 1976).

ACV Administration Regimens and Blood Sampling

ACV was administered every 6–8 h intravenously (Acyclovir Recordati®, Biologici Italia Laboratories S.r.l., Masate, Milan, Italy) over 60 min infusion, with median (range) starting daily doses equal to 40.7 (15.6–136.7) mg/kg/day. The prescribed doses refer to the local protocols and depend on the age, treatment indication, and renal clearance. These differences in dosage reflect systematic changes in institutional practice over the ten years covered by the study. The first pharmacokinetic assessment (two samples) was performed after at least four days of ACV administration when the steady state was achieved. In particular, blood samples were withdrawn 10 min before (trough levels, C_{min}) or 30 min after IV infusion (maximum concentration, C_{max}). For some patients, blood samples were collected on several occasions. Blood samples were centrifuged, and plasma concentrations of ACV were measured by a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) performed in multiple reaction monitoring mode (Kanneti et al., 2009). Briefly, after deproteinization, calibration standards, quality controls (both prepared in human blank plasma) and patients' plasma samples (using fluconazole as internal standard, IS) were prepared by solid phase extraction (Oasis HLB Vac Cartridge, Waters, Milford, CT, United States). Acyclovir and IS were eluted through a C18 column with a mobile phase consisting of 0.1% formic acid solution and methanol (30:70 v/v) at a flow rate of 0.8 ml/min in isocratic conditions. The LC-MS apparatus worked in positive ion detection, and quantification was performed in multiple reactions monitoring (MRM) of transition ions m/z 226.3 > 152.1 and m/z 306.9 > 219.9 for ACV and IS, respectively.

Pharmacokinetic Modeling and Simulation

The population pharmacokinetics (POP/PK) analysis was performed on the available plasma concentrations using a nonlinear mixed effect modeling approach using NONMEM software vers. 7.4 (ICON, Dublin, Ireland)[1] and the packages PsN and Xpose (Jonsson and Karlsson, 1999; Lindbom et al., 2004). One- and two-compartment models with first-order elimination were tested, while the residual error was assayed as an additive, proportional and mixed model. Interoccasion variability (IOV) was evaluated for pharmacokinetic

[1]ICON plc. NONMEM®. [Accessed 1 September 2021]. https://www.iconplc.com/innovation/nonmem/.

TABLE 1 | Characteristics of the 120 patients enrolled in the present study. There were not statistically significant differences between males and females.

	All patients (*n* = 120)	
	Mean ± SD	**Median**
Age (years)	8.6 ± 5.0	9.5
Weight (kg)	32.4 ± 19.1	27.8
Height (cm)	128.2 ± 30.0	131.5
Serum creatinine (mg/dl)	0.361 ± 0.206	0.320
eGFR (ml/min/1.73 m^2)	228.1 ± 79.7	209.4
BSA (m^2)	1.058 ± 0.426	0.998

eGFR, estimated glomerular filtration rate; BSA, body surface area.

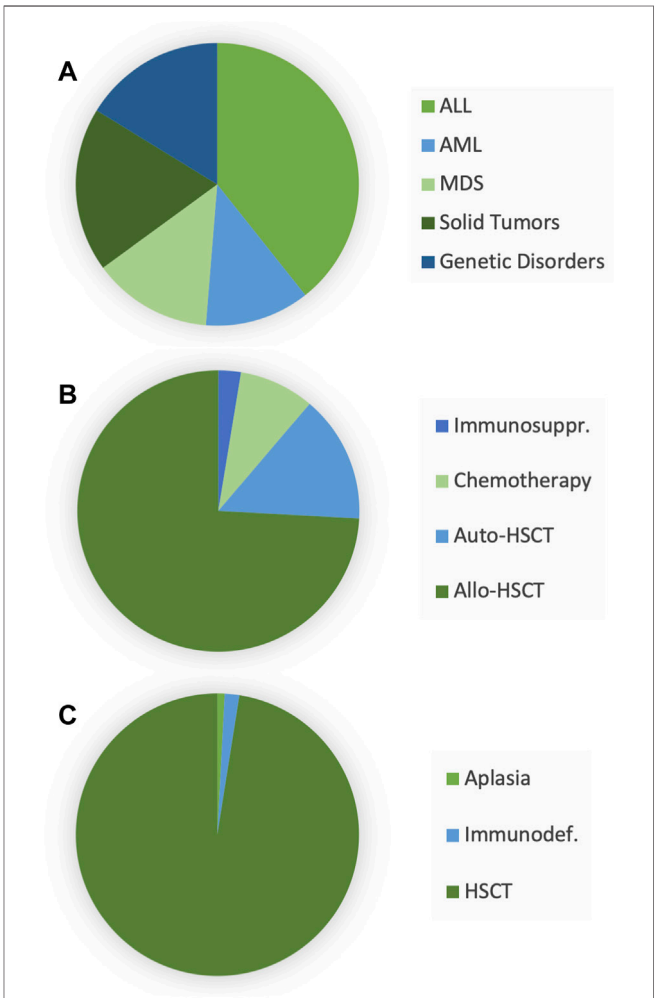

FIGURE 1 | Characteristics of the 120 enrolled patients: initial diagnosis **(A)**, first-line therapy **(B)**, and reasons for acyclovir administration **(C)**. Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; HSCT, hematopoietic stem cell transplantation.

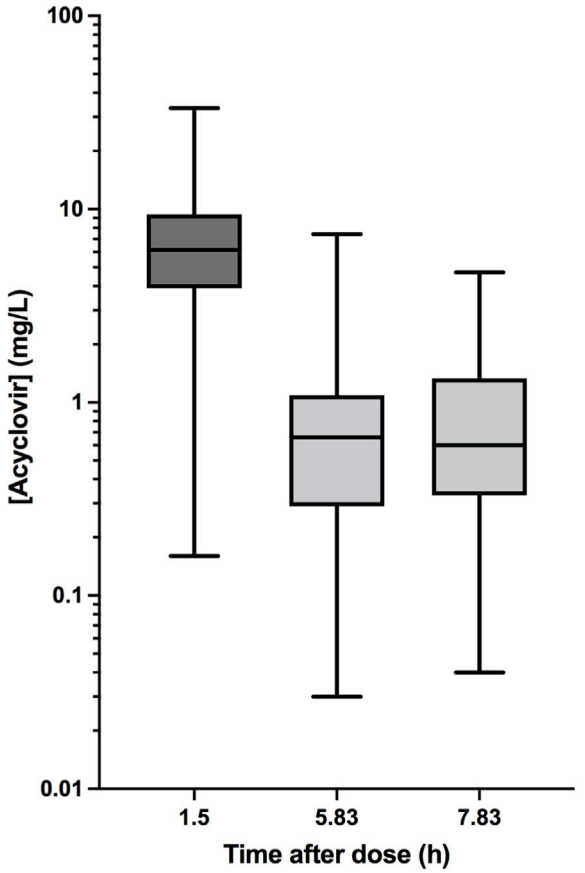

FIGURE 2 | Box-and-whiskers plot of single measured plasma concentrations of acyclovir 30 min after the end of the infusion (1.5 h) and 10 min before the next dose in 6-h (5.83 h) and 8-h (7.83 h) regimens.

parameters. The introduction of covariates within the model was guided by their range of values in the dataset and their possible mechanistic involvement in ACV pharmacokinetics. Overall, a decrease in the objective function value (OFV) greater than 3.84 points ($p < 0.05$) and 6.63 points ($p < 0.01$) in the forward inclusion and backward exclusion, respectively, were considered during the model development. The conditional weighted residuals (CWRES) were calculated (Hooker et al., 2007), and the goodness-of-fit (GOF) plots, the precision of parameter estimates, η- and ε-shrinkage values were evaluated for each model. A bootstrap analysis and a prediction-corrected visual predictive check (pcVPC) were used to judge the final model (Bergstrand et al., 2011). The terminal elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/k_{el}$, where k_{el} is the individual empirical Bayes estimate of the ACV elimination constant.The software NONMEM was used to simulate different drug administration schedules based on the final POP/PK model. The simulation included dosages in the range 15–30 mg/kg administered as standard (i.e., 1 h), prolonged (i.e., 2 and 3 h) and continuous IV infusions every 6 and 8 h in 1,000 individuals for each dose level. C_{min} values higher than 0.56 or 1.156 mg/L in at least 50% of patients or C_{max} values > 25 mg/L in less than one-fourth of patients were considered as the desired pharmacokinetic targets in patients grouped according to the presence of augmented renal clearance (ARC) or not (i.e., eGFR >250 or ≤250 ml/min/1.73 m^2, respectively) (Abdalla et al., 2020).

TABLE 2 | Parameter values of the final model and bootstrap results obtained in 1,000 resampled databases.

	Final model		Bootstrap	
	Value	SE	Median	95% CI
OFV	889.024		873.384	756.091–994.564
CL (L/h)	6.184	1.630	6.186	2.965–9.985
V (L)	18.942	2.135	19.129	14.945–24.130
EGFR on CL	1.627	0.269	1.595	0.769–2.097
IIV_{CL}	45.2%	25.4%	44.9%	23.9%–57.8%
IIV_V	56.7%	33.8%	57.2%	13.0%–76.6%
IOV_{CL}	20.0%	9.6%	19.9%	15.2%–31.5%
Residual variability	0.181	0.050	0.177	0.061–0.369
Shrinkage	η_1 shrinkage	19.2	η_2 shrinkage	24.7

Note: final model equations: CL (L/h) = $[6.184 + (eGFR/209.4)^{1.627} + (WGT/27.8)^{0.75}](\eta 1 + IOV)$ and V (L) = $[18.942 + (WGT/27.8)^1]\ \eta 2$.

SE, standard error; 95% CI, 95% confidence intervals; OFV, objective function value; CL, systemic clearance; V, volume of distribution; eGFR, estimated glomerular filtration rate; IIV, interindividual variability; IOV, interoccasion variability; WGT, body weight.

Statistical Analysis

Data are presented as mean ± standard deviation (SD), median and minimum-maximum range, or 95% confidence interval (95% CI) according to the parameter described. Statistical computations (i.e., unpaired Student's t-test with Welch's correction, Mann-Whitney test, ANOVA, Fisher exact test) were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, United States) after checking for normal distribution of values (when appropriate) by the Kolmogorov-Smirnov test, and the significance level was set at $p < 0.05$.

RESULTS

Patients and Acyclovir Monitoring

The current database included 73 boys and 47 girls (age, mean ± SD, 8.0 ± 5.2 and 9.5 ± 4.6 years, respectively; **Table 1**). Most patients were affected by hematological malignancies and addressed to allogenic HSCT (**Figure 1**). Twenty-six patients received ACV to treat a viral infection caused by HSV or VZV, whereas in the remaining 94 children the drug was administered for prophylaxis. Along with the treatment, blood samples were withdrawn to perform ACV monitoring of plasma concentrations in 120, 54, and 13 patients on a first, second, and third occasion, respectively. On the first occasion, measured C_{max} accounted for 7.6 ± 5.4 mg/L, while C_{min} values were 1.0 ± 1.1 and 0.85 ± 1.3 mg/L for the 6-h and 8-administration schedule of ACV, respectively, without significant differences across occasions (**Figure 2**). The dose was changed in 47 children (increased in 39) and 8 patients (increased in 7) at the second and third occasions, respectively.

In 26 patients (21.7%) who received ACV to treat herpetic infections, doses were 381.3 ± 199.2 mg (median, 400 mg) on

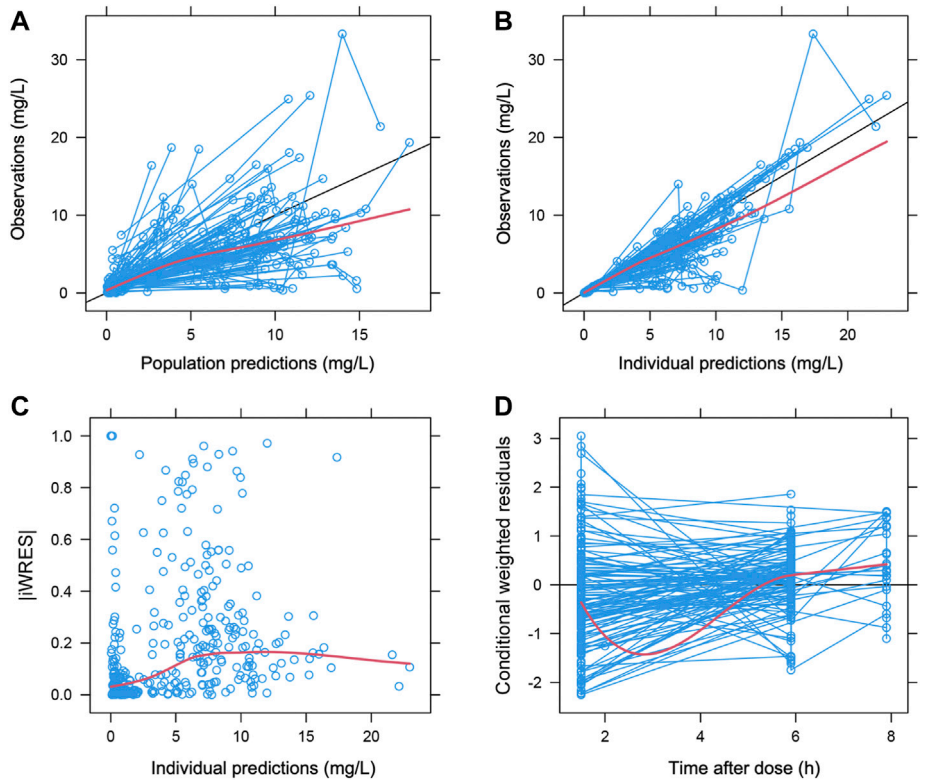

FIGURE 3 | Goodness-of-fit plots. Population **(A)** and individual **(B)** prediction values vs. observations. **(C)**, absolute values of individual weighted residuals (iWRES) error vs. individual predictions and **(D)** conditional weighted residuals vs. time. Symbols, individual plasma concentrations of acyclovir. Red lines, regression, and Loess lines. Black line, line of identity.

the first occasion. In 11 and 4 patients, a second and a third occasions were available, with doses of 432.5 ± 150.9 mg (median 400 mg) and 500.0 ± 163.3 mg (median 500 mg), respectively. Among the measured ACV plasma concentrations, 15 and 11 patients had at least one C_{min} value > 0.56 and >1.156 mg/L, respectively. ACV administration was followed by a complete recovery, improvement, or infection control in approximately 75% of patients. In comparison, in the remaining individuals, the records showed a worsening of symptoms and signs (14.3% of patients) or the emergence of a further viral infection (i.e., cytomegalovirus, 9.5% of individuals). Measured C_{min} values in 3 out of 4 patients with poor response to therapy were greater than 1 mg/L, while in the fourth child, the C_{min} value accounted for 0.78 mg/L.

POP/PK Modeling

A total of 187 pairs (120, 54, and 13 for the first, second, and third occasion, respectively) of ACV plasma concentrations for a total of 374 plasma concentration values were available for model development that started with a 1-compartment model with additive residual error (OFV, points 1,372.979). The residual variability was best described by a proportional error model (−141.269 points), while interindividual (IIV) and interoccasion variability (IOV) in systemic clearance (CL)

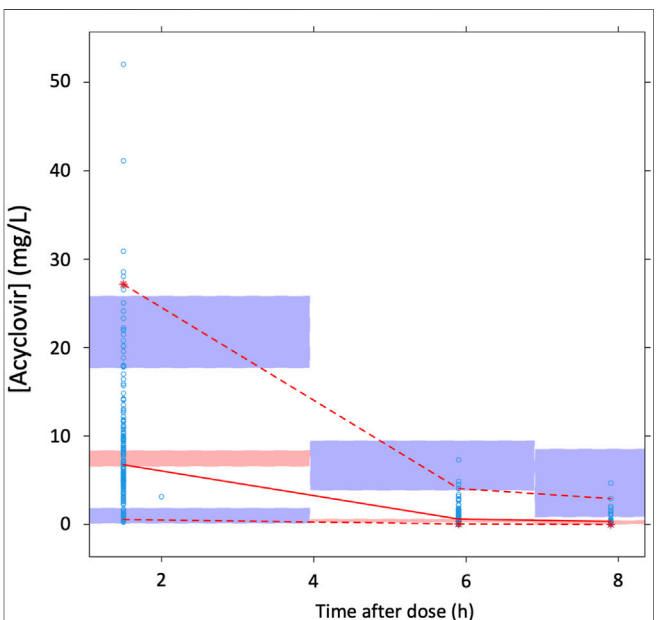

FIGURE 4 | The prediction-corrected visual predictive check based on the present data. Open circles, individual plasma concentrations of acyclovir together with their median and 5th-95th percentiles (solid and dashed red lines, respectively). The 95% confidence intervals of the simulated median (pale pink) and 5th-95th percentiles (pale blue) are showed.

TABLE 3 | Values of the pharmacokinetic parameter obtained in the present 120 children by the final POP/PK model. There were not statistically significant differences between males and females.

	All patients (*n* = 120)	
	Mean ± SD	**Median**
Dose (mg)	334.0 ± 158.2	350.0
CL (L/h)	14.0 ± 5.5	14.6
AUC (hxmg/L)	26.0 ± 13.2	23.3
V (L)	25.4 ± 8.7	26.1
k_{el} (h^{-1})	0.574 ± 0.188	0.558
$t_{1/2}$ (h)	1.364 ± 0.614	1.237

CL, systemic clearance; AUC, area under the time-concentration curve; V, volume of distribution; kel, constant of elimination; t1/2, terminal elimination half-life.

significantly decreased the OFV value (−197.363 and −49.596 points, respectively). Furthermore, the introduction of IIV on the volume of distribution (V) ameliorated the model (−47.866 points), whereas IOV did not likely because changes in body weight across occasions were not significant (i.e., 32.2 ± 19.1, 30.9 ± 19.8, and 32.8 ± 22.7 kg, respectively at the first, second, and third occasion) and it was not considered in the following modeling.

Among covariates with a possible effect on acyclovir pharmacokinetics, only eGFR on CL (−12.386 points) and body weight (with allometric scaling) on both CL and V (−22.811 and −12.664 points, respectively) did significantly decrease OFV; hence they were retained in the final model (**Table 2**). Among the GOF plots (**Figure 3**), the CWRES versus time after dose graph revealed an overprediction during the first few hours after the dose, which was likely dependent on the schedule of blood withdrawal, while the pcVPC plot did not entirely fit the lowest C_{min} and highest C_{max} values (**Figure 4**). However, the bootstrap analysis (with 1,000 resampled databases) resulted in good performance of the final model (**Table 2**), with nearly 90% of runs that ended successfully.

The analysis of PK parameters of the final model (showed in **Table 3**) did not demonstrate significant gender-based differences. The terminal $t_{1/2}$ accounted for 1.364 ± 0.614 h, while IIV_{CL} and IOV_{CL} values were 46.4% and 20.0%, respectively.

The present CL and V values for a typical individual do match those previously obtained (Abdalla et al., 2020), despite the higher eGFR values calculated in the present patients (median, 209.4 ml/min/1.73 m^2) with respect to previous ones (164 ml/min/1.73 m^2).

More interestingly, the analysis of PK parameters between the first and the second occasion did not reveal significant differences for any pharmacokinetic parameter considered (**Table 4**). In agreement with these results, an additional analysis of ACV PK in 13 patients did not show significant differences across the three occasions (**Table 5**).

POP/PK Simulation

The present simulation was based on the median values of covariates that were included in the final model. In particular, individual values of eGFR were randomly calculated from a distribution similar to that obtained from the present population of patients (i.e., mean and standard deviation values of 228.1 and 79.7 ml/min/1.73 m^2, respectively), while body weight was fixed at 27.8 kg.

In agreement with a previous study (Abdalla et al., 2020), an ACV dose of 20 mg/kg every 6 h may ensure a C_{min} value > 0.56 and >1.125 mg/L in approximately 62.5% and 50.9% of the patients, respectively, while 28.6% of patients may experience a C_{max} value > 25 mg/L when the eGFR was ≤250 ml/min/1.73 m^2 (**Table 6**). On the contrary, at eGFR values > 250 ml/min/1.73 m^2, standard dosing regimens had a lower probability of target attainment, especially when the time interval between doses was 8 h (**Table 6**). The increase in dose to improve target attainment may expose the patients to an augmented risk of high peak plasma concentrations.

The following simulation of prolonged 2- and 3-h IV infusions resulted in an increased probability of ACV C_{min} values > 0.56 and >1.125 mg/L (**Figure 5**; **Table 7**). Of note, the reduced rate of infusion resulted in a lower probability of C_{max} values > 25 mg/L. In line with these results, simulated continuous infusions at doses of 10 mg/kg every 8 h were associated with C_{min} values > 0.56 mg/L in all patients regardless of the eGFR value. When the desired C_{min} threshold was set at 1.125 mg/L, 96.1% and 90.5% of patients achieved the target when the eGFR was ≤250 (C_{min} = 3.18 ± 1.69 mg/L) and >250 ml/min/1.73 m^2 (C_{min} = 2.51 ± 1.26 mg/L), respectively.

DISCUSSION

The present study was performed in a homogeneous population of oncologic paediatric patients receiving IV ACV for prophylaxis or to treat HSV and VZV infections. The final findings of the

TABLE 4 | Pharmacokinetics of ACV across two occasions. Values are presented as mean ± SD (median) values.

Occasion	Dose (mg)	CL (L/h)	AUC (hxmg/L)	V (L)	$t_{1/2}$ (h)
1	334.0 ± 158.2	14.0 ± 5.5	26.1 ± 13.2	25.4 ± 8.7	1.364 ± 0.614
(73M + 47F)	(350.0)	(14.1)	(23.3)	(25.4)	(1.237)
P	*0.213***	*0.440**	*0.755***	*0.300**	*0.924***
2	367.9 ± 167.7	14.7 ± 5.4	26.1 ± 12.4	27.0 ± 9.4	1.356 ± 0.516
(37M + 17F)	(350.0)	(14.6)	(23.9)	(28.0)	(1.238)

*Note: *, Student's t test; **, Mann-Whitney test.*

CL, systemic clearance; AUC;area under the time-concentration curve; V, volume of distribution; $t_{1/2}$, terminal elimination half-life.

TABLE 5 | Pharmacokinetics of ACV across three occasions in 13 patients.

Occasion	Dose (mg)	CL (L/h)	AUC (hxmg/L)	V (L)	$t_{1/2}$ (h)
1	199.2 ± 157.8 (123.0)	12.5 ± 5.3 (13.3)	17.4 ± 11.8 (13.1)	22.5 ± 10.9 (20.2)	1.310 ± 0.501 (1.100)
2	283.1 ± 188.6 (250.0)	11.74 ± 4.7 (12.5)	26.7 ± 21.4 (20.0)	24.4 ± 11.5 (25.5)	1.533 ± 0.816 (1.425)
3	298.5 ± 177.6 (300.0)	12.3 ± 4.0 (12.3)	24.3 ± 11.5 (24.4)	24.4 ± 11.5 (25.5)	1.441 ± 0.661 (1.319)
ANOVA	*0.500*	*0.989*	*0.832*	*0.618*	*0.321*

CL, systemic clearance; AUC, area under the time-concentration curve; V, volume of distribution; $t_{1/2}$, terminal elimination half-life.

TABLE 6 | Results from simulated regimens consisting in ACV dose ranging from 10 up to 30 mg/kg infused in 1 h (standard regimen). The percentages of patients who achieved C_{min} values > 0.56 and >1.125 mg/L or C_{max} values > 25 mg/L are showed according to an eGFR threshold of 250 ml/min/m^2. Each regimen consisted of 1,000 simulated patients.

	1-h infusion	Time interval between doses: 6 h				
	Dose (mg/kg)	10	15	20	25	30
eGFR ≤ 250	C_{min} > 0.56	50.9	57.8	62.5	65.7	67.9
	C_{min} > 1.125	35.2	45.1	50.9	54.6	57.8
	C_{max} > 25	1.0	10.1	28.6	49.1	67.3
eGFR > 250	C_{min} > 0.56	37.8	44.8	50.6	54.0	59.0
	C_{min} > 1.125	22.2	32.4	37.9	42.5	44.8
	C_{max} > 25	0.6	5.7	21.3	36.5	55.6
		Time interval between doses: 8 h				
eGFR ≤ 250	C_{min} > 0.56	31.2	39.7	44.4	47.2	50.1
	C_{min} > 1.125	19.1	26.4	31.2	35.0	39.6
	C_{max} > 25	1.0	8.9	25.0	44.2	63.8
eGFR > 250	C_{min} > 0.56	20.1	27.1	32.8	35.7	37.6
	C_{min} > 1.125	11.1	15.6	20.1	24.8	26.8
	C_{max} > 25	0.6	5.4	20.6	34.9	52.4

eGFR, estimated glomerular filtration rate; C_{min}, minimum plasma concentration; C_{max}, maximum plasma concentration.

POP/PK analysis demonstrate a high variability between and within individuals that warrants the adoption of therapeutic drug monitoring. Furthermore, the simulation suggested that prolonged IV infusions could increase concentrations in the therapeutic range while reducing the risk of toxic peak concentrations.

ACV is a fundamental agent for prophylaxis and treatment of herpes virus infections, especially in HSCT patients or those who received high-dose antineoplastic chemotherapy like those enrolled in the present study. However, appropriate use of ACV depends on maintaining effectiveness through plasma concentrations above the threshold of sensitivity of known viral strains while reducing the risk of toxic concentrations identified in plasma levels >25 mg/L (Abdalla et al., 2020). The achievement of those goals may be severely influenced by patients' characteristics, especially in children. Indeed, previous paediatric studies have clearly demonstrated that the variability in ACV pharmacokinetics is better explained by the renal function rather than dose (De Miranda and Blum, 1983), meaning that including plasma creatinine or eGFR within the model reduced IIV_{CL} and predicted the individual values with respect to observations (Zeng et al., 2009). In addition to this, the variability in both CL and V_d was significantly associated with body weight (Zeng et al., 2009; Abdalla et al., 2020). The present findings do confirm those conclusions in the largest population of enrolled patients published so far, showing a large interpatient and intrapatient variability that requires the adoption of TDM protocols. In the present study the unexplained IIV_{CL} (46.3%) was accompanied by a IOV_{CL} that accounted for 20.0%. Together, these values strengthen the benefit to include IOV in the estimation of individual PK parameters (Karlsson and Sheiner, 1993), and do sustain the monitoring of ACV concentrations during chemotherapy, because the time-varying covariates help to explain the variability across different occasions. Since it is possible that "the magnitude of IOV increases with the time between study occasions" (Karlsson and Sheiner, 1993), the mean/median interval time between two consecutive occasions in the present study was 16.5/8 days (minimum-maximum range, 2–165 days), likely explaining the lower IOV_{CL} with respect to IIV_{CL}.

Some characteristics of the present study have to be pointed out. First of all, the number of enrolled patients did allow the analysis of patients who received IV ACV only, thus overcoming the variability associated with the oral administration of ACV or its prodrug valacyclovir. Second, the database used to develop the POP/PK model included plasma concentrations obtained at fixed time points that corresponded to those adopted in TDM protocols (Di Paolo et al., 2013) instead of dense blood sampling (Zeng et al., 2009), or random time points (Abdalla et al., 2020). The choice of the sampling scheme may depend on several factors, but ultimately the present POP/PK model returned PK estimates very similar to those published by other studies (Zeng et al., 2009; Abdalla et al., 2020). For example, the present mean values of CL (0.54 L/h/kg) and V (0.97 L/kg) were comparable to those previously reported in children with a mean bodyweight of 20 kg (Eksborg et al., 2002; Nadal et al., 2002). Analogous conclusions can be drawn for terminal $t_{1/2}$ (De Miranda and Blum, 1983; Zeng et al., 2009) and the IOV_{CL} value (20.0%), which was in agreement with the value (19.2%) found in a previous study (Zeng et al., 2009). Even the simulation part of the present study showed a concordance with previous findings. For example, ACV doses of 10–20 mg/kg administered as a conventional 1-h IV infusion every 6 h showed almost identical percentages of toxic concentrations (Abdalla et al., 2020). Moreover, the present findings are suggesting that a 20-mg/kg dose every 6 h may achieve effective concentrations in approximately 50% of patients, while the dose should be increased in children with ARC.

The study's novelty resides in the simulation of alternative regimens of acyclovir administration. With a short terminal half-life, the maintenance of effective C_{min} values is guaranteed by higher doses of acyclovir (i.e., 20 mg/kg) or more frequent dosing (i.e., every 6 h instead of 8 h). However, those high-dose-intensity regimens may

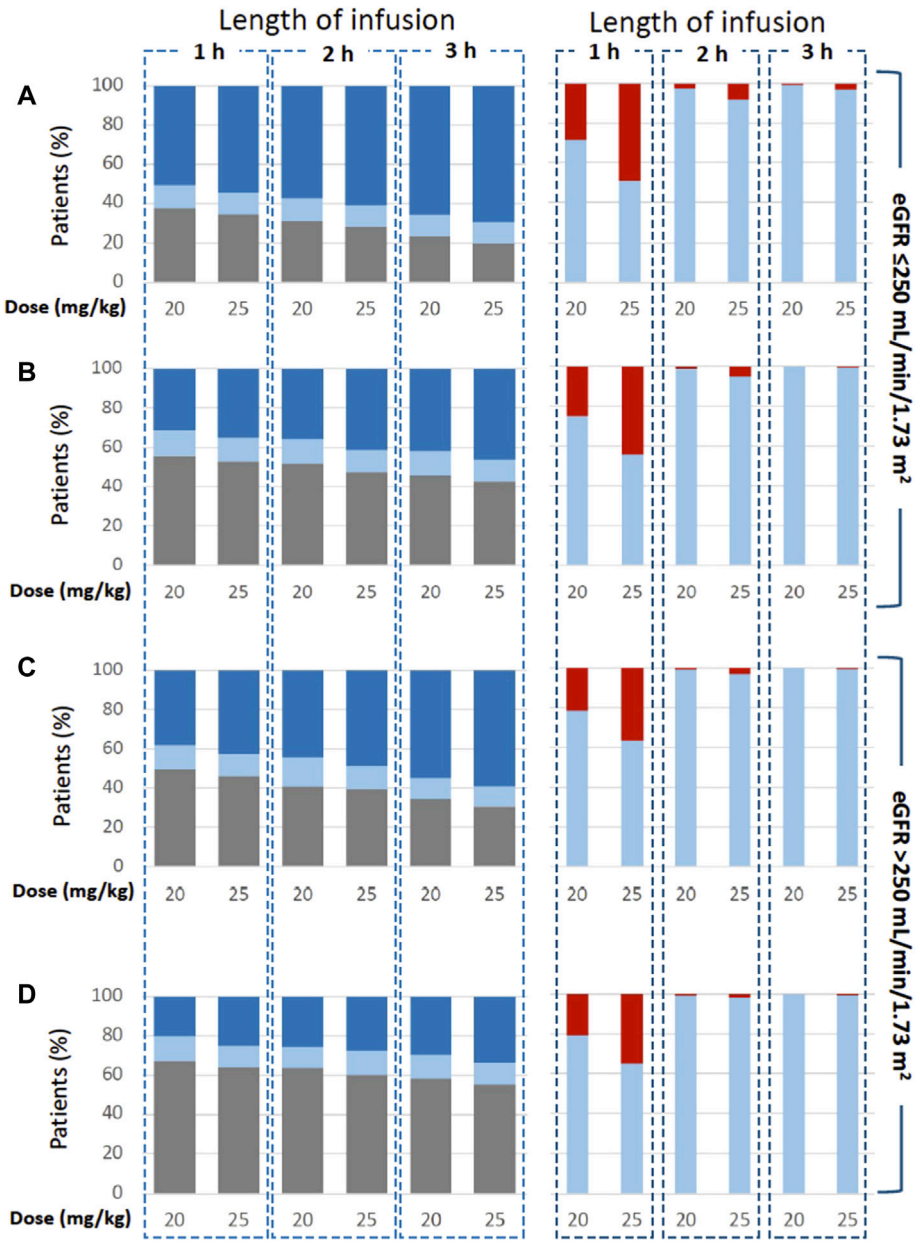

FIGURE 5 | Simulated regimens consisting in ACV 20 or 25 mg/kg administered as 1-h, 2-h and 3-h infusion every 6 h [graphs **(A,C)**] and 8 h [graphs **(B,D)**]. Plots show the probability of target attainment (on the left) according to C_{min} values < 0.56 (grey), ≥0.56-<1.125 (pale blue) and >1.125 mg/L (blue). Moreover, graphs show the probability of achieving C_{max} values < 25 (pale blue) and >25 mg/L (red).

result in high C_{max} values that could expose the patients to the risk of toxicities, while ineffective through concentrations may be measured especially in patients with ARC (Abdalla et al., 2020). As observed for other antimicrobial drugs that have a short plasma half-life, a prolonged (i.e., 2 or 3 h) or continuous infusion together with an appropriate dose increase may allow the achievement of effective target plasma concentrations. Indeed, the simulation of a prolonged infusion of a 20 mg/kg dose resulted in an increased percentage of patients achieving the predefined PK targets for the standard 1-h IV infusion. The percentage of patients who achieve effective C_{min} values sharply increased when the simulation considered a continuous infusion, even with a low dose-intensity regimen, consisting of 10-mg/kg doses every 8 h. Noteworthy, fragile patients affected by severe HSV infections were successfully cured with continuous IV infusions of acyclovir at higher doses (up to 30 mg/kg) (Engel et al., 1990; Kim et al., 2011). Interestingly, in most individuals "clinical response was seen within 72 h of continuous ACV administration" that "was well tolerated, even in patients with renal insufficiency." Indeed, the authors did not observe any sign of toxicity, including hematological adverse reactions or deterioration in renal function.

TABLE 7 | Results from simulated regimens consisting in ACV doses of 20 and 25 mg/kg infused in 1 h (standard regimen) or in prolonged infusions (2 and 3 h). The percentages of patients who achieved C_{min} values > 0.56 and >1.156 mg/L or C_{max} values > 25 mg/L are showed according to an eGFR threshold of 250 ml/min/m^2. Each regimen consists of 1,000 simulated patients.

		Time interval between doses: 6 h					
		1-h infusion		2-h infusion		3-h infusion	
	Dose (mg/kg)	20	25	20	25	20	25
eGFR ≤ 250	C_{min} > 0.56	62.5	65.7	69.3	72.1	76.9	79.9
	C_{min} > 1.125	50.9	54.6	57.4	61.0	66.0	69.6
	C_{max} > 25	28.6	49.1	2.5	8.0	0.6	3.2
eGFR > 250	C_{min} > 0.56	50.6	54.0	59.2	60.5	65.6	69.4
	C_{min} > 1.125	37.9	42.5	44.3	48.7	54.8	58.9
	C_{max} > 25	21.3	36.5	0.6	2.9	0.0	0.3
		Time interval between doses: 8 h					
eGFR ≤ 250	C_{min} > 0.56	44.4	47.2	48.3	5 2.6	54.3	57.3
	C_{min} > 1.125	31.2	35.0	35.5	41.2	41.9	46.2
	C_{max} > 25	25.0	44.2	1.0	5.1	0	0.6
eGFR > 250	C_{min} > 0.56	32.8	35.7	36.3	39.9	41.8	44.7
	C_{min} > 1.125	20.1	24.8	25.5	27.7	29.6	33.6
	C_{max} > 25	20.6	34.9	0.6	1.6	0	0.3

eGFR, estimated glomerular filtration rate; C_{min}, minimum plasma concentration; C_{max}, maximum plasma concentration.

Further studies have demonstrated that continuous infusions would prolong the time during which the ACV concentrations exceed the IC_{50} value for HSV and VZV and therefore may be considered a valid alternative to intermittent IV dosing (Spector et al., 1982; O'Leary et al., 2020). It is worth noting that in some cases, continuous ACV infusion was adopted to treat neonatal HSV encephalitis (Kakisaka et al., 2009; Cies et al., 2015; O'Leary et al., 2020). In particular, plasma concentrations were maintained above 3 mg/L (Cies et al., 2015) or even higher (5.5–8 mg/L) (O'Leary et al., 2020) to ensure cerebrospinal fluid concentrations of at last 1 mg/L.

Interestingly, a former study that enrolled 13 patients included 4 children who received continuous i.v., infusions of acyclovir at doses of 6.1–9.7 mg/kg/h from 7 up to 13 days (Fletcher et al., 1989). Plasma concentrations were ≥4.5 mg/L (up to 22.1 mg/L in some patients) and one child developed neutropenia, whereas none of the patients experienced renal insufficiency. In agreement with those observations, another study did not report signs or laboratory findings of systemic toxicity in 3 adolescents who received continuous i.v., infusions of ACV at doses of 7.2–28.8 mg/kg/day (Spector et al., 1982). Finally, the present simulation showed that a 10 mg/kg dose of ACV administered as a continuous infusion every 8 h allowed the achievement of therapeutic plasma concentrations in more than 90% of patients, regardless the eGFR value.

Those results do sustain the adoption of prolonged infusions (or continuous ones) because the reduced rate of drug infusion may be advantageous to decrease the risk of toxic C_{max} values. In particular, the percentage of patients at risk of higher peak plasma concentrations is abated, moving from a standard 1-h infusion to prolonged or continuous infusions. Additionally, a loading dose consisting of a 30-min infusion may precede the continuous infusion to ensure the achievement of therapeutic C_{min} values.

Although these premises of greater efficacy and good tolerability, the nephrotoxic effects of acyclovir in children have been associated with a variety of causes, as well as the concomitant administration of nephrotoxic drugs, a reduced eGFR at baseline, hypertension, older age, and obesity (Schreiber et al., 2008; Richelsen et al., 2018; Yalçınkaya et al., 2021). Therefore, these risk factors should be carefully evaluated, especially when dose-intense regimens were adopted (Fletcher et al., 1989).

In conclusion, the present findings confirm the high variability of ACV pharmacokinetics in immunocompromised children undergoing HSCT or myelotoxic chemotherapies, hence TDM protocols are recommended to adjust drug dosing. Indeed, standard dosing regimens seems adequate to achieve effective plasma concentrations of ACV, despite the drug could be ineffective in a variable percentage of some patients. The further increase in dosage may expose patients to an augmented risk of toxicities. Noteworthy, alternative regimens based on prolonged IV infusions (i.e., 20 mg/kg as a 3-h infusion every 6 h) or even continuous infusions (i.e., 10 mg/kg every 8 h) may increase the efficacy of ACV while reducing the risk of highest plasma peaks and sparing patients from severe toxicities. Prospective trials adopting TDM protocols are required to confirm the present findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the IRCCS Burlo Garofolo (reference no. IRB RC 10/20). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NM, DN, GL, RS, EP, LS, EB and AP developed the concept and designed the study. NM, DN, EP and LS. provided study material or participants. GL, RS and AP verified the data and performed the statistical analyses. NM, EB and AP wrote the initial draft of the manuscript. All authors provided critical comments and editing, contributed to the data interpretation, reviewed the analyses of this manuscript, and approved its final version.

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The Efficacy and Safety of Bivalirudin Versus Heparin in the Anticoagulation Therapy of Extracorporeal Membrane Oxygenation: A Systematic Review and Meta-Analysis

Min Ma[1,2†], Shichu Liang[1†], Jingbo Zhu[3], Manyu Dai[4], Zhuoran Jia[4], He Huang[1] and Yong He[1*]*

[1]Department of Cardiology, West China Hospital of Sichuan University, Chengdu, China, [2]Department of Cardiology, The Sixth People's Hospital of Chengdu, Chengdu, China, [3]Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4]Department of Cardiology, The First Affiliated Hospital of Anhui Medical University, Hefei, China

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***Correspondence:**
He Huang
huanghe@wchscu.cn
Yong He
heyong_huaxi@163.com

†These authors have contributed equally to this work

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Background: Bivalirudin is a direct thrombin inhibitor (DTI) that can be an alternative to unfractionated heparin (UFH). The efficacy and safety of bivalirudin in anticoagulation therapy in extracorporeal membrane oxygenation (ECMO) remain unknown.

Methods: This study followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. A systematic literature search was performed in PubMed, EMBASE, and The Cochrane Library databases to identify all relevant original studies estimating bivalirudin's efficacy and safety versus UFH as anticoagulation therapy in ECMO. The time limit for searching is from the search beginning to June 2021. Two researchers independently screened the literature, extracted data and evaluated the risk of bias of the included studies. The meta-analysis (CRD42020214713) was performed *via* the RevMan version 5.3.5 Software and STATA version 15.1 Software.

Results: Ten articles with 847 patients were included for the quantitative analysis. Bivalirudin can significantly reduce the incidence of major bleeding in children (I^2 = 48%, p = 0.01, odd ratio (OR) = 0.17, 95% confidence interval (CI): 0.04–0.66), patient thrombosis (I^2 = 0%, p = 0.02, OR = 0.58, 95% CI: 0.37–0.93), in-circuit thrombosis/interventions (I^2 = 0%, p = 0.0005, OR = 0.40, 95% CI: 0.24–0.68), and in-hospital mortality (I^2 = 0%, p = 0.007, OR = 0.64, 95% CI: 0.46–0.88). Also, comparable clinical outcomes were observed in the incidence of major bleeding in adults (I^2 = 48%, p = 0.65, OR = 0.87, 95% CI: 0.46–1.62), 30-day mortality (I^2 = 0%, p = 0.61, OR = 0.83, 95% CI: 0.41–1.68), and ECMO duration in adults (I^2 = 41%, p = 0.75, mean difference (MD) = −3.19, 95% CI: −23.01–16.63) and children (I^2 = 76%, p = 0.65, MD = 40.33, 95% CI: −135.45–216.12).

Conclusions: Compared with UFH, bivalirudin can be a safe and feasible alternative anticoagulant option to UFH as anticoagulation therapy in ECMO, especially for heparin resistance (HR) and heparin-induced thrombocytopenia (HIT) cases.

Keywords: extracorporeal membrane oxygenation, heparin, bivalirudin, meta-analysis, systematic review

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a life-supporting system that provides circulatory and/or pulmonary support for patients suffering from severe, life-threatening disease (Karagiannidis et al., 2016), including refractory acute heart failure, ST-segment elevation myocardial infarction (STEMI), or acute respiratory distress syndrome (ARDS). Moreover, ECMO is applied in severe conditions, such as heart transplantation and shock, as well. With the development of medical technology, ECMO complications have reduced significantly, with greatly improved survival rates. In recent studies, ECMO proved its superiority in reducing the mortality in patients with severe respiratory failure from COVID-19 (Shaefi et al., 2021). However, during ECMO treatment, coagulation-related complications (i.e., bleeding or thrombosis) remain the main factors affecting morbidity and mortality. Therefore, clinical researches has focused on the avoidance of those complications.

Blood's exposure to a foreign surface may render patients vulnerable to thromboembolic events, which can be prevented by the heparinization of blood (Finley and Greenberg, 2013). For decades, unfractionated heparin (UFH) has been the most common anticoagulant and mainstay antithrombotic in ECMO. Nevertheless, its clinical use may be restricted by UFH-related complications, such as heparin resistance (HR), caused by the consumptive deficiency of antithrombin (AT III), and heparin-induced thrombocytopenia (HIT). This devastating event may occur with heparin exposure (Ortel, 2009; Koster et al., 2013). Therefore, replacement of anticoagulation therapy appears crucial.

Bivalirudin is an alternative anticoagulant option. As an oligopeptide analog of hirudin, bivalirudin is a parenteral direct thrombin inhibitor (DTI), inherently independent of AT III. Moreover, bivalirudin is a bivalent DTI that binds specifically to thrombin at two sites without a cofactor (Warkentin et al., 2008). Furthermore, the reversible and transient binding to thrombin makes it a mainstream anticoagulant in the cardiac catheterization room (Warkentin et al., 2008). However, there are no large-scale, randomized controlled trials (RCTs) reporting the incidences of major bleeding, thrombosis, and mortality of bivalirudin versus UFH in the treatment of ECMO. Therefore, we believe it is worthwhile to carefully conduct a meta-analysis to evaluate the efficacy and safety of bivalirudin versus UFH in ECMO anticoagulation therapy.

METHODS

Study Design and Literature Search

This is a registered meta-analysis on PROSPERO (https://www.crd.york.ac.uk/prospero/). The registration number is CRD42020214713.

The participants, intervention, comparison, outcome, and study design approach (PCIOS) were used to select clinical studies (**Table 1**). Reviews, meta-analyses, non-human studies, case reports, and conferences were excluded. Studies that did not compare the clinical outcomes between UFH and bivalirudin were excluded as well. Two authors (S. Liang and J. Zhu) independently searched the PubMed, EMBASE, and The Cochrane Library databases for articles published from inception until 1 June 2021, using the heading terms "heparin," "unfractionated heparin," "bivalirudin," "extracorporeal membrane oxygenation," "ECMO," "ECMO treatment," "ECLS," or "ECLS treatment". No language restrictions were used. The references of relevant literature were also searched to look for more eligible studies.

Data Extraction and Quality Assessment

Data were extracted by the same two independent readers (S. Liang and J. Zhu) who performed the literature search and study selection; the researchers were not blinded to the authors and institutions of included studies. Disagreements were solved by a third reader (M. Ma). Y. He supervised the whole process. This meta-analysis followed the guidelines for preferred reporting items for systematic reviews and meta-analyses (PRISMA) (Shamseer et al., 2015). The two reviewers extracted the following information independently: the first author, published year, study design (prospective/retrospective), study duration, total patients and number of patients in the bivalirudin and UFH groups, the doses in the bivalirudin and UFH group, and the incidence of thromboses, major bleeding, and mortality (per-patient).

For the observational studies, the Newcastle-Ottawa Scale (NOS) was used to assess the risk of bias. The NOS ranges from 0 (lowest) to 9 (highest), and studies with scores ≥6 are considered high quality. For RCTs, the modified Jadad quality scoring scale is used for the quality assessment, which includes the generation of random sequences, distribution methods, randomized concealment, double-blinding, withdrawals and dropouts. The Jadad score among four to seven is considered as good quality.

Sensitivity analysis of the included studies was conducted via a one-by-one elimination method to evaluate the meta-analysis's stability. A Galbraith plot was used to find the cause of heterogeneity. Egger's test was used to test the publication bias via Stata version 15.1 Software (The StataCorp LP, Texas City, United States).

Statistical and Meta-Analysis

For studies describing the results via median and interquartile range (IQR), Standard deviations (SDs) of the mean differences (MDs) were obtained as described by former researches (Wan et al., 2014; Luo et al., 2018). The RevMan version 5.3.5 Software (The Cochrane Collaboration, Copenhagen, Denmark) and Stata version 15.1 Software (The StataCorp LP, Texas City, United States) was used for all statistical analyses. Statistical

TABLE 1 | ''PICOS'' approach for selecting clinical studies in the systematic search.

PICOS	
1 Participants	The patients (both adult and pediatrics) receiving the treatment of ECMO despite differences in ECMO indication and configuration, concurrent medications, and presence of HIT and HR.
2 Intervention	The patients who took bivalirudin during the treatment of ECMO.
3 Comparison	The patients who took UFH during the treatment of ECMO.
4 Outcomes	The incidence rate of major bleeding, thrombosis, and mortality
5 Study design	Prospective and retrospective observational studies; RCTs

ECMO, extracorporeal membrane oxygenation; HR, heparin resistance; HIT, heparin-induced thrombocytopenia; UFH, unfractionated heparin; RCT,randomized controlled trials.

heterogeneity was assessed by using the Cochrane Q and the I square statistics. Heterogeneity was interpreted as absent (I^2: 0–25%), low (I^2: 25.1–50%), moderate (I^2: 50.1–75%), or high (I^2: 75.1–100%) (Higgins et al., 2003). The use of a random-effects model was also considered when the number of studies was relatively small, and a random-effects model was applied to estimate the continuous outcome data for data with a p-value ≤0.1 and an I^2-value >50%, which indicated statistical heterogeneity (Higgins et al., 2003). Otherwise, a fixed-effects model was used. The overall log with its 95% CI was used as the summary of the overall effect size. A p-value <0.05 was considered statistically significant.

RESULTS

Literature Search and Study Selection

The literature search produced 125 total findings (101 on EMBASE, 4 on The Cochrane Library, and 20 on PubMed); 80 full texts were retrieved after duplicates were removed. The titles and abstracts of studies were screened, after which 53 articles were excluded due to the following reasons: systematic reviews (n = 2), reviews, letters and editorials (n = 34), case reports (n = 17). A total of 27 full-text articles were reviewed, and 17 were excluded later because they lacked the comparison between UFH and bivalirudin (n = 15) or they are only with abstracts (n = 2). Finally, ten unique retrospective observational studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Macielak et al., 2019; Brown et al., 2020; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) with 847 patients were included for the quantitative analysis. All articles were published before 1 June 2021. The literature screening process is presented in **Figure 1**.

Data Extraction and Quality Assessment

Table 2 shows basic information from the included studies; **Table 3** shows group definition of the bivalirudin group and clinical outcomes. Generally, these included studies met most NOS quality indicators. However, the control group of all the studies did not meet the standard of "community controls" and "no history of diseases" as the controls was from a hospital. Moreover, as the included studies were all case-control retrospective studies, they were not blinded to the case/control status. According to the NOS, all the included studies were considered as high quality (**Supplemental Table S1**).

Major Bleeding

Nine studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Macielak et al., 2019; Brown et al., 2020; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021) reported the incidence of major bleeding. Two studies (Macielak et al., 2019; Brown et al., 2020) were not included due to the different ways of expression (per ECMO day). The incidence rate of major bleeding is 0.223 and 0.139 per ECMO day in the UFH and bivalirudin group in Macielak et al. (Macielak et al., 2019)'s study, 0.308 and 0.062 per ECMO day in Brown et al. (Brown et al., 2020)'s study, respectively. Regarding Ljajikj et al. (Ljajikj et al., 2017)'s study, we considered both delayed chest closure and intracranial bleeding as major bleeding because the authors thought that delayed chest closure might also be the result of diffuse persisting bleeding.

Seven studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021) were included in the meta-analysis, and moderate heterogeneity was observed (I^2 = 59%, p = 0.02), therefore a subgroup analysis was conducted. Five studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Kaseer et al., 2020) were included in the adults group, whilst two studies (Hamzah et al., 2020; Schill et al., 2021) were include in the children group (**Figure 2**). Low heterogeneity was observed in both adults and children group (I^2 = 48% and 48%, p = 0.10 and 0.16, respectively), therefore a fixed-effects model was used. The results showed that the difference of pooled incidence of major bleeding was significantly reduced in children (I^2 = 48%, p = 0.01, odd ratio (OR) = 0.17, 95% confidence interval (CI): 0.04–0.66) in the bivalirudin group, but not in adults (I^2 = 48%, p = 0.65, OR = 0.87, 95% CI: 0.46–1.62). The heterogeneity decreased after subgroup analysis, which indicates that the age maybe one of the sources of heterogeneity.

The sensitivity analysis of the incidence of major bleeding of the included studies showed that all studies' estimate was within 95% CI of the total effect except for Berei et al. (Berei et al., 2018)'s study, which means the analytical stability may be affected (**Supplementary Figure S1**). After removing the study the difference of pooled incidence of major bleeding was still not significantly reduced in the adult group (I^2 = 0%, p = 0.05, OR = 0.40, 95% CI: 0.16–0.99).

TABLE 2 | Basic information of the included studies.

Study	Duartion	Total Patients (Pediatric Patients)	VV/VA-ECMO	Indication of ECMO (Number of patients)	Heparin bolus	Bivalirudin group		Heparin group	
						Dose	Number (Pediatric Patients)	Dose	Number (Pediatric Patients)
Ranucci2011	January 2008-April 2011	21 (9)	NR	Postcardiotomy ECMO procedure (21)	100U/kg	0.03–0.05 mg/kg/h	13 (4)	5–10 U/kg/h	8 (5)
Pieri2012	January 2008-March 2011	20 (0)	10/10	NR	NR	0.025 mg/kg/h	10 (0)	3 U/kg/h	10 (0)
Ljajikj2017	March 2012-March 2016	57 (0)	NR	Left ventricular assist device implantation(57)	10 000 U	APTT>160s: 0.25 mg/kg/h APTT<160s: 0.5 mg/kg/h	21 (0)	NR	36 (0)
Berei2018	January 2012 -September 2015	72 (0)	6/66	Cardiogenic shock (51) Septic shock (11) Respiratory shock (4) Mixed shock (6)	80U/kg	0.04 mg/kg/h	44 (0)	8–12 U/kg/h	28 (0)
Macielak2019	January 2012 -June 2017	110 (0)	NR	Emergency salvafe (61) Cardiogenic shock (46) ARDS (29) Respiratory insufficiency (29) Failed to wean from CRB(23) Others (12)	50–100U/kg	0.01–0.1 mg/kg/h	10 (0)	12 U/kg/h	100 (0)
Brown2020	March 2014-January 2018	15 (0)	7/5(3 peripheral RVAD)	NR	80U/kg	NR	NR	NR	NR
Hamzah2020	October 2014-May 2018	32 (32)	3/29	Heart transplantation (32)	50–100U/kg	Ccr>60 ml/min: 0.3 mg/kg/h renal impairment: 0.15 mg/kg/h	16 (16)	open chest: 10U/kg/h <12M: 18U/kg/h 1Y-12Y: 16U/kg/h >12Y: 14U/kg/h	16 (16)
Kaseer2020	January 2013-September 2018	52 (0)	24/28	Cardiogenic shock (13) Respiratory failure (13) Heart and/or lung transplant (9) Others (1)	NR	0.1 mg/kg/h	19 (0)	10.4 U/kg/h	33 (0)
Schill2021	June 2018-December 2019	48 (0)*	16/32*	Postcardiotomy shock (16)* Respiratory failure (17)* Cardiogenic shock (15)*	50–100U/kg	Typical: 0.15 mg/kg/h Ccr<30 ml/min: 0.075 mg/kg/h Receiving CRRT: 0.1 mg/kg/h	14 (0)*	20 U/kg/h or 28 U/kg/h	34 (0)*

(Continued on following page)

TABLE 2 | (*Continued*) Basic information of the included studies.

Study	Duartion	Total Patients (Pediatric Patients)	VV/VA-ECMO	Indication of ECMO (Number of patients)	Heparin bolus	Bivalirudin group		Heparin group	
						Dose	Number (Pediatric Patients)	Dose	Number (Pediatric Patients)
Seelhammer2021	January 2014-October 2019	422 (89)	64/358	Post cardiotomy (162) Cardiac (100) Respiratory (86) Extracorporeal Cardiopulmonary Resuscitation (69) Post transplant (5)	1000U/kg	NR	134 (24)	NR	288 (65)

*ARDS, acute respiratory distress syndrome; CPB, cardiopulmonary bypass; CRRT, continuous renal replacement therapy; VV-ECMO, Venovenous ECMO; VA-ECMO, Venoarterial ECMO; NR, Not reported; RVAD, right ventricular assist device. *runs.*

TABLE 3 | Definition of the bivalirudin group and clinical outcomes.

Study	Definition		
	Bivalirudin group	Thrombosis	Major Bleeding
Ranucci2011	Non-HIT patients	NR	NR
Pieri2012	Non-HIT patients	Thrombosis could be attributed either to the patient (ie, venous or arterial occlusion with clinical signs and symptoms or evident at the radiologic examination) or the oxgenator	NR
Ljajikj2017	HIT patients	NR	NR
Berei2018	Non-HIT patients	Clinically documented venous or arterial thromboembolism or thrombus within the ECMO circuit	Any bleeding event associated with a drop in hemoglobin of at least 3 mg/dl within the prior 24 h
Macielak2019	Non-HIT patients	Requirement for oxygenator exchange, requirement for circuit exchange, laboratory values indicating acute hemolysis (pfHg>50 mg/dl or LDH>1,000U/L), or systemic thromboembolism including VTE, intra-cardiac thrombus, or ischemic stroke	Clinically overt bleeding associated with a hemoglobin fall of at least 2 g/dl in a 24-h period or a transfusion requirement of one or more 10 ml/kg PRBC transfusions over that same time period
Brown2020	Non-HIT patients	Ischemic cerebral vascular accidents, ischemic digits, visceral ischemia, or pump failure due to suspected thrombosis	Intracranial hemorrhage, decrease in hemoglobin by 3 g/dl over 24 h in the setting of a bleed with overt source, hemodynamic instability with associated blood transfusion, fatal bleeding, and bleeding requiring an intervention such as epistaxis requiring nasal packing, GI bleeding with cauterization or clipping, washoutetc.
Hamzah2020	Non-HIT patients	Significant thrombosis is defined as thromboembolic events to the brain, visceral organs, or extremities. Circuit thrombosis that leads to circuit change is considered significant thrombosis	Bleeding associated with a decrease in the measurement of hemoglobin by 2 g/d or transfusion of packed RBCs at a rate greater than 20 ml/kg over 24 h. CNS bleeding or bleeding that requires surgical intervention would also be considered a significant bleeding event
Kaseer2020	Non-HIT patients and HIT patients	Composite thrombotic events postdecannulation defined as arterial and/or venous thromboembolism within 72 h of ECMO decannulation	Any bleed with a drop in hemoglobin of ≥3 mg/dl within 24 h
Schill2021	Non-HIT patients	Patients with a history of thrombophilia, arterial or venous thromboses, or circuit thromboses were considered to have high thrombotic risk	Patients with intracranial hemorrhage, bleeding requiring surgical intervention or massive transfusion were considered high bleeding risk
Seelhammer2021	Non-HIT patients	Ischemic complication (stroke, deep vein thrombosis, pulmonary, myocardial infarction, mesenteric ischemia)	NR

CNS, central nervous system; ECMO, extracorporeal membrane oxygenation; GI, gastro intestinal; HIT, heparin-induced thrombocytopenia; LDH, lactate dehydrogenase; NR, Not reported; PRBC, packed red blood cell; pfHg, plasma free hemoglobin; VTE, venous thromboembolism.

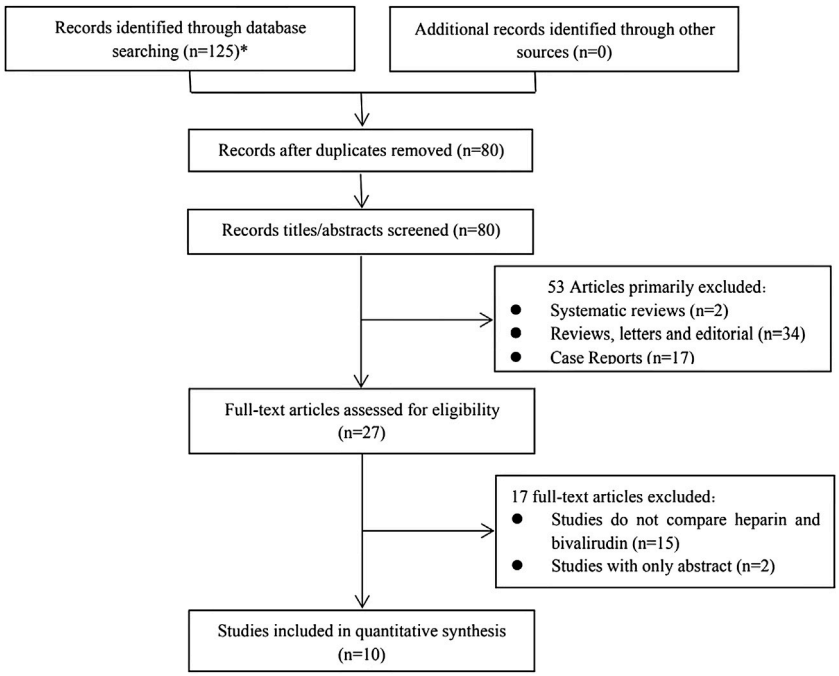

FIGURE 1 | Flow chat of study selection (*101 from Embase, 20 from Pubmed and 4 from The Cochrane Library).

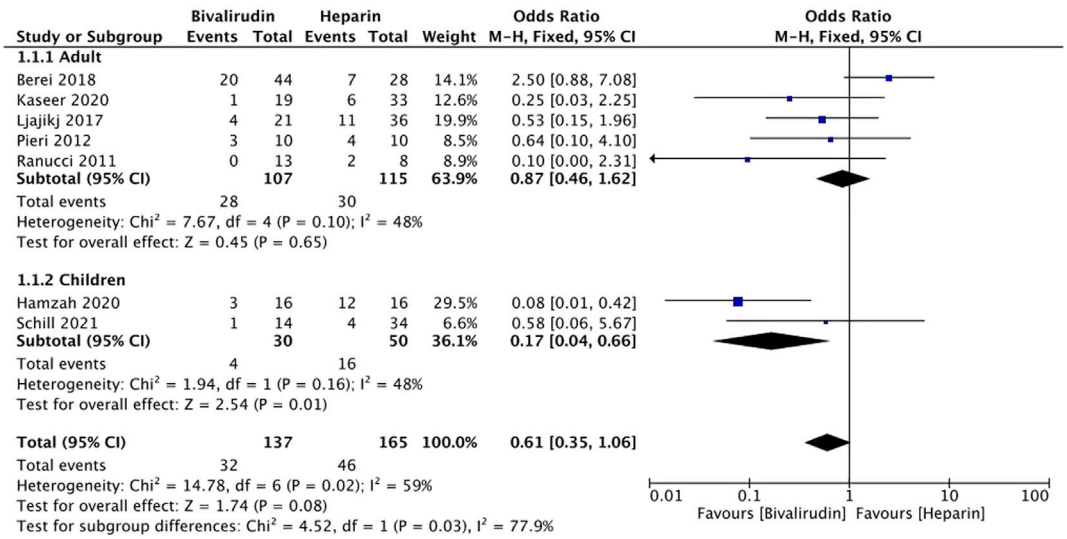

FIGURE 2 | The incidence of major bleeding between the bivalirudin group and the heparin group.

Thrombosis

Ten studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Macielak et al., 2019; Brown et al., 2020; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) reported the incidence of thrombosis. Two studies (Macielak et al., 2019; Brown et al., 2020) were not included due to the different ways of expression (per ECMO day). The incidence rate of thrombosis is 0.207 and 0.089 per ECMO day in the UFH and bivalirudin group in Macielak et al. (Macielak et al., 2019)'s study, 0.043 and 0 per ECMO day in Brown et al. (Brown et al., 2020)'s study, respectively.

Thrombosis can be divided into patient thrombosis and in-circuit thrombosis/interventions. Eight studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) reported the incidence of patient thrombosis and six studies

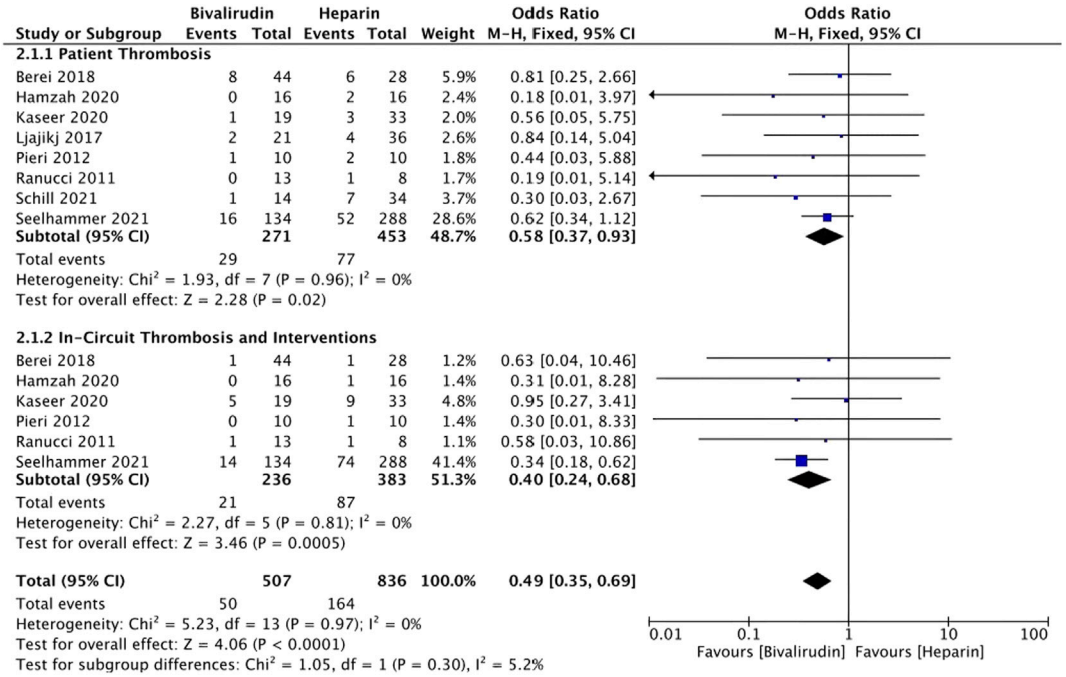

FIGURE 3 | The incidence of thrombosis between the bivalirudin group and the heparin group.

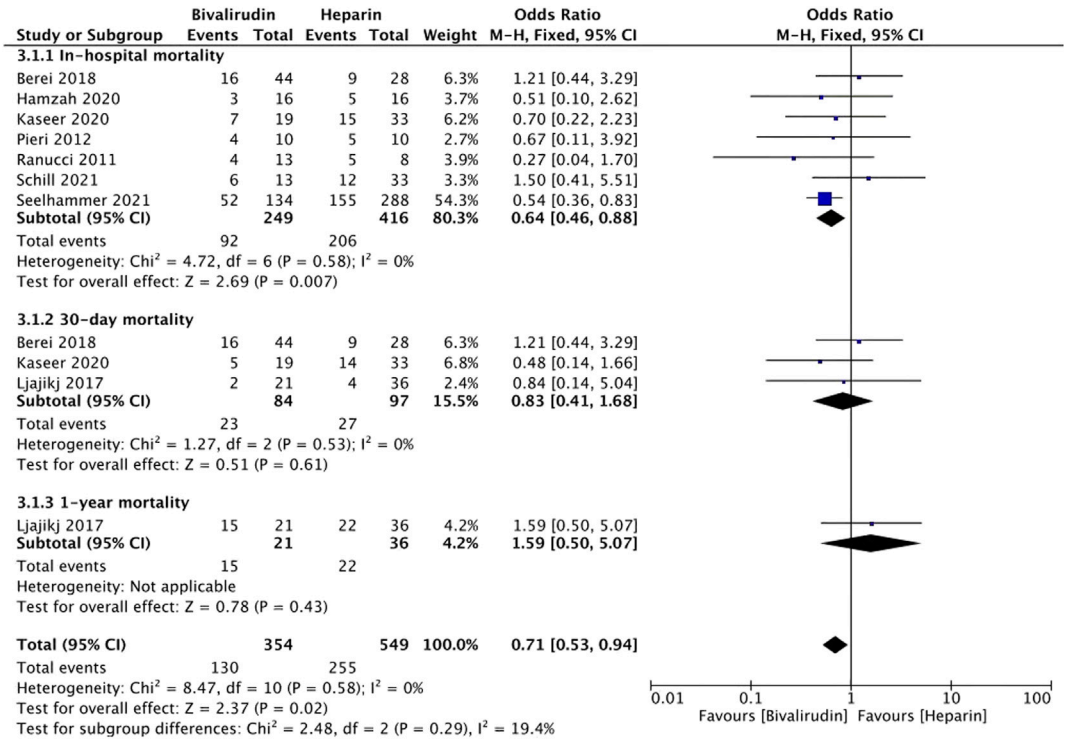

FIGURE 4 | The incidence of mortality between the bivalirudin group and the heparin group.

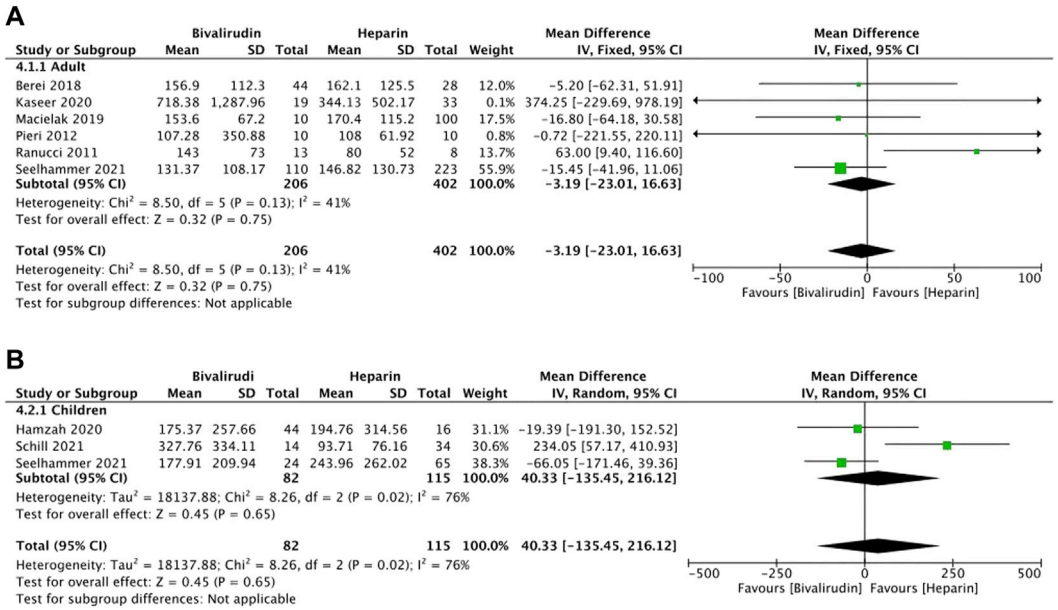

FIGURE 5 | The ECMO duration between the bivalirudin group and the heparin group. **(A)** adult group; **(B)** children group.

(Ranucci et al., 2011; Pieri et al., 2013; Berei et al., 2018; Hamzah et al., 2020; Kaseer et al., 2020; Seelhammer et al., 2021) reported the incidence of in-circuit thrombosis/interventions group (**Figure 3**). Low heterogeneity was observed in both group ($I^2 = 0\%$, $p = 0.96$ and 0.81, respectively), therefore a fixed-effects model was used. The results showed that both the difference of pooled incidence of patient thrombosis ($I^2 = 0\%$, $p = 0.02$, OR = 0.58, 95% CI: 0.37–0.93) and in-circuit thrombosis/interventions ($I^2 = 0\%$, $p = 0.0005$, OR = 0.40, 95% CI: 0.24–0.68) was significantly reduced in the bivalirudin group. The sensitivity analysis showed that all studies' estimate was within 95% CI of the total effect, which means the analytical stability was not affected (**Supplementary Figure S2**)

Mortality

Seven studies (Ranucci et al., 2011; Pieri et al., 2013; Ljajikj et al., 2017; Berei et al., 2018; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) reported the incidence of mortality. Mortality can be divided into in-hospital mortality, 30-day mortality, and 1-year mortality. Seven studies (Ranucci et al., 2011; Pieri et al., 2013; Berei et al., 2018; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) were included in the in-hospital mortality group, three studies (Ljajikj et al., 2017; Berei et al., 2018; Kaseer et al., 2020) were included in the 30-day mortality group, and only one study (Ljajikj et al., 2017) was included in the 1-year mortality group (**Figure 4**). Low heterogeneity was observed in both in-hospital mortality and 30-day mortality group ($I^2 = 0\%$, $p = 0.58$ and 0.53, respectively), therefore a fixed-effects model was used. The results showed that the difference of pooled incidence of in-hospital mortality was significantly reduced in the bivalirudin group ($I^2 = 0\%$, $p = 0.007$, OR = 0.64, 95% CI: 0.46–0.88), but the difference of pooled incidence of 30-day mortality was not significant ($I^2 = 0\%$, $p = 0.61$, OR = 0.83, 95% CI: 0.41–1.68). The sensitivity analysis showed that all studies' estimate was within 95% CI of the total effect, which means the analytical stability was not affected (**Supplementary Figure S3**)

ECMO Duration

Eight studies (Ranucci et al., 2011; Pieri et al., 2013; Berei et al., 2018; Macielak et al., 2019; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) reported the ECMO duration, five of which (Pieri et al., 2013; Hamzah et al., 2020; Kaseer et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) described the ECMO duration between the bivalirudin group and the heparin group *via* IQR (**Figure 5**). Six studies (Ranucci et al., 2011; Pieri et al., 2013; Berei et al., 2018; Macielak et al., 2019; Kaseer et al., 2020; Seelhammer et al., 2021) were included in the adult group and three studies (Hamzah et al., 2020; Schill et al., 2021; Seelhammer et al., 2021) were included in the children group. Low heterogeneity was observed in the adult group ($I^2 = 41\%$, $p = 0.13$), therefore a fixed-effects model was used. The results showed that the MD of pooled ECMO duration was not significant between the two groups ($I^2 = 41\%$, $p = 0.75$, MD = -3.19, 95% CI: -23.01–16.63).

High heterogeneity was observed in the children group ($I^2 = 76\%$, $p = 0.02$), therefore a random-effects model was used. The results showed that the MD of pooled ECMO duration was not significant between the two groups ($I^2 = 76\%$, $p = 0.65$, MD = 40.33, 95% CI: -135.45–216.12).

After removing the study conducted by Schill et al. (2021), the heterogeneity of this outcome decreased significantly ($I^2 = 0\%$, $p = 0.68$), which indicated the main source of heterogeneity. Therefore, a fixed-effects model was used. The results showed that the MD of pooled ECMO duration was not significant between the two groups ($I^2 = 0\%$, $p = 0.25$, MD = -53.30, 95% CI: -143.16–36.56). However, a directional change occurred after removing Schill et al. (2021), indicating that the results of this meta-analysis maybe not that

stable, more studies should be included. For adults' ECMO duration, the sensitivity analysis showed that all studies' estimate was within 95% CI of the total effect, which means the analytical stability was not affected (**Supplementary Figure 4A**). For children's ECMO duration, the sensitivity analysis showed that two studies' (Schill et al., 2021; Seelhammer et al., 2021) estimate was not within 95% CI of the total effect, which means the analytical stability maybe affected (**Supplementary Figure 4B**).

Publication Bias

Egger's and Begg's tests suggested no significant publication bias of the incidence of major bleeding (Egger $p = 0.093$ and Begg $p = 0.368$), patient thrombosis (Egger $p = 0.116$ and Begg $p = 0.035$), circuit thrombosis (Egger $p = 0.503$ and Begg $p = 0.452$), in-hospital mortality (Egger $p = 0.551$ and Begg $p = 0.764$), 30-day mortality (Egger $p = 0.757$ and Begg $p = 1$), ECMO duration in adults (Egger $p = 0.156$ and Begg $p = 0.133$) and children (Egger $p = 0.282$ and Begg $p = 0.296$).

DISCUSSION

Though no large-scale clinical trials have compared the prognosis of anticoagulation therapy with bivalirudin or UFH, bivalirudin has become the first-line anticoagulant therapy strategy for patients with HR, HIT, or those who need surgery. It is widely used in patients undergoing high-risk percutaneous coronary intervention (PCI) and transcatheter aortic valve implantation (TAVI) (Stone et al., 2006; Kastrati et al., 2008; Stone et al., 2008; Han et al., 2015; Ahmad et al., 2017; Villablanca et al., 2017). To our knowledge, this is the first registered meta-analysis exploring the efficacy and safety of bivalirudin versus UFH in anticoagulation therapy in ECMO. The results showed that bivalirudin can significantly reduce the incidence of major bleeding in children, thrombosis in both patients and pumps, and in-hospital mortality. Also, comparable clinical outcomes were observed in the incidence of major bleeding in adults, 30-day mortality, and ECMO duration.

There are great challenges in treating patients receiving ECMO, and finding the balance between anticoagulation therapy and hemorrhagic complications is essential. Major bleeding is one of the most common complications of ECMO, often affecting the mortality of the patients. We found that bivalirudin can reduce the incidence of major bleeding in children, this is the same in Hamzah et al. (Hamzah et al., 2020)'s study. This phenomenon may due to the reason that children's livers are immature, and their anticoagulant proteins are defective. What is more, children are more prone to develop HR. Hamzah et al. (Hamzah et al., 2020) observed a shorter time to reach treatment anticoagulation levels and fewer bleeding events in the bivalirudin group than that in the UFH group. As an anticoagulant, UFH can stimulate platelet activation *in vivo*, while bivalirudin can be used as an inhibitor of thrombin-dependent platelet activation and collagen-induced platelet procoagulant activity (Busch et al., 2009; Kimmelstiel et al., 2011). Bivalirudin has better antithrombotic and anticoagulant effects than UFH, with less platelet activation and consumption (Burstein et al., 2019). This may explain children's lower tendency of major bleeding in the bivalirudin group.

Although low-dose UFH seems to safely reduce the risk of major bleeding and not increase the risk of thrombosis (Carter et al., 2019; Wood et al., 2020), it may not be practical in patients with HIT and HR. As a way to reduce the UFH dose, a heparin-coating circuit can reduce coagulation activation and the inflammatory reaction, protect platelets and coagulation factors, improve biocompatibility, avoid high-dose systemic heparinization, and reduce the dosage of UFH. Nevertheless, studies have reported that the release of UFH from the circuit may also be responsible for HIT, even in small quantities (Pappalardo et al., 2009). In some department protocols, the heparin-coating circuit's use was continued even after the diagnosis of HIT (Koster et al., 2000). These findings highlight the pitfalls of UFH and the strengths of bivalirudin. The consumption of platelet and thrombin may lead to consumption coagulopathy, which causes intravasular and extravascular thrombosis. Additionally, the complexity of pharmacokinetic parameters may increase due to the increase of volume distribution and random adsorption of drugs on different parts of the pump, which requires continuous dose titration of UFH (Kato et al., 2021). Compared with UFH, bivalirudin has a more predictable pharmacokinetic profile, a greater reduction in thrombin, and no associated incidence of HIT (Netley et al., 2018). HIT can leads to death in some severe cases (Zhong et al., 2020), which greatly affects the in-hospital mortality. This explains the lower in-hospital mortality in the bivalirudin group.

The activated partial thromboplastin time (APTT) value reflects anticoagulation condition: the higher the values, the higher the risk of bleeding. Kaseer et al. found that compared with UFH, the percentage of time that the APTT was within the therapeutic range was higher with bivalirudin (50 vs. 85.7%; $p = 0.007$), which means that bivalirudin more consistently maintained the APTT within the therapeutic range in comparison to UFH. Bivalirudin appears to be a reliable alternative anticoagulation option in patients with pediatric ECMO who have failed UFH (Cuker et al., 2018). The researchers recommended an initial bivalirudin dose of 2.5 mcg/kg/min for all patients, checking the APTT 2 hours after the initial dose and then every 4 hours after that (Netley et al., 2018). However, the optimal monitoring strategy remains to be explored (Ryerson et al., 2020). To monitor bivalirudin therapy, APTT hepzyme (HPTT), intrinsic coagulation pathways with heparinase (HEPTEM), and measurement of the clotting time is recommended (Teruya et al., 2020).

Economic factors should also be taken into consideration as comparable clinical outcomes of the incidence of major bleeding in adults, 30-day mortality, and ECMO duration in both groups. For patients with acute myocardial infarction, treatment with bivalirudin may be a cost-effective option rather than heparin plus glycoprotein IIb/IIIa inhibitor (Schwenkglenks et al., 2011; Schwenkglenks et al., 2012). This cost-effectiveness may translate into the ECMO population as well. In ECMO anticoagulation therapy, although bivalirudin is much more expensive than UFH (reportedly $1170 per vial) (Kaseer et al., 2020), the total cost might be lower due to less frequent monitoring, platelet transfusion, etc (Hamzah et al., 2020; Kaseer et al., 2020). Furthermore, Ranucci et al. also reported that the bivalirudin group lost less blood ($p = 0.015$), and therefore required fewer platelet concentrates ($p = 0.008$), fresh frozen plasma ($p = 0.02$), and purified antithrombin ($p = 0.048$). Thus, the daily cost of ECMO

was significantly lower in the patients in the bivalirudin group (Ranucci et al., 2011).

Our study indicated that bivalirudin may provide superior anticoagulation therapy in ECMO compared to that of UFH. For the incidence of major bleeding and thrombosis, patients who received bivalirudin or any other DTI may have done so because of the HIT potential, a hypercoagulable syndrome already predisposing patients to worse outcomes, especially regarding potential thrombotic sequela. For mortality, the underlying cause of patients requiring ECMO is likely the major determinant of outcomes and is already associated with an extremely high risk of adverse events. For the ECMO duration, weaning from ECMO differs between centers, and there is no specific information about standardized weaning protocols (Lüsebrink et al., 2020), more studies are truly requested. From our perspective, to rationally use bivalirudin in ECMO, the baseline APTT value, the presence of renal and/or liver insufficiency, the use of other drugs (e.g., argatroban) (Geli et al., 2021), the possibility of bivalirudin resistance, and the methods of operation should all be taken into consideration.

Strengths and Limitations

Compared with the former systematic review (Sanfilippo et al., 2017), our study introduced new clinical studies and expanded the sample size, and we conducted the first meta-analysis. However, there are still some limitations in our study. Initially, the studies included were retrospective small-size studies, which means the argumentation intensity may not be strong enough, and only a hypothesis can be generated. We hope that there will be more large-scale RCTs in the future. Secondly, though sensitivity and subgroup analyses were performed, the patients' variable character may lead to heterogeneity, which may affect the stability of the results. Future research is essential to ensure the homogeneity of the population as much as possible. Finally, the lack of specific results or available research data may restrict our subgroup analysis, such as the use of bivalirudin versus UFH in a different type of ECMOs (VV or VA), and different indications (STEMI, ARDS, heart transplantation, and so forth); these can be further investigated in future studies and enrolled in the sensitivity analysis. Despite these limitations, our meta-analysis provides valuable insight into the use of bivalirudin in the anticoagulation therapy of ECMO.

CONCLUSION

Bivalirudin can significantly reduce the incidence of major bleeding in children, patient thrombosis, in-circuit thrombosis/interventions, and in-hospital mortality. Though comparable clinical outcomes were observed in the incidence of major bleeding in adults, 30-day mortality, and ECMO duration, the incidence of the aforementioned complications is seemingly lower in the bivalirudin group. Compared with UFH, bivalirudin is safer, more practical, and dependable, which can be a safe and feasible alternative anticoagulant option to UFH as anticoagulation therapy in ECMO, especially for patients at risk for HR and HIT.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

MM and SL contributed equally to this work. MM, SL, JZ, ZJ, MD, HH, and YH conceived the study, participated in the design, collected the data, performed statistical analyses and drafted the manuscript. SL and JZ performed statistical analyses and helped to draft the manuscript. SL and JZ collected the data and revised the manuscript critically for important intellectual content. ZJ and MD helped for the language editing and data revision. MM, HH, and YH helped to revise the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.771563/full#supplementary-material

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REVIEW
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The Inflammation in the Cytopathology of Patients With Mucopolysaccharidoses-Immunomodulatory Drugs as an Approach to Therapy

Anna-Maria Wiesinger[1,2], Brian Bigger[2,3], Roberto Giugliani[4], Maurizio Scarpa[2,5], Tobias Moser[6], Christina Lampe[2,7], Christoph Kampmann[8] and Florian B. Lagler[1,2]*

[1]Institute of Congenital Metabolic Diseases, Paracelsus Medical University, Salzburg, Austria, [2]European Reference Network for Hereditary Metabolic Diseases, MetabERN, Udine, Italy, [3]Stem Cell and Neurotherapies, Division of Cell Matrix Biology and Regenerative Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom, [4]Department of Genetics, Medical Genetics Service and Biodiscovery Laboratory, HCPA, UFRGS, Porto Alegre, Brazil, [5]Regional Coordinating Center for Rare Diseases, University Hospital Udine, Udine, Italy, [6]Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University, Salzburg, Austria, [7]Department of Child and Adolescent Medicine, Center of Rare Diseases, University Hospitals Giessen/Marburg, Giessen, Germany, [8]Department of Pediatric Cardiology, University Hospital Mainz, Mainz, Germany

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Edited by:
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***Correspondence:**
Anna-Maria Wiesinger
anna.wiesinger@pmu.ac.at

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Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases (LSDs), characterized by the accumulation of glycosaminoglycans (GAGs). GAG storage-induced inflammatory processes are a driver of cytopathology in MPS and pharmacological immunomodulation can bring improvements in brain, cartilage and bone pathology in rodent models. This manuscript reviews current knowledge with regard to inflammation in MPS patients and provides hypotheses for the therapeutic use of immunomodulators in MPS. Thus, we aim to set the foundation for a rational repurposing of the discussed molecules to minimize the clinical unmet needs still remaining despite enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT).

Keywords: mucopolysaccharidoses, MPS, immunomodulation, inflammation, cytopathology, drug discovery

1 INTRODUCTION

Mucopolysaccharidoses (MPSs) are a heterogeneous group of congenital lysosomal storage disorders (LSDs) caused by the deficiency in one of the enzymes involved in the degradation of glycosaminoglycans (GAGs). These macromolecules provide structural support to the extracellular matrix (ECM) and are involved in the cellular regulation and communication processes (Sepuru and Rajarathnam, 2019). MPSs are classified into 7 main types and several subtypes, related to 11 specific enzyme deficiencies (Neufeld and Muenzer, 2001). Although the clinical features exhibited by MPS patients differ depending on species of GAGs accumulated, reduced life expectancy is present in all types. Clinical symptoms, age of presentation onset, diagnosis, treatment and complications vary from one MPS to another and even within the spectrum of the same MPS type. For this reason, transdisciplinary work to diagnose, treat and support is required.

At this point, only enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) are available for the treatment of patients in a limited number of MPS

types. Although ERT and HSCT are causal therapies, neither are curative and show several limitations (Lagler, 2018). ERT effects, particularly on bone and CNS pathology is very limited, as bioavailability in these target tissues is low (Chen et al., 2019). HSCT is only confirmed to be effective in the CNS in early detected cases of MPS I. For patients with other MPS types, evidence of efficacy is still limited or absent (Beck, 2007). There is no approved therapeutic option for patients with MPS IIIA-D, MPS IVB or MPS IX. Thus, additional therapeutic strategies are urgently needed.

Inflammation, especially neuroinflammation, has been reported in several MPSs (Parker and Bigger, 2018; Viana et al., 2020).

Immunomodulators are promising treatment options that may be used to cover clinical needs which are unmet with ERT. These molecules are not directed towards correction of the enzymatic defects and the causative gene mutation, but rather targeted to pathways that are secondarily altered in the MPS. This dysregulation may be pharmacologically addressed by anti-inflammatory therapies to be used along with ERT or as monotherapy in types where approved ERTs are missing.

Acetylsalicylate and prednisolone have been tested in the treatment of inflammation in MPS. Although in both animal studies a significant reduction in cytokines and oxidative stress have been observed, such high doses of anti-inflammatory drugs are hardly feasible in humans, given the adverse effects of long-term use of steroids or COX inhibitors (DiRosario et al., 2009; Arfi et al., 2011).

Therefore, a better understanding of the underlying cell mechanism might improve the current knowledge about MPS and set the path for emerging treatments.

In vivo and *in vitro* studies have shown, that the toll-like receptor-4 (TLR4) pathway with downstream activation of the myeloid differentiation primary response 88 (MyD88) adaptor protein triggers neuroinflammatory processes in the brain of MPS patients. This results in production and release of pro-inflammatory cytokines (e.g., TNF-α and IL-1β) and chemokines (e.g., CXCR4 and MIP-1α) (Ausseil et al., 2008; Goodall et al., 2014). This inflammatory mechanism is mainly triggered by accumulation of the GAG heparan sulfate (HS) e.g., in MPS I, II and III but secondary storage molecules such as GM gangliosides may also trigger these pathways (Parker et al., 2020). It is likely to have a prominent impact on inflammatory processes in neurons and astrocytes (Goodall et al., 2014; Parker and Bigger, 2018). Therefore, in these forms of MPS, HS storage and TLR4 appear to be key drivers of neuropathy.

Moreover, an accumulation of the other GAGs namely DS (dermatan sulfate), KS (keratan sulfate), C6S (chondroitin 6 sulfate), C4S (chondroitin 4 sulfate) and HA (hyaluronan) might contribute to lysosomal dysfunction and secondary events, such as abnormal vesicle and plasma membrane trafficking, impaired autophagy, mitochondrial dysfunction, oxidative stress, impaired Calcium (Ca^{2+}) homeostasis with membrane permeabilization, lysosomal disruption and ultimately activation of the NLRP3 inflammasome (Parker and Bigger, 2018). The latter appears to have a dominating role in the induction of inflammation in MPS.

The scope of this article is to review current knowledge on inflammatory immune response in MPS and to deduct possible treatment strategies. We focus on market-approved drugs, which may be repurposed to be used in MPS patients.

1.1 Hurler/-Hurler Scheie/-Scheie Syndrome (MPS I) and Hunter Syndrome (MPS II)

1.1.1 Metabolic Dysfunction

MPS I is caused by a deficiency of the enzyme α-L-iduronidase (IDUA) leading to the accumulation of DS and HS in lysosomes and the extracellular matrix (ECM) (Muenzer, 2011; Campos and Monaga, 2012). MPS I presents as a spectrum of phenotypes from attenuated to severe with many phenotypes in between. Three MPS I subtypes have been classified that differ in severity and onset of disease, usually classified as attenuate (Scheie Syndrome), intermediate (Hurler-Scheie Syndrome) and severe (Hurler Syndrome) (Zhou et al., 2020). The severe form, Hurler Syndrome, is related to absence or extremely low functional levels of IDUA activity, associated with genotypes such as deletions and nonsense mutations (Ahmed et al., 2014).

MPS II is caused by a deficiency of the enzyme iduronidate-2-sulfatase (IDS), ultimately leading to the accumulation of the same GAGs as in MPS I—DS and HS (Muenzer, 2011). Depending on the residual functional IDS activity, MPS II can present as a neuronopathic and a non-neuronopatic form. Two thirds of the affected MPS II patients present the severe neuronopathic form (Neufeld and Muenzer, 2001).

Due to the fact, that an accumulation of the same GAGs in MPS I and MPS II is present, the cytopathology and appearance of clinical manifestations is comparable within these two types of MPS. Therefore, biochemical mechanisms as well as the inflammatory immune response refer in MPS I and MPS II to the prominent role of HS and DS and its proteoglycans (Parker and Bigger, 2018).

On a microscopic scale, MPS is characterized by an existence of foamy (GAG-laden) macrophages, fibroblasts, bone, muscle cells and neural cells. Murine models show substantial accumulation of HS and DS in the liver, kidney, spleen, heart, and to a less extent in the CNS cells, already during the fetal period (Wang et al., 2010). The brain GAG storage may occur later and slower than in other tissues. This has been suggested by Holley et al. (2011), due to the measurement of HS levels in IDUA deficient mice tissues, whereby a minor storage in the brain compared with the liver was reported.

Deposits of extracellular GAG and foamy cells leading to an higher absorption of water in the tissue, thus the tissue become inflated. These also interfere with the structure of fibers like collagen and elastin (Hampe et al., 2020) with abnormal collagen IV deposition in basement membranes of adenoid and tonsillar tissues seen in patients resulting in ECM remodeling (Pal et al., 2018). Perturbations of the balance of DS against other ECM components is likely one of the underlying reasons for airway remodeling seen in these diseases.

DS-containing proteoglycans, decorin and biglycan, have collagen binding sequences and controlling functions in

morphology, size, growth and content of collagen fiber (Reed and Iozzo, 2002). Decorin limits the diameter of collagen fibrils and a deficiency is associated with fragile skin and thin dermis, weak tendons, decreased airway resistance, slow wound healing process and delayed angiogenesis (Young et al., 2002; Maccarana et al., 2009). Biglycan has the ability to modulate bone-morphogenic protein 4 (BMP-4)-induced osteoblast differentiation and blocks BMP-4 activity, thus biglycan appears as an essential regulator in skeletal growth (Xu et al., 1998; Moreno et al., 2005). Moreover, biglycan affects the Wnt signaling pathway (Berendsen et al., 2011). A dysregulation of the Shh and Wnt/β-catenin signaling causing abnormal heart development and atrioventricular valve formation, demonstrated by a MPS II zebrafish model (Costa et al., 2017). DS-containing proteoglycans may be linked as well to cardiac manifestations and other vascular clinical features, due to a disruption of elastin fibers, resulting in elastin which is reduced in content and aberrant in structure (Hinek and Wilson, 2000).

HS proteoglycans are associated with the cell surface, syndecan and glypican, or the peri-cellular matrix, betaglycan and perlecan. ECM-associated HS proteoglycans interact with growth factors, growth factor receptors, collagen and other ECM proteins and are essential for the structural constituent of basal lamina. Syndecan and glypican interact with ECM components or cytoskeleton, including collagen and fibronectin *via* its extracellular GAG unit. Furthermore, they regulate the biological activity of ligands and act as a co-receptor to catalyze the interaction between ligand and receptor. Therefore, HS and its proteoglycans are significantly involved in the regulation of chemokine and cytokine gradients produced by cells that have been stimulated *via* pro-inflammatory cytokines (Bernfield et al., 1999; Pasquale and Pavone, 2019).

Severe signs of CNS disease in MPS I and MPS II patients, like cognitive decline, loss of speech, and behavior changes such as hyperactivity, directly correlate with HS levels in urine, blood and fibroblasts. Based on that, studies have shown higher HS levels for neuronopathic MPS I and MPS II patients (Wilkinson et al., 2012; Bigger et al., 2018). Thus, HS may be the key driver for neuroinflammation.

1.1.2 Inflammatory Immune Responses

The accumulation of these substrates has been associated with cell-to-cell and cell-to-ECM adhesion leading to widespread inflammation and tissue damage (Hampe et al., 2020). The inflammatory reactions within the CNS and joints may be triggered primary and secondary storage of undegraded substrates. Several complex processes lead to lysosomal disruption and the final initiation of the NLRP3 inflammasome: 1) activation of the TLR4 pathway, 2) sequestering of immune cells in the ECM, 3) abnormal vesicle trafficking, 4) impaired autophagy, 5) mitochondrial dysfunction, 6) oxidative stress, 7) impaired Ca^{2+} homeostasis and membrane permeabilization.

These mechanisms are illustrated in **Figure 1** and primarily resemble the pathomechanism in MPS I and II. However, some aspects apply for all MPS types. In the sections about the other types, the main specificities described are the ones which differ from MPS I and II. The final activation of the inflammasome describes the instigation of long-term chronic inflammatory processes and cell death *via* pyroptosis.

In numerous LSDs, including MPSs, secondary storage of substances that is not described by the primary lysosomal defect has been consistently recognized and published (Parenti et al., 2021). The secondary storage of HS also occurs in the ECM and in the Golgi secretory pathway and acts as a positive regulator of HS-sulfation with the consequent accumulation of abnormal HS molecules at non-lysosomal sites. Viana et al. (2020) conducted a comprehensive analysis of brain cortex tissues from eight post-mortem autopsy sample of patients with MPS I, MPS II and MPS III and age-matched controls *via* high performance liquid chromatography (HPLC) together with histochemical staining of fixed tissues. A significant increase of HS and an accumulation of secondary substrates including GM2 and GM3 gangliosides has been described. The altered metabolism of gangliosides occurs very early in the course of the disease and may constitute a causal factor determining the progress of the CNS dysfunction. The accumulation of GM2 and GM3 gangliosides, as well as free cholesterol has been recognized to occur in patient's neurons and animal models of MPS. In general, cholesterol and gangliosides are co-localized to specialized membrane micro domains, known as rafts, which are essential for cell signaling supposed. Their co-sequestration reportedly, could severely impact neuronal function and disease pathogenesis (Walkley, 2004; McGlynn et al., 2004). GM gangliosides are able to activate TLR4, and cholesterol is able to activate the inflammasome (Parker et al., 2020).

The mechanisms leading to secondary storage are not yet clarified. Basically, secondary storage might be caused by an inhibition by primary substrates of other lysosomal enzymes, changes of the lysosomal environment (e.g., pH changes) or an impairment of vesicle trafficking through the endosomal/lysosomal system and the autophagic pathway (Sobo et al., 2007; Fecarotta et al., 2020). Current studies suggest that secondary storage plays a major role in the pathophysiology of MPS and that this pathological mechanism is common for all MPS types, due to the fact that the post-mortem results by Viana et al. (2020) are similar to those in mouse models.

Especially the induction of TLR4 seems to play a major part in the pathogenic pathway of MPS. HS chains and proteoglycans are able to promote an inflammatory response *via* TLR4 activation, requiring CD44 and Myd88 (Simonaro et al., 2010; Parker and Bigger, 2018). Goodall et al. (2014) identified soluble HS fragments, which are released from the ECM, as TLR4 agonists, due to their LPS similar structure. Many aspects of HS interactions with TLR4 remain unclear. However, it is obvious that HS has a prominent role in facilitating innate immune responses, including a production of pro-inflammatory cytokines and chemokines (Parker and Bigger, 2018). The role of inflammatory and immune processes in the pathophysiology of CNS-, osteoarticular- and cardiovascular symptoms may largely been driven *via* TLR4 induction (Wilkinson et al., 2012; Opoka-Winiarska et al., 2013; Khalid et al., 2016; Hampe et al., 2020).

Based on these assumptions, Raymond et al. (2016) evaluated the cerebrospinal fluid (CSF) of 25 consecutive patients with MPS

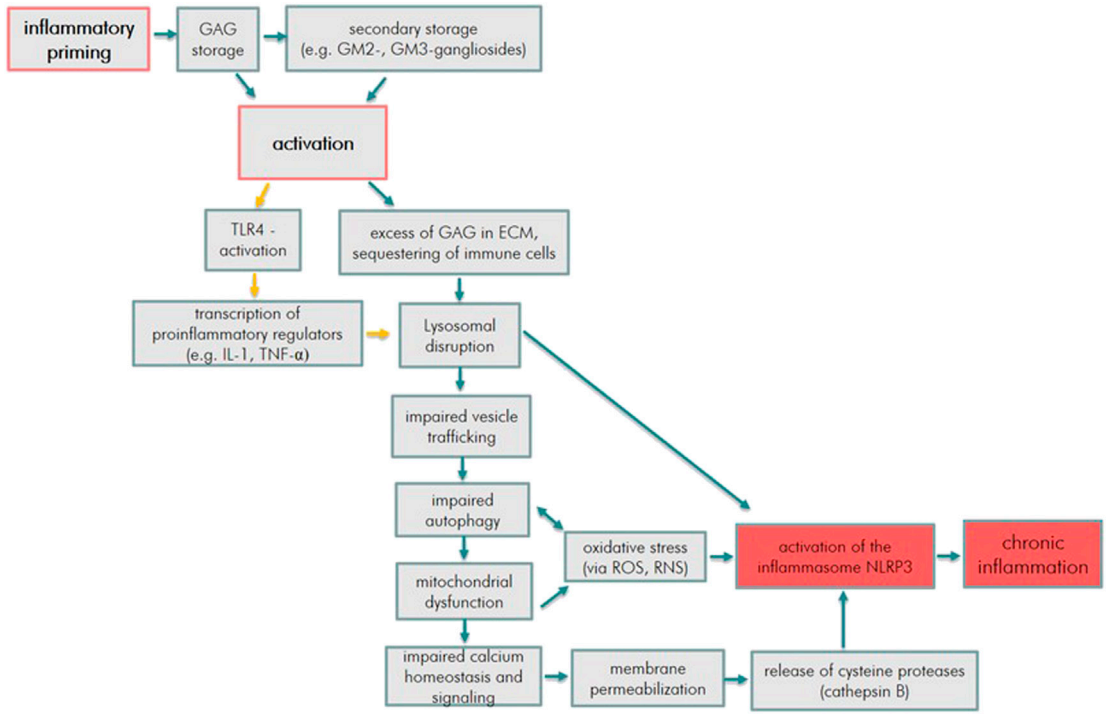

FIGURE 1 | Inflammatory pathway cue to GAG storage in MPS (orange arrows indicate the process mainly due to HS accumulation, blue arrows indicate processes due to HS, DS, C4S, C6S, KS, and HA accumulation).

I Hurler. The cytokine analyses demonstrated a significant elevation of inflammatory markers including: IL-1β, TNF-α, MCP-1, SDF-1α, IL-1Ra, MIP-1β, IL-8, and VEGF in comparison to unaffected children.

Fujitsuka et al. (2019) measured for the first time eight biomarkers which were significantly elevated in untreated MPS II patients, compared to normal controls: EGF, IL-1β, IL-6, HS0S, HSNS, DS, mono-sulfated KS, and di-sulfated KS. Therefore blood samples have been collected from 46 MPS II patients. To understand the CNS pathology in the neuronopathic form of MSP II Bhalla et al. (2020) utilized a mouse model. In addition to the accumulation of CSF GAGs, neuronopathic MPS II patients showed elevated levels of lysosomal lipids, neurofilament light chain (NfL), and other biomarkers of neuronal damage and degeneration. Furthermore, they suggest that these biomarkers of downstream pathology are strongly correlated with HS. This is in agreement with the observation mentioned above, that plasma and CSF from MPS I patients showed significantly elevated inflammatory cytokines, including IL-1β, TNF-α, MCP-1, SDF-1α, IL-1Ra, MIP-1β, IL-8, and VEGF (Fujitsuka et al., 2019; Fecarotta et al., 2020).

It is assumed that TNF-α is the major driver and controller of peripheral inflammation in MPS. However, in the brain TNF-α is subordinated to other cytokine responses, such as IL-1. Nevertheless, there is no doubt that peripheral inflammation can influence central events. Therefore, targeted pharmacological strategies should address both central and peripheral inflammation (Parker and Bigger, 2018).

Several factors contribute to signaling dysregulation and sequestering of immune cells in the ECM. One of them is the synthesis of aberrant GAGs that disrupts physiologic GAG interactions with different receptors. The accumulation of GAGs and its incomplete degradation is not restricted to the lysosomes, but also occurs in the ECM. Furthermore, HS storage has also been detected in the Golgi secretory pathway, acting as a positive regulator of HS-sulfation and increasing the N-sulfotransferase activity of HS-modifying N-deacetylase/N-sulfotransferase enzymes (Campos and Monaga, 2012). Partially degraded HS with abnormal sulfation patterns has an impact on leukocyte and immune cell migration, further exacerbating inflammation (Hampe et al., 2020). These non-natural HS molecules, which contain increased sulfation and a non-reducing end, could impair cell response to different growth factors or cytokines, such as the fibroblast growth factors (FGFs) or bone morphogenetic proteins (BMPs) (Pan et al., 2005; Ballabio and Gieselmann, 2009; Holley et al., 2011). Significantly increased amounts and sulfation patterning of HS have been observed in brains of MPS I, MPS II and MPS III mice (Holley et al., 2011; Wilkinson et al., 2012; Bigger et al., 2018).

These GAGs were shown to modulate BMP-4 signaling activity in MPS I cells and to affect FGF2-HS interactions and FGF signaling in multipotent adult progenitor cells derived from MPS I patients (Pan et al., 2005). Dysregulated FGF-2 signaling was also found in MPS I chondrocytes, together with transformed GAG (Kingma et al., 2016).

ECM proteins, such as biglycan, fibromodulin, PRELP, type I collagen, lactotransferrin, and SERPINF1 were significantly reduced in the mouse model of MPS I, and further analysis identified several dysregulated mRNAs (e.g., Adamts12, Aspn, Chad, Col2a1, Col9a1, Hapln4, Lum, Matn1, Mmp3, Ogn, Omd, P4ha2, Prelp, and Rab32) (Heppner et al., 2015; Fecarotta et al., 2020). These modifications in the key structure may have a great impact in the pathogenesis of MPS I patients. FGF-2 acts as a proliferative agent and protector of several cell types, including neurons and their precursor cells (Alzheimer and Werner, 2003).

Sequestering hematopoietic stem cells to the ECM of bone marrow cells and limiting their migration has been demonstrated in MPS I and may occur due to an excessive 2-O-sulfatasion of HS and increased binding to the chemokine CXCL12 (Wilkinson et al., 2012). As all chemokines and cytokines have possible HS binding sites, this might has an impact on chemokine and cytokine signaling in the brain (Bigger et al., 2018).

In MPS II partially degraded HS has an increased level of sulfation on the carbohydrate backbone (Węgrzyn et al., 2010). Therefore, experts hypothesized that these modifications interfere differently with neuronal functioning, leading to differences in behavioral problems. However it is more likely that overly sulfated HS results in increased TLR4 activation and consequential activation of downstreaming signaling pathways to achieve this (Bigger et al., 2018).

Mitochondrial dysfunction caused by impaired autophagy has been recognized in several LSDs, such as sphingolipidoses (Gaucher disease, Niemann-Pick disease type C, Krabbe disease), gangliosidoses, multiple sulfatase deficiency and neuronal ceroid lipofuscinoses (Parenti et al., 2021) and proposed as one of the mechanisms underlying MPS neurodegeneration (Saffari et al., 2017; Annunziata et al., 2018). Findings were observed post-mortem in a MPS I patients brain, in particular in the cerebral limbic system, central gray matter and pons (Kobayashi et al., 2018). Another link between autophagy and MPS has been reported recently. Mutations in the *VPS33A* gene, encoding a protein which is involved in autophagy, resulted in an MPS-like disorder characterized by elevated levels of HS in plasma and urine, and in the main phenotypical MPS features (Kondo et al., 2016).

Impaired autophagy appears to be associated with dysregulation of the mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated kinase (AMPK) signaling (Lim et al., 2017; Pasquale et al., 2020; Stepien et al., 2020). It has been proven that autophagy controls IL-1β secretion by targeting pro-IL-1β for degradation and it has been extensively proven that there is a progressive block of autophagy in lysosomal storage disorders, including MPS (Azambuja et al., 2020). Furthermore, it has been observed that IL-1β and TNF-α correlate with each other. The correlation observed in this study by Jacques et al. (2016) suggests a possible involvement of NO in the induction and maintenance of inflammatory states in MPS II patient. Their results indicate that, at some extent, inflammatory processes, oxidative and nitrative imbalances are predominant in MPS patients—even during long-term ERT.

Oxidative stress has been identified as consequence of defects in mitophagy and mitochondrial dysfunction. These processes have been implicated in the pathogenesis of many neurodegenerative diseases, which share several features of neuroinflammation and cell death (Rego and Oliveira, 2003). An elevation of reactive oxygen and nitrogen species (ROS, RNS) by phagocytes and an accumulation of damaged mitochondria has been studied in MPS I animal models (Donida et al., 2015) and MPS I (Pereira et al., 2008) and MPS II (Filippon et al., 2011) blood samples. The patient samples demonstrated oxidative damage to proteins and lipids, increased catalase activity and reduced total antioxidant status—even under ERT. Interestingly, elevated levels of glutathione, responsible for the elimination of toxic peroxides and malondialdehyde, an indicator of lipid peroxidation, compared with control subjects have been presented (Pereira et al., 2008). This in turn, may contribute to inflammatory processes by a misidentification and autoimmune response to proteins damaged by oxidation—maybe even in the pathophysiology of bone and joint disease in MPS.

Unfolded protein response (UPR) and consecutive endoplasmatic reticulum (ER) stress may further contribute to cell disruption (Filippon et al., 2011; Pierzynowska et al., 2021).

Moreover, mitochondrial dysfunction can impair lysosomal functions, like acidification by the acidic pump V-ATPase, relying on the ATP generated by the mitochondria (Stepien et al., 2020). The mechanism inducing the rise in Ca^{2+} channel expression is still unclear, however it might be caused by an incomplete cellular glucose availability impairing mTORC1 activity of and resulting in increased Ca^{2+} channel gene transcription (Lim et al., 2015). Abnormal Ca^{2+} signaling may trigger the permeabilization of lysosomal membranes and allows an elevation of pH in the organelles impacting the activity and release of other lysosomal hydrolases into the cytoplasm, such as cathepsin B. (Boya and Kroemer, 2008; Pereira et al., 2010). This may trigger inflammatory signaling pathways, especially inflammasome activation and mitochondria dependent processes.

The relationship between autophagy and inflammation has recently been linked to inflammasome interactions in different medical situation and a subsequent release of the highly pro-inflammatory cytokine IL-1β (Abdelaziz et al., 2015). The activation of the NLRP3 inflammasome is mediated by the innate immune response to cellular stress signals such as lysosomal dysfunction, impaired ion homeostasis, free radicals, oxidative stress and other stimuli like ATP, leading to the maturation and release of IL-1β (Latz et al., 2013). An activation of ATP and most other NLRP3 activators leading to an efflux of K^+. The intracellular Ca^{2+} increase, from intracellular stores or *via* membrane transporters, might be related to an activation of the NLRP3 inflammasome and the production and release of ROS might be another potential driver of inflammasome activation. Lastly, Bigger and Parker postulated that particulate matter taken up *via* phagocytosis can lead to lysosomal membrane permeabilization, the leakage of cathepsin B and other cysteine proteases and NLRP3 inflammasome activation (Parker and Bigger, 2018).

Gonzalez and colleagues have already demonstrated an overexpression of cathepsin B in MPS I mice (Gonzalez et al., 2018). In addition to that, Azambuja et al. (2020) observed in

MPS II high levels of cathepsin B in the brain tissue and a leakage of the enzyme IDS, which is a known activator of the inflammasome. Furthermore, the author studied MPS II mice brains and demonstrated elevated activity of Caspase-1 and IL-1β, confirming that this pathway is indeed altered. However, no increase in NLRP3 levels was seen, it may be mediated either by other inflammasome proteins (such as NRLP1) or even *via* other pathways.

The interplay of these processes add up to the inflammation-induced cytopathology as illustrated in **Figure 1**.

1.2 Sanfilippo Syndrome (MPS III)

1.2.1 Metabolic Dysfunction

MPS III is caused by a deficiency of one out of four different enzymes: N-sulfoglucosamine sulfohydrolase (MPS IIIA), α-N-acetylglucosaminidase (MPS IIIB), heparan-α-glucosaminide-N-acetyltransferase (MPS IIIC), N-acetylglucosamine-6-sulfatse (MPS IIID). The deficiency of any of these enzymes leads to the accumulation of HS in lysosomes and ECM (Muenzer, 2011). Subtype A is the most frequent subtype in European and North American countries. Therefore, more studies are linked to MPS IIIA.

In contrast to MPS I and II, in MPS III DS is only slightly accumulated. This dominance of the neurotropic accumulation of HS is in-line with the primary neurologic manifestation in MPS III. As mentioned above, HS accumulation causes a modification in the lysosomal environment. The high surplus of undegraded substances can bind to various enzymes, like hydrolases reducing their activity and causing secondary storage of gangliosides and other GAGs. This might be a major contributor to the CNS pathology (Walkley, 2004; Berendsen et al., 2011).

Therefore, a correlation between disease severity and the plasma concentration of HS and urinary total GAGs level has also been studied for MPS III (Ruijter et al., 2013). However, Ruijter et al. (2012) also observed elevated DS levels in in the newborn MPS III dried blood spots. Similar increases have been observed in the liver from MPS III mice (Holley et al., 2018). This accumulation was identified as a result of IDS activity inhibition by MPS III HS (Parker and Bigger, 2018) and is relatively modest compared to HS accumulation.

Throughout the brain of MPS III animal models both microglia and astrocyte activation are present (Villani et al., 2007; Arfi et al., 2011)—similar to MPS I and MPS II. Neuronal loss in MPS III is not consistently observed *via* animal models, but it has been detected in patients with magnetic resonance imaging and autopsy (Sharkia et al., 2014). This may demonstrate the phenotypical variability between human and mice.

The fact, that the CNS has a limited capability of regeneration, a high sensitivity to damage and a necessity of extended cellular survival might explain the severe neural pathology in MPS III patients—concerning both, the CNS and the peripheral nervous system (Benetó et al., 2020).

1.2.2 Inflammatory Immune Response

Secondary storage within neurons has been described by means of a canine model of MPS IIIA. Jolly et al. (2000) interpreted the outcome as accumulation of GAGs and gangliosides. McGlynn et al. (2004) also demonstrated in MPS III animal models, that an accumulation of GM2 and GM3 gangliosides as well as cholesterol play a role in the neuroinflammatory response. Li et al. (2002) demonstrated in a MPS IIIB mouse model important alterations in the expression of several genes involved in HS degradation. The levels of mRNAs for FGF-1 and FGF-2 were lower in the brain regions tested. These alterations may be responsible for the lack of response to acute injury, the insufficiency of neural cell genesis and the capacity for plasticity.

Several other substances, associated with neurodegenerative diseases like Alzheimer or Parkinson disease, have also been shown to accumulate in neurons of MPS III. Increased levels of protein markers, such as lysozyme, hyperphosphorylated tau, phosphorylated tau kinase, GSK3B and amyloid-β are all evident in the brains of MPS III mice (Settembre et al., 2008; Fraldi et al., 2016). Therefore, several experts suggest a possible association between MPS III and neurodegenerative diseases. MPS IIIC mouse brains showed elevations in these markers although there is typically a lower storage than similarly aged MPSIIIA and MPSIIIB mice (Ohmi et al., 2011; Beard et al., 2017). Furthermore, Hamano et al. (2008) showed that α-synuclein aggregation, a specific characteristic for Parkinson's disease, is present in MPS IIIA and MPS IIIB patients neurons. In MPS IIIA a relation between lysosomal disruption and presynaptic maintenance is assumed to be mediated by a simultaneous loss of α-synuclein and cysteine string protein-α (CSP-α) at nerve terminals. The relative loss of the function of α-synuclein by its abnormal autophagy can be assumed as a major contributor to neuronal degeneration (Sambri et al., 2017). VAMP2, important for neurotransmitter release by docking and fusion of vesicles at the synaptic junction was also shown to be reduced and abnormally distributed in synapses (Wilkinson et al., 2012).

Increased levels of markers for metabolic stress (e.g., glypican) and proteins involved in autophagy (e.g., LC3) have also been studied in MPS mice brains and are much more abundant in MPS III than MPS I or MPS II (Ohmi et al., 2011; Martins et al., 2015). This may contribute to the differences in disease phenotypes (Bigger et al., 2018). Ohmi et al. (2011) suggests that the increased levels of the proteoglycan glypican in MPS III are significant for the brain abnormalities, as glypican is the precursor of the glycan HS and might be metabolized otherwise in MPS III compared to MPS I or MPS II.

Parker and Bigger assume that there is an even more increased production of highly sulfated HS in MPS III, due to an increase of the chain modification enzyme N-Deacetylase/N-Sulfotransferase (NDST). Exocytosed and proteoglycan-bound-HS may interact with TLR4, propagating an inflammatory response. Furthermore, these highly sulfated fragments may be released intracellularly due to lysosomal destabilization and induce the TLR4 pathway and may also directly activate the NLRP3 inflammasome (Parker and Bigger, 2018).

Increased NDST enzyme activity has already been observed by Holley et al. (2011) in MPS I murine brain. Therefore, we hypothesize that levels of this enzyme are higher in MPS III subtypes, subsequently leading to an enhanced TLR4 induction and activation of the NLRP3 inflammasome. Taking into consideration, that IL-1 and cathepsin B expression has shown

to be upregulated in brains of MPS III animal models (Arfi et al., 2011). Linking HS, its proteoglycans and fragments to the degree of the neurological disease pathology in MPS.

However, Ausseil et al. (2008) have demonstrated that in MPSIIIB mice deficient TLR4, neurodegeneration can occur autonomously of microglial activation by HS, assuming that inflammation *via* this pathway is not the main reason for pathology in MPS III. The subsequent discovery of the inflammasome and demonstration that alternate substrates and pathologies may also eventually lead to inflammasome activation (Parker et al., 2020) with elevated levels of pro-inflammatory cytokines, such as IL-1, TNF-α, MCP-1, and MIP-1α, all provide support for this (Ausseil et al., 2008; Arfi et al., 2011; Wilkinson et al., 2012). A recent murine model of MPS IIIA described that a restoration of the lysosomal pathway was associated with reduced neuroinflammation and improvement of cognitive decline (Monaco et al., 2020).

Furthermore, it has been proven, that abnormal autophagy is one of the key drivers regarding the inflammatory immune response of MPS IIII. Original studies on the impairment of autophagy in LSDs were performed in a MPS IIIA mouse model inter alia. The disruption of the autophagic pathway was reported by Settembre et al. (2008) and might be linked to an inefficient degradation of exogenous aggregate-prone proteins. Consequently, this results in massive accumulation of dysfunctional mitochondria, as well as polyubiquitinated proteins. The role of autophagy in the pathophysiology of the disease, in connection with phenotypical appearance has been further studied by Webber et al. (2018) by means of an MPS IIIA *Drosophila* model. These MPS IIIA flies showed a progressive defect in climbing ability—a hallmark of neurological dysfunction. Autophagy-related proteins (Atg1 and Atg18), superoxide dismutase enzymes (Sod1 and Sod2), as well as heat shock protein (HSPA1) have been identified *via* genetic screen as prominent factors for modifying the climbing phenotype. Moreover, a decreasing HS biosynthesis significantly worsens the behavioral phenotype.

Abnormal mitochondrial numbers and morphology have been observed in MPS IIIC mice and could subsequently lead to oxidative stress (Martins et al., 2015). Pathological findings have been described in detail in an animal model of MPS IIIC by Pshezhetsky (2016). The progressive accumulation of pleomorphic, swollen mitochondria containing disorganized or reduced cristae has been reported as one of the most prominent pathological changes in MPS III neurons. This finding has been observed in all parts of the brain. Neurons containing swollen mitochondria are present as early as at 5 months of age and by the age of 12 months the mitochondrial dysfunction can be identified in the major part of neurons. The author speculates that cytokines have the ability to cause mitochondrial impairment, due to the release of ROS/RNS and oxidative stress eventually leading to neuroinflammation and cell death (Pshezhetsky, 2016).

While some experts suggest that oxidative stress may play an important role in early stages of disease (Villani et al., 2009; Villani et al., 2012), others assume that oxidative stress is not a consequence, but rather a cause of neuroinflammation, as oxidative stress is present at an early stage in the human brain (Trudel et al., 2015).

In 2017 Roca et al. (2017) generated for the first time a MPS IIID animal model, due to the fact that the subtypes D is the most infrequent one of these four. However, each mouse model of MPSIIIA-D demonstrated variations in the time at onset and severity of the disease pathology, such as GAG build-up, degree of lysosomal distention, severity of neuroinflammation, onset of behavioral abnormalities and lifespan.

As mentioned above, several experts hypothesize that behavioral differences might be due to the localization and or to the amount of GAG storage, as well as variances in GAG chain length, sulfation patterning or chemical modifications at the non-reducing terminus of the partially degraded HS (Węgrzyn et al., 2010; Wilkinson et al., 2012; Bigger et al., 2018). Therefore, the N-sulfate (MPS IIIA), amino (MPS IIIC) or N-acetyl (MPS IIIB and MPS IIID) moieties may be the decisive factors for phenotypical presentation and severity of neurological symptoms in MPS III.

1.3 Morquio Syndrome (MPS IV)

1.3.1 Metabolic Dysfunction

MPS IV is caused by a deficiency of the enzymes galactosamine-6-sulfatase (MPS IVA) or β-galactosidase (MPS IVB). The deficiency of the respective enzyme leads to the accumulation of KS and/or C6S in lysosomes and ECM (Muenzer, 2011). In MPSIV, skeleton is severely affected, liver and spleen might be slight enlarged, while the CNS is spared.

The prominent role of KS fulfils a number of specific biological functions, including tissue hydration, cellular recognition of protein ligands, axonal guidance, cell motility, and embryo implantation (Weyers et al., 2013). Shimada et al. (2015) demonstrated, that di-sulfated KS is supposed to be a novel biomarker for MPS IV, however di-sulfated KS levels are higher in MPS IVA than in MPS IVB. Compared with MPS IVA patients, the patients with MPS IVB usually show a milder skeletal dysplasia phenotype.

KS level in MPS IVA patients varies with age and clinical severity (Tomatsu et al., 2004). Blood KS levels in patients with severe MPS IVA are higher than in patients with the attenuated form. Both blood and urine KS are reliable biomarkers in younger patients. However, the synthesis of KS decreases after adolescence and KS levels in MPS IVA patients are naturally normalized or near normalized by the age of 20 years, as the growth plate is closed or damaged (Tomatsu et al., 2015; Khan et al., 2017).

Besides the storage of KS, there is also an accumulation of another GAG, called C6S, documented in MPS IVA patients. C6S accumulates in heart valves and aorta and related foam cells/macrophages contain C6S rather than KS (Yasuda et al., 2013). The KS accumulation is assumed as the primary driver for bone dysplasia. The role of C6S in MPS IVA is still uncertain (Khan et al., 2017).

Nonetheless, the study by Tan and Tabata (2014) came to the conclusion that C6S attenuates the inflammatory response in murine model *via* a significantly reduced production of IL-6 and TNF-α. Therefore, a more detailed analysis of tissue distribution pattern of C6S is urgently needed to reveal pathogenic roles of this GAG in MPS IVA. C6S may play a considerable part in the biochemical pathology or even contribute the intensification of

the inflammatory effects, leading to skeletal abnormalities and short stature, triggered by KS.

1.3.2 Inflammatory Immune Response

Several MPS IVA murine models have already been studied (Tomatsu, 2003; Tomatsu et al., 2005; Tomatsu et al., 2007). Overall, none of these model mice have the same phenotypical feature seen in human patients, even though abundant storage materials do accumulate in multiple tissues. The major reason may be that rodents, including mice, synthesize far less KS compared to human—up to 100 folds lower (Khan et al., 2017).

Oxidative stress may be a hallmark in MPS IV, according to results by Donida et al. (2015) and Fujitsuka et al. (2019). Their results showed high lipid and protein oxidative impairement, reduced antioxidant defenses and elevated levels of inflammatory markers.

Pro-inflammatory cytokines as well as GAG levels and oxidative stress parameters have been analyzed by Donida et al. (2015) in urine and blood samples from MPS IVA patients under ERT and in healthy matched controls. Patients affected by MPS IVA demonstrated decreased antioxidant defense levels, evaluated *via* glutathione content and superoxide dismutase activity. The damage of lipids and proteins has been evaluated *via* urine isoprostanes and di-tyrosine levels and plasma sulfhydryl groups. MPS IVA patients compared to controls presented a higher DNA damage with an origin in pyrimidine and purine bases. Furthermore, the pro-inflammatory cytokine IL-6 was increased in MPS IVA patients and presented an inverse correlation with glutathione levels—consistent with studies involving animal models of MPS I and MPS III (Ohmi et al., 2003; Arfi et al., 2011).

Fujitsuka et al. evaluated the levels of 8 pro-inflammatory factors (EGF, IL-1β, IL-6, MIP-1α, TNF-α, MMP-1, MMP-2, and MMP-9), collagen type II, and DS, HS (HS0S, HSNS), and KS (mono-sulfated, di-sulfated) in blood samples of MPS II, MPS IVA and MPSIVB patients. Eight biomarkers were significantly elevated in untreated MPS IVA patients as well: EGF, IL-1β, IL-6, MIP-1α, MMP-9, HSNS, mono-sulfated KS, and di-sulfated KS, and four biomarkers were elevated in MPS IVA patients under ERT: IL-6, TNF-α, mono-sulfated KS, and di-sulfated KS. Two biomarkers were significantly elevated in untreated MPS IVB patients: IL-6 and TNF-α. Reversely, collagen type II levels were significantly reduced in untreated and ERT-treated MPS II patients and untreated MPS IVA patients (Fujitsuka et al., 2019). Previous animal models have shown that enhanced apoptosis of MPS chondrocytes leads to a reduction of proteoglycans and total collagen in the cartilage (Simonaro et al., 2001). It is essential to identify if the increase of collagen type II correlates with specific clinical improvements, like skeletal dysplasia.

Overall, three pro-inflammatory factors (IL-6, TNF-α, and MMP-1) showed significantly different levels in untreated MPS IVA patients compared to ERT treated MPS IVA patients. However, there was no decrease of KS in the ERT-treated group (Fujitsuka et al., 2019). This striking discrepancy between biomarkers and blood KS level cannot declared by the currently limited published data.

These data presented suggest that an inflammatory immune response, leading to skeletal abnormalities, may occur in MPS IV due to impaired autophagy and the subsequent mitochondrial damage. The data presented showed a possible relation between inflammation and oxidative stress in MPS IV disease (even under ERT in MPS IVA).

Secondary storage as well as membrane permeabilization has so far not been studied, but should not be ruled out. An induction of TLR4 is unlikely due to the structure of the respective GAGs (KS and C6S). Indeed C6S may even reduce inflammation *via* suppression of NF-κB translocation (Tan and Tabata, 2014).

1.4 Maroteaux-Lamy Syndrome (VI)

1.4.1 Metabolic Dysfunction

MPS VI is caused by a deficiency of the enzyme N-acetylgalactosamine-4-sulfatase (ARSB) leading to an accumulation of DS in lysosomes and ECM (Muenzer, 2011). Due to the fact, that an accumulation of the same GAGs as in MPS I and MPS II is present, but without a storage of HS, the cytopathology and appearance of clinical manifestations is quite similar, though without any neurological involvement. As a consequence of DS accumulation, cartilage apoptosis, synovial hyperplasia, recruitment of macrophages, transformed connective tissue matrices and inflammatory joint destruction have been identified.

1.4.2 Inflammatory Immune Response

Secondary storage has been described by a feline model of MPS VI. Pyramidal neurons were demonstrated to contain abnormal quantities of GM2 and GM3 gangliosides and unesterified cholesterol. Some animals evaluated in this study also received HSCT, but no variations in neuronal storage were reported between treated and untreated animals. The study demonstrated that deficiency of ARSB activity may cause a metabolic abnormality in neurons of CNS in cats. These changes cannot be straightforwardly corrected by HSCT. Given the close pathological and biochemical similarities between feline and human, it is conceivable that MPS VI patients have similar neuronal involvement (Walkley, 2004). An accumulation of gangliosides may trigger changes in dendrite and axon morphology. This is supposed to cause synaptic dysfunction, neuronal cell death and neurodegeneration in MPS VI patients brain (Bigger et al., 2018).

In DS storage diseases such as MPS VI, there is no evidence of CNS involvement, although MPS VI is characterized by similar secondary storage, pathology and inflammation (Bigger et al., 2018). However, it should be pointed out that structural brain abnormalities have also been reported for MPS types that are not typically associated with neurocognitive impairment. For example, Azevedo et al. (2013) described in patients affected by MPS VI enlarged perivascular spaces and white matter lesions, without IQ correlation.

The important role of TLR4 signaling in MPS bone and joint disease has been revealed by Simonaro and colleagues by an MPS VI mouse model. An elevation in the expression of genes

encoding TLR4 (e.g., LBP, MyD88, CXCR4, and MIP-1a) and matrix metalloproteinase (e.g., MMP-1, MMP-2, MMP-9, and MMP-13) was found in synovial cells and cartilage. An elevated expression of the CD44 adhesion receptor was detected in fibroblasts and chondrocytes of MPS VI rats—adequate for the TLR4 activation (Simonaro et al., 2008; Simonaro et al., 2010). This work suggests, that an inflammatory immune response *via* TLR4 induction can also be triggered by DS.

However, we assume, that the increased production and release of inflammatory markers is mainly initiated by mitochondrial dysfunction. The structure of DS could be taken to imply that an activation of TLR4 is hardly possible.

Tessitore et al. (2009) studied several anomalies along with inflammation, in association with DS storage in MPS IV and assume abnormal autophagy as key driver. Mitochondrial dysfunction in fibroblasts from MPS VI patients with an accumulation of polyubiquitinated proteins and overproduction of ROS/RNS, due to impaired autophagy has been observed. Furthermore, they showed similar anomalies, along with inflammation and apoptosis, in association with DS accumulation in the visceral organs of MPS VI rats, but not in their CNS where DS storage is absent. They assume that DS storage disrupts the capability of lysosomes in order to completely and correctly degrade substances from the autophagic pathway, consequently leading to cell toxicity.

1.5 Sly Syndrome (MPS VII)

1.5.1 Metabolic Dysfunction

MPS VII is caused by a deficiency of the enzyme β-glucuronidase, leading to an accumulation of several GAGs in lysosomes and ECM (Muenzer, 2011). HS and DS are the main GAG accumulating in MPS VII, a storage of C4S and C6S has also been proven.

As already pointed out above, DS accumulation is characteristically associated with somatic and skeletal involvement in MPS I, MPS II, MPS VI, and MPS VII, while HS storage may be the key driver for neurodegeneration in MPS I, MPS II, MPS III, and MPS VII. The storage of CS derivatives (here C4S and C6S) is linked to skeletal abnormalities and short stature, an accumulation of C6S has also been mentioned in MPS IVA.

Depending on whether there is a complete absence or higher levels of the lysosomal enzyme MPS VII can present as severe or mild with or without neurological involvement. Furthermore, nearly half of all reported cases had non-immune hydrops fetalis *in utero* or at delivery (Holtz et al., 2020).

1.5.2 Inflammatory Immune Response

Simonaro et al. (2010) have also proven an activation of the TLR4 pathway in MPS VII. Interestingly, an inactivation of TLR4 in MPS VII mice had a significant positive effect on their growth, greater length of the bone, unchanged bone density, improved growth plates, and normalization of TNF-α level. Furthermore the author assumed, that peripheral inflammation may largely be driven and controlled by TNF-α (Simonaro et al., 2005; Simonaro et al., 2008; Simonaro et al., 2010). However, Parker and Bigger put forward the hypothesis that this is not the case in the brain, where TNF-α might be secondary to other pro-inflammatory factors, such as IL-1. Nevertheless, it is unquestionable that peripheral inflammation has the ability to influence central events (Parker and Bigger, 2018).

Inflammatory immune responses have also been proven *via* gene expression profile analysis in brains from MPS VII mice. Genes related to the immune system and inflammation were upregulated, while major oligodendrocyte genes were downregulated. Specific brain areas may be more vulnerable to inflammation than others, as patterns of gene expression dysregulation appeared specific for different brain regions (Parente et al., 2016).

Walton and Wolfe demonstrated similar neural results by a canine MPS VII model, as Li et al. (2002) *via* MPS IIIB mouse model. Neuronal cells mature less than normal ones, although these differences were only present an early phase after isolation. This study was carried out with neural progenitor cells, since they have a prominent role for brain development and function (Walton and Wolfe, 2007).

Studies completed in a MPS VII canine model showed an impaired secondary ossification initiation phase and a possible dysregulation of signaling pathways modulating bone development and bone ossification. An aberrant persistence of Sox9 protein was detected in chondrocytes and these cells were incapable to properly transit from proliferation to hypertrophy (Oguma et al., 2007). Another recently conducted work by Peck et al. (2019) has been focused on signaling pathways essential in the regulation of endochondral ossification. The osteoactivin gene was highly upregulated in MPS VII. However, key factors of the osteogenic pathways, like Wnt/β-catenin or BMP signaling were unaltered, suggesting that important bone formation pathways are not activated.

The osteopenia described in MPS VI and MPS VII is possible due to early chondrocyte death and decreased activity of osteoblasts. This could result in incomplete and disorganization of the growth plate (Opoka-Winiarska et al., 2013). This has so far not been described in MPS I and MPS II.

Bartolomeo et al. (2017) demonstrated, that an impaired autophagy may also be present in MPS VII. Their murine model has proven that deregulation of mTORC1 signaling adversely affects bone growth in LSDs. Lysosomal dysfunction induces a significant mTORC1 activation in chondrocytes. Consequently, chondrocytes are unable to successfully secret collagens. The autophagic rescue resulted in restored levels of collagen in the cartilage and improvements of the bone phenotype.

1.6 Natowicz Syndrome (MPS IX)

1.6.1 Metabolic Dysfunction

MPS IX is caused by a deficiency of the enzyme hyaluronidase, leading to an accumulation of hyaluronan (HA) in lysosomes and ECM (Muenzer, 2011). HA is defined as GAG, although it differs from other GAG members as it is not sulfated or protein-linked. Furthermore, HA is synthesized at the cell membrane and not in the Golgi apparatus. HA is an important component of the ECM of connecting tissues (Triggs-Raine, 2015).

TABLE 1 | MPS classification.

Type	Eponym	Enzyme (deficit)	GAG (storage)	Clinical features	Gen (–locus)	ERT/HSCT	Incidence per 100,000 live births
MPS I	Hurler (severe)	α-L-iduronidase	DS, HS	Coarse facial features, short stature, cognitive decline, skeletal abnormalities (=dystosis multiplex) corneal opacity, cardio respiratory disease, hepatosplenomegaly frequent airway and ear infections	IDUA (4p16.3)	✓✓ Laronidase, Aldurazyme®	0.69–1.66
	Hurler-Scheie (intermediate)			Coarse facial features short stature moderate cognitive decline cardio respiratory disease, hepatosplenomegaly, corneal opacity, skeletal abnormalities (=dystosis multiplex) frequent airway and ear infections			
	Scheie (mild)			Coarse facial features, no cognitive decline, cardio respiratory disease, skeletal abnormalities (=dystosis multiplex) hepatosplenomegaly, corneal opacity frequent airway and ear infections			
MPS II	Hunter (severe/ mild)	Iduronate-2-sulfatase	DS, HS	Coarse facial features, short stature, skeletal abnormalities (=dystosis multiplex), cardio respiratory disease, hepatosplenomegaly, (cognitive decline) Hearing loss frequent airway and ear infections	IDS (Xq28)	✓(✓) Idursulfase, Elaprase®	0.30–0.71
MPS III	Sanfilippo A	N-sulfoglucosamine sulfohydrolase	HS	Cognitive decline, hearing loss mild skeletal abnormalities (=dysostosis multiplex)	SGSH (17q25.3)	×	0.29–1.89
	Sanfilippo B	α-N-acetylglucos-aminidase		hepatosplenomegaly	NAGLU (17q21.2)		0.42–0.72
	Sanfilippo C	Heperan-α-glucosaminide-N acetyltransferase			HGSNAT (8p11.21)		0.07–0.21
	Sanfilippo D	N-acetylglucosamine 6-sulfatase			GNS (12q14.3)		0.1
MPS IV	Morquio A	Galactosamine-6-sulfatase	KS, C6S	Skeletal abnormalities (=dysostosis multiplex), short stature frequent airway	GALNS (16q24.3)	✓(✓) Elosulfase-α,Vimizim®	0.22–1.3
	Morquio B	β-galactosidase	KS	and ear infections	GLB1 (3p22.3)	×	0.02–0.14
MPS VI	Maroteaux-Lamy (severe/mild)	N-acetylgalactos-amine-4-sulfatase	DS	Coarse facial features, short stature skeletal abnormalities (=dysostosis multiplex), corneal opacity cardio respiratory disease, hepatosplenomegaly frequent airway and ear infections	ARSB (5q14.1)	✓ Galsulfase, Naglazyme®	0.36–1.3
MPS VII	Sly (hydrops fetalis/severe/ mild)	β-glucuronidase	DS, HS, C4S, C6S	Coarse facial features, short stature, cardio respiratory disease, skeletal abnormalities (=dystosis multiplex), corneal opacity, (cognitive decline)	GUSB (7q11.21)	✓(✓) Vestronidase- α, Mepsevii®	0.05–0.29
MPS IX	Natowicz	Hyaluronidase	HA	Short stature, frequent ear infections	HYAL1 (3p21.31)	×	4 case reported

MPS XI research has been conducted to a lesser extent, due to the fact, that this MPS type is the rarest of all. Therefore, neither the biochemical pathology nor the inflammatory immune response (see below) is fully understood.

1.6.2 Inflammatory Immune Response

Polydisperse HA fragments, with an average molecular weight of 200 kDa, have been described to stimulate chemokines, cytokines, growth factors, proteases and ROS/RNS by macrophages. HA fragments include various inflammatory effects, such as activation of macrophages and dendritic cells as well as stimulating the transcription of inflammation-related genes, including TNF-α, IL-12, IL-1β, and MMPs (Termeer et al., 2000).

Multiple studies have shown that HA fragments can also be protective, although studies regarding low-molecular-weight HA-fragments initially illustrated a pro-inflammatory response. The strongest TLR4 induction was observed with HA of 35 kDa (Hill et al., 2012). Simonaro et al. (2010) also assumed that an activation of the TLR4 pathway may be linked to HA.

Martin et al. (2008) characterized a Hyal1 null mouse model of MPS IX and compared the phenotype with the human disease. An increased number of chondrocytes displaying intense HA

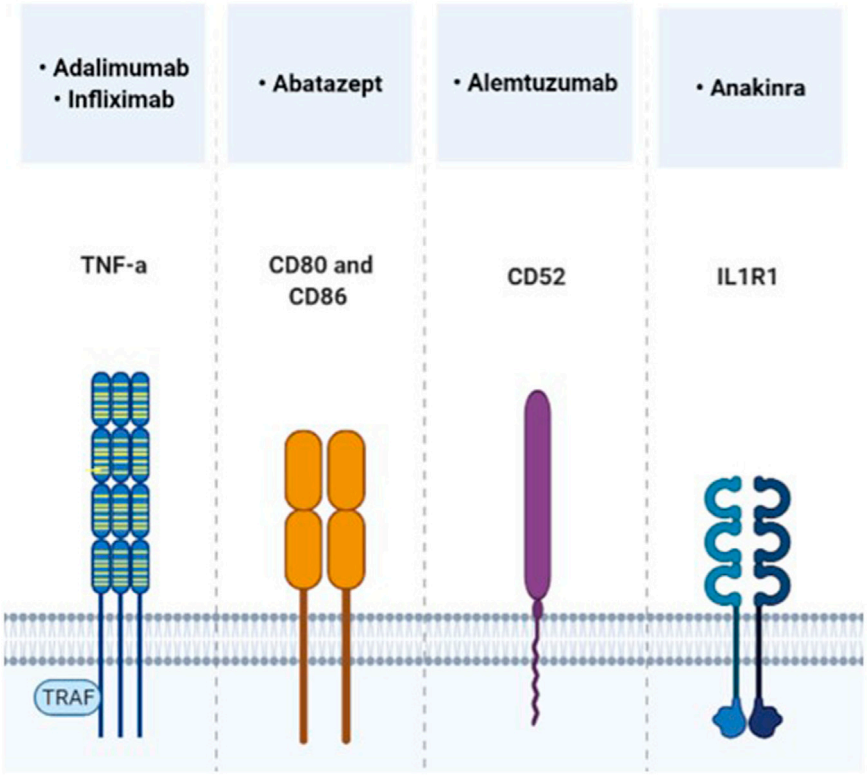

FIGURE 2 | Direct immunomodulatory molecules, which target the cytokine receptor pathway and have already been tested in MPS trials.

staining in the epiphyseal and articular cartilage of Hyal1 null mice, demonstrating an HA storage. Increased levels of HA have not been detected—neither in the serum nor in the non-skeletal tissues. This finding indicates that osteoarthritis is the key clinical feature in MPS IX.

An overview of all MPS types and subtypes is given in **Table 1** below.

2 REPURPOSING: IMMUNOMODULATORY MOLECULES

The observation that substantial inflammation induced cytopathology takes place in progressive manner, even in patients under ERT, directs us towards a role of inflammation as a key contributor to the unmet clinical need in MPS. In the following section, we summarize the available evidence on immunomodulation in MPS and give an overview on ongoing (pre-) clinical trials with immunomodulatory molecules utilizing this treatment strategy.

Currently there are different types of immunomodulatory molecules under investigation.

The characterization of cellular inflammatory processes, which can trigger the pathophysiology of MPS, is now providing evidence to address the limitations of ERT and HSCT and to identify novel therapeutic targets. **Figure 1**, aforementioned, shows the inflammatory immune response with several promising target points to intervene.

Due to the current knowledge on inflammation, these processes seem feasible to intervene in the activation of the innate immune response:

(1) TLR4 induced inflammation, with TNF-α as key driver.
(2) Activation of the NLRP3 inflammasome, with IL-1β as key driver.
(3) Perturbed autophagy and secondary mitochondriopathy.
(4) Increased oxidative stress.
(5) Impaired Ca^{2+} homeostasis and signaling.
(6) Increased lysosomal membrane permeability with release of cysteine proteases.

In the following, the focus is set on relevant target points that can be addressed by approved immunomodulatory drugs, which have already been studied in MPS in preclinical or clinical setting (**Figures 2**, **3**). Evidence supports a therapy with biologicals and antioxidants. Perhaps several different targets have to be addressed simultaneously to receive a beneficial effect, especially in the CNS. To date treatment combinations have only been studied in MPS with ERT.

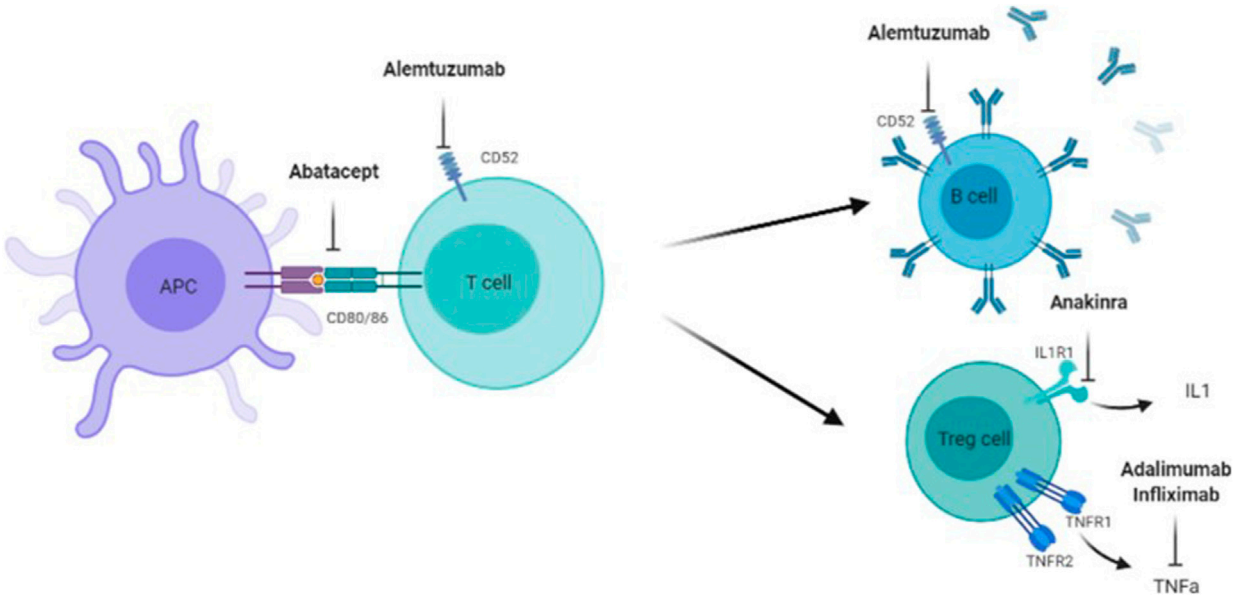

FIGURE 3 | Target points of Abatacept, Alemtuzumab, Anakinra, Adalimumab, and Infliximad. Adaliminab and infliximab block the action of INF-a, Anakinra inhibits the ILI receptor and a following ILI release, Alemtuzumab targets mature B- and T-cells *via* CD52, Abatacept prevents full T-cell activation *via* CD80 and CD86 between antigen presenting cell (APC) and T-cell.

2.1 TLR4 Induced Inflammation, With TNF-α as Key Driver

Promising treatment strategies targeting TLR4 pathways include biologicals as well as Pentosan Poylsulfate (PPS). These directly target the transcription and release of pro-inflammatory regulators. Furthermore, TAK242 a TLR4 inhibitor is currently under investigation by Takeda (Rice et al., 2010).

2.1.1 Adalimumab

Adalimumab, a human monoclonal antibody that inhibits TNF-α, has been proven effective and safe by Polgreen et al. (2017) for patients with MPS I and II in a 32-week, randomized, double blind, placebo-controlled, crossover study (NCT02437253; NCT03153319). Two patients, one with MPS I and one with MPS II, completed the clinical trial. There were no serious adverse events reported. Data from this small pilot study suggest that Adalimumab may improve pain, physical and neurogenic function in children with MPS I or II. This trial was based on encouraging results by Simonaro et al. (2010) and Eliyahu et al. (2011). Both evaluated the efficiency of another TNF-α inhibitor, Infliximab, based on an MPS animal model. Their work has underscored the importance of the TLR4/TNF-α inflammatory pathway in the skeletal pathology of MPS. Moreover, they demonstrated that combining ERT with anti-TNF-α drugs improves the outcome. They further validated the use of TNF-α and other immunomodulatory molecules as potential biomarkers for MPS.

2.1.2 Abatacept

Abatacept, a disease modifying anti-rheumatic drug, blocks the co-stimulatory signal mediated by CD28^{-}CD80/86 engagement. This pairing is required for T-cell activation. A single arm phase I study on Abatacept is currently running (NCT01917708). This substance is combined with cyclosporine and mycophenolate mofetil as graft versus host disease prophylaxis in 10 children undergoing unrelated HSCT for serious non-malignant diseases. Patients affected by MPS I may also be enrolled. Participants receive 4 doses of Abatacept 10 mg/kg/dose i.v. (days −1, +5, +14, +28) and are followed for 2 years. This trial aims to assess the tolerability and the immunological effects of Abatacept. First results may be posted in the year 2021, as the actual study completion data is given as 19th September 2019.

2.1.3 Alemtuzumab

Alemtuzumab is a monoclonal antibody used as immune reconstitution therapy for patients with aggressive multiple sclerosis (MS). Alemtuzumab targets CD52 leading to profound but transient peripheral immunodepletion (Moser et al., 2020). Alemtuzumab has been tried in phase II studies in MPS patients (type I and/or type II and/or type III and/or type VI). Alemtuzumab was used after HSCT and combined with other interventions (e.g., Busulfan) as graft versus host prophylaxis. Three of a total of 44 patients enrolled were affected by MPS. The MPS patients consisted of one with Hurler syndrome, who underwent transplant at 22 months of age and two children with Hunter syndrome who underwent transplant at 10 months and 1.25 years of age, respectively. They continued to achieve and improve skills, but showed mild developmental delay (Vander Lugt et al., 2020).

The high molecular weight (MW) of monoclonal antibodies indicates a low CNS bioavailability, but analog to MS, effects on the peripheral immune system might secondarily impact CNS inflammation. In addition, Torres-Acosta et al. (2020) observed

CNS effects induced by systemic TNF-α lowering in a mouse model on Alzheimer's disease. Thus indicating promising beneficial effects in neuronopathic MPS patients. The ability to lower peripheral inflammatory responses *via* antibodies may also impact directly on the CNS, as most cytokines (the effectors of the immune system) are produced at greater volume in the periphery rather than the brain, but cytokines can cross the blood brain barrier and exert their effects in both compartments.

2.1.4 Pentosan Polysulfate

Pentosan Polysulfate (PPS), an anti-inflammatory and pro-chondrogenic molecule, has shown to supress the activation of TLR4 in MPS in preclinical and clinical settings. PPS has been tested *in vitro* and *in vivo* studies. Early s.c., PPS therapy presented a decrease in neuroinflammation and—degeneration in a MPS IIIA mouse brain (Guo et al., 2019). Injected PPS shows an enhanced delivery to tissues like bone and cartilage, than orally administered PPS and has shown beneficial aspects in an osteoarthritis MPS VI mouse model (Frohbergh et al., 2014). Simonaro et al. (2016) observed PPS to decrease pro-inflammatory markers and GAG accumulation in the urine and importantly, a significant cytokine reduction in the CSF of MPS I dogs.

An open label, randomized, monocentric phase II study administering s.c., PPS in a dose of 1 or 2 mg/kg in four MPS I patients. PPS was injected weekly for 12 weeks, afterwards biweekly for 12 weeks. The treatment was well tolerated and the results showed a significant decrease of urinary GAG excretion and an improvement of the skeletal pathology—in both, the 1 and 2 mg/kg group. In patients with mild pain, the pain intensity remained stable, however in those with severe pain a significant amelioration has been monitored (Hennermann et al., 2016). In a pilot clinical safety study three male adults suffering from MPS II received weekly PPS injections for 12 weeks. Results showed decreases in the inflammatory cytokines TNF-α and macrophage migration inhibitory factor (MIF) (Orii et al., 2019). Nevertheless, the exact inflammatory mechanism by which PPS affects GAG storage and stimulates chondrocyte formation is yet unclear (Schuchman et al., 2013; Simonaro et al., 2016).

2.2 Activation of the NLRP3 Inflammasome, With IL-1β as Key Driver

2.2.1 Anakinra

The induction of TLR4 has been linked to the production and release of IL-1β, as well as the relationship between autophagy and inflammation by the inflammasome (Parker and Bigger, 2018).

IL-1β is a key driver regarding the activation and upregulation of the immune system. On this account, a further immunomodulatory drug is currently under investigation, with focus on chronic brain inflammation.

Anakinra, a human IL-1R antagonist, is presently being investigated in an open-label, single center, pilot study of 20 MPS III patients, aged ≥4 years (NCT04018755). After an initial 8 week-observational period, patients receive 100 mg s.c., of Anakinra for 36 weeks. The endpoints include, but are not limited to, changes in behavior, sleep, stooling, communication, mood, and gait; Other key outcome parameters addressed are seizure frequency, disordered movement and fatigue.

Brain exposure of systematically dosed biologics is naturally challenging to predict in both clinical and pre-clinical settings. One reason is, that the composition of the CSF does not entirely reflect the contents of the brain interstitium and reverse. Moreover, samples of the brain interstitial fluid are not easily available. Large proteins, like monoclonal antibodies are estimated to enter the brain compartment in a range of 0.1%–1% only. Sjöström et al. (2021) recently reported, that Anakinra passes the human brain-like endothelial monolayer at 4.7-fold higher rate than the monoclonal antibodies tested. Therefore, Anakinra may reach the brain compartment at clinically relevant levels, encouraging the utility of Anakinra for treatment of neuroinflammatory diseases.

2.3 Perturbed Autophagy and Secondary Mitochondriopathy

Several studies performed in animal models have linked impaired autophagy and oxidative stress to MPS. Therefore, rescuing the autophagic flux and mitochondrial dysfunction may be beneficial for the unmet medical need MPS patients, as described in several animal models. Altered autophagy has been reported in MPS II (Fiorenza et al., 2018), MPS IIIA (Webber et al., 2018), MPS IIIC (Pshezhetsky, 2016), MPS VI (Tessitore et al., 2009) and MPS VII (Bartolomeo et al., 2017).

It has already been demonstrated, that an inhibition of autophagy can improve the efficacy of several cancer therapies—preclinical evidence is growing. To date, hydroxychloroquine (HCQ) is the only approved drug, which targets autophagy. However, studies of pharmacodynamics reported that high doses of HCQ (up to 1,200 mg/day) produce only modest inhibition *in vivo* and might be inconsistent (Shi et al., 2017). Furthermore, HCQ fails to inhibit autophagy in an acid milieu, as the cellular uptake of HCQ is significantly reduced. The inhibitory interaction of HCQ with the lysosome might be contributing to the lysosomal disruption in MPS and beyond. However, preclinical studies are still lacking and due to the potency of the drug other autophagy inhibitors are needed. To date, multiple molecules are in the pre-clinical stage of investigation (e.g., 3-Methyladeninin, Wortmannin, LY294002, SBI-0206965, Spautin-1, SAR405, NSC185058, Verteporfin, Lys05, ROC325, and Spautin-1) (Chude and Amaravadi, 2017).

2.4 Increased Oxidative Stress

Targeting the increased oxidative stress, consequences of defects in mitophagy and mitochondrial dysfunction, may also be a promising treatment option. Oxidative imbalance has been described even in the early stages of the disease course and studied in MPS IIIA (Arfi et al., 2011), MPS IIIB (Trudel et al., 2015), MPS IVA (Donida et al.,

TABLE 2 | Preclinical- and clinical development status of innovative immunomodulatory therapies that have been tested in MPS (NCT clinicaltrials.gov identifier number).

MPS type / Biologicals	Preclinical study *in vitro*	Preclinical study animal model	Clinical trial (completed/ recruiting/active)	Reference
Adalimumab				
MPS I			Phase I and II: NCT02437253; NCT03153319	Polgreen et al. (2017)
MPS II			Phase I and II: NCT02437253; NCT03153319	Polgreen et al. (2017)
Infliximab				
MPS VI		Infliximab in MPS VI mice		Simonaro et al. (2010), Eliyahu et al. (2011)
Abatacept				
MPS I			Phase I: NCT01917708	
Alemtuzumab				
MPS I			Phase II: NCT01962415 (terminated) Phase II: NCT00668564 Phase II: NCT01043640	Miller et al. (2011), Vander Lugt et al. (2020)
MPS II			Phase II: NCT01962415 (terminated) Phase II: NCT01043640	Vander Lugt et al. (2020)
MPS III			Phase II: NCT00383448	Miller et al. (2011)
MPS VI			Phase II: NCT00668564 Phase II: NCT01043640	Miller et al. (2011)
MPS VII			Phase II: NCT00668564	Miller et al. (2011)
Anakinra				
MPS III			Phase II and III NCT04018755	
Pentosane Polysulfate (PPS)				
MPS I		PPS in MPS I dogs and mice	Phase II: (EudraCt 2014-000,350-11)	Schuchman et al. (2013), Hennermann et al. (2016), Simonaro et al. (2016), Guo et al. (2019)
MSP II			Phase II	Orii et al. (2019)
MPS III		PPS in MPS IIIA mice		Guo et al. (2019)
MPS VI		PPS in MPS VI mice		Frohbergh et al. (2014)
Antioxidant therapy				
Resveratrol				
MPS VII		Resveratrol in MPS VII *Drosophila m.* model		Bar et al. (2018)
Coenzyme Q10				
MPS III	CoQ10 in MPS IIIA and B cells			Matalonga et al. (2014)

2015) animal models and MPS I (Pereira et al., 2008) and MPS II (Filippon et al., 2011) blood samples.

Novel therapeutic strategies for MPS should not only be focused on lowering the GAG level, but also on ameliorating the whole spectrum of cellular process, causing reduction of symptoms. Antioxidant therapy may have a major impact of disease progression. Although these approaches are not expected to be curative, they may help in improving quality of life. Resveratrol and Coenzyme Q10 are promising treatment options, which have already been tested in a preclinical MPS setting.

2.4.1 Resveratrol

Resveratrol, a natural phenol and phytoalexin appears as a potential candidate, as its mechanism of action in autophagy stimulation is pleiotropic. Antioxidant, anti-inflammatory and autophagy-modulating properties of Resveratrol are widely reported (Rintz et al., 2020). The only research published so far with the use of Resveratrol in MPS has been conducted with a *Drosophila melanogaster* model. This MPS VII animal model has demonstrated that Resveratrol treatment improved behavior and crossed the BBB in flies (Bar et al., 2018). Resveratrol has been verified safe and well tolerated in various animal and human studies, including neurodegenerative disorders (e.g., Alzheimer disease, Parkinson disease). Potential adverse events are considered as rather unlikely (Rintz et al., 2020).

2.4.2 Coenzyme Q10

Coenzyme Q10 (CoQ10) levels are reduced in patients suffering from MPS III. Cultured fibroblasts from MPS IIIA and B have been treated with 50 μmol/L CoQ10 and a mixture of antioxidants. Increased enzymatic activity was especially observed in MPS IIIB cell lines and decreased GAG storage was noticed in some MPS IIIA and MPSIIIB fibroblasts, especially the ones presenting enhanced exocytosis (Matalonga et al., 2014). A nutritional study in nine MPS III patients explored the nutritional status by analyzing various vitamins and micronutrients in blood and in CSF. CoQ10 plasma concentrations were significantly deficient in 8 of 9 participants (Yubero et al., 2016).

2.5 Impaired Ca^{2+} Homeostasis and Signaling

To date there is no effective therapy concerning membrane permeabilization and the respective release of cysteine proteases.

Caspase antagonists, such as Balnacasan (VX765) targeting Caspase 1 and Pralnacasan (VX-740) targeting IL-1β -converting-enzyme (ICE), inhibit the release of LPS induced IL-1β and IL-18 (Xu et al., 2019; Flores et al., 2020; Hook et al., 2020). Balnacasan has already proven to be safe for several medical applications, including treatment of epilepsy, arthritis, heart attack, but is less effective and therefore not yet approved. A Cathepsin B inhibitor (CA074) is

currently under investigation for several diseases—from mercury induced autoimmunity to cancer. A subsequent attenuation of the systemic adaptive immune response has been reported in preclinical studies (Toomey et al., 2014).

Furthermore, there are several chemicals, which are capable to inhibit cysteine proteinases, like iodacetamine. Nevertheless, none of these are considered to be safe enough as pharmaceutical for human usage.

Table 2 summarizes immunomodulatory therapies, which are already under investigation in MPS.

3 CONCLUSION

Lysosomal storage appears to have a prominent impact on the inflammatory cytopathology of MPS. Primary and secondary accumulation of undegraded substrates and probably many more factors may initiate a self-propagating innate immune response in MPS—finally leading to inflammation and clinical deterioration. We face a complex interplay between multiple processes on a cellular and humoral level.

Patients affected by MPSs show a wide spectrum of clinical features and disease severity. Understanding the pathomechanism in each MPS type and subtype can reveal insights on how to better treat and manage the disease.

The complexity of the pathogenic cascade in MPS disease provides a number of potential clinical intervention points. Perhaps several different targets have to be addressed together to receive a benefit in clinical features, especially concerning CNS pathology. Combining therapies which target different aspects of the pathogenic cascade has shown neuroprotective effects in a neurodegenerative mouse model of the neurodegenerative LSD Niemann Pick disease type C1 (Williams et al., 2014). This approach may be valuable in MPSs as well. Currently, several promising therapeutic approaches are under investigation, including biologicals and small molecules. Immunomodulatory drugs, within the meaning of repurposing, are emerging tools for specific and/or adjuvant use in patients with MPS disease.

For this purpose, additional and larger clinical trials are needed to investigate benefits of different immunomodulatory agents in MPS. On the other hand, clearly structured and evidence-based individual treatment trials are required, as each MPS patient differs phenotypically among another.

Therefore, the understanding of the role of inflammation in the cytopathology of patients with MPS is necessary to gain new (adjuvant) treatment approaches, causing reduction of symptoms and enhancing quality of life.

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A-MW and FL designed and wrote the manuscript. BB, RG, CK, MS, CL, and TM reviewed and critically read the manuscript. All authors read and approved the submitted manuscript.

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GLOSSARY

AMPK AMP-activated kinase
APC antigen presenting cell
BBB blood brain barrier
BMP bone morphogenetic proteins
CNS central nervous system
CoQ10 Coenzyme Q10
CSF cerebrospinal fluid
C4S chondroitin 4 sulfate
C6S chondroitin 6 sulfate
CSPα cysteine string protein α
DS dermatan sulfate
ECM extracellular matrix
EGF epidermal growth factor
ER endoplasmatic reticulum
ERT enzyme replacement therapy
FGF fibroblast growth factor
GAG glycosaminoglycan
HA hyaluronan (hyaluronic acid)
HCQ hydroxychloroquine
HS heparan sulfate
HSCT hematopoietic stem cell transplantation
IL interleukin
I.V. intravenous
JIA juvenile idiopathic arthritis
KS keratan sulfate
LSD lysosomal storage disease
MCP monocyte chemotactic proteins
MPS mucopolysaccharidoses
MIP macrophage inflammatory protein
MIF macrophage migration inhibitory factor
MMP metalloproteinases
mRNA messenger ribonucleic acid
mTORC1 mechanistic target of rapamycin complex 1
MW molecular weight
MYD88 myeloid differentiation primary response 88
PC pharmacological chaperones
P.O. per oral
PP pentosane polysulfate
RNS reactive nitrogen species
ROS reactive oxygen species
rRNA ribosomal ribonucleic acid
SCRT stop codon read through therapy
SRT substrate reduction therapy
S.C. sub cutaneous
TLR4 toll like receptor 4
TNF tumor necrosis factor
tRNA transfer ribonucleic acid
UPR unfolded protein response

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Improving Therapy of Pharmacoresistant Epilepsies: The Role of Fenfluramine

Gianluca Dini[1]*, *Eleonora Tulli*[1], *Giovanni Battista Dell'Isola*[1], *Elisabetta Mencaroni*[1], *Giuseppe Di Cara*[1], *Pasquale Striano*[2,3] and *Alberto Verrotti*[1]

[1]Department of Pediatrics, University of Perugia, Genoa, Italy, [2]Pediatric Neurology and Muscular Diseases Unit, IRCCS "G. Gaslini" Institute, Genoa, Italy, [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy

Epilepsy is among the most common neurological chronic disorders, with a prevalence of 0.5–1%. Despite the introduction of new antiepileptic drugs during recent years, about one third of the epileptic population remain drug-resistant. Hence, especially in the pediatric population limited by different pharmacokinetics and pharmacodynamics and by ethical and regulatory issues it is needed to identify new therapeutic resources. New molecules initially used with other therapeutic indications, such as fenfluramine, are being considered for the treatment of pharmacoresistant epilepsies, including Dravet Syndrome (DS) and Lennox-Gastaut Syndrome (LGS). Drug-refractory seizures are a hallmark of both these conditions and their treatment remains a major challenge. Fenfluramine is an amphetamine derivative that was previously approved as a weight loss drug and later withdrawn when major cardiac adverse events were reported. However, a new role of fenfluramine has emerged in recent years. Indeed, fenfluramine has proved to be a promising antiepileptic drug with a favorable risk–benefit profile for the treatment of DS, LGS and possibly other drug-resistant epileptic syndromes. The mechanism by which fenfluramine provide an antiepileptic action is not fully understood but it seems to go beyond its pro-serotoninergic activity. This review aims to provide a comprehensive analysis of the literature, including ongoing trials, regarding the efficacy and safety of fenfluramine as adjunctive treatment of pharmacoresistant epilepsies.

Keywords: fenfluramine, pharmacoresistant epilepsy, Dravet syndrome, Lennox-Gastaut, anti-seizure medication (ASM)

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***Correspondence:**
Gianluca Dini
gianlucadini90@gmail.com

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INTRODUCTION

Epilepsy is one of the most common chronic neurological disorder, with a significant socioeconomic and psychological impact worldwide. It affects over 70 million people, one-third of which is drug-resistant (Löscher et al., 2020; Fattorusso et al., 2021). The definition for drug resistant epilepsy (DRE) is not unique, but it is defined by the International League Against Epilepsy (ILAE) as failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom (Kwan et al., 2010). DRE reduces quality of life and it is a potential life-threatening condition. Nowadays, despite the advances in the field of epilepsy and the recent approval of new antiseizure medications (ASMs), DRE still represents a major problem (Yoo and Panov 2019; Fattorusso et al., 2021). The exact

incidence and prevalence of DRE are uncertain, due to the non-univocal definitions and misdiagnosis (Dalic and Cook 2016). In a recent epidemiological systematic review by Kaliani et al. the pooled prevalence of DRE among epileptic patients was 30%. The pooled incidence proportion was 15% in children and 34% in adults, with an overall pooled incidence of 20% (Kalilani et al., 2018). These results were consistent with those frequently reported in the literature. Several risk factors for DRE have been identified, for example age at epilepsy onset (<1 year) or epilepsy aetiology. Patients with symptomatic epilepsy had 3 times-increased risk for DRE compared with patients with idiopathic epilepsy (Chen et al., 2018; Kalilani et al., 2018). Not surprisingly Lennox-Gastaut syndrome (LGS), Dravet Syndrome (DS), early infantile epileptic encephalopathy or Rasmussen encephalitis are almost pharmacoresistant (Dalic and Cook 2016). Also the coexistence of neuropsychiatric disorders such as intellectual disability or Attention Deficit Hyperactivity Disorder (ADHD) is related to the risk of DRE (Matricardi et al., 2020). Other risk factors include a history of febrile seizure, status epilepticus, abnormal EEG, abnormal neuroimaging test results (Kalilani et al., 2018) or an inadequate response to the initial ASM therapy and time to achieving seizure freedom (Kwan and Brodie 2000; Schmidt 2007). Sex and seizure type were not associated with risk of DRE, although focal seizures were suggested to have a higher risk than generalized seizures (Kalilani et al., 2018). Family history of epilepsy is a controversial risk factor of DRE (Fattorusso et al., 2021). The heterogeneity of seizure types and epileptic syndromes, the presence of comorbidities, the multifactorial genesis and the difficulty to understand its exact causal mechanism make DRE management and treatment extremely challenging. This is particularly true in pediatric patients. Indeed, epileptic syndromes like LGS or DS, that are often associated with pharmacoresistant epilepsy, occur in pediatric age. Moreover, ASMs are often used in an off-label manner in children due to the lack of clinical trials in this population. Treatment options available for DRE patients are polytherapy, surgical therapy or alternative therapy, as vagus nerve stimulation or ketogenic diet (López González et al., 2015; Löscher et al., 2020; Verrotti et al., 2020; Fattorusso et al., 2021). Polytherapy should be considered as first line of treatment. When choosing the most appropriate ASMs combination several factors should be kept in consideration, as efficacy, mechanism of action, pharmacokinetics, potential synergic interaction (for example Valproate plus Lamotrigine) and the risk of an additive adverse event profile. The addition of a fourth drug should be avoided (López González et al., 2015; Park et al., 2019; Verrotti et al., 2020). Recently new molecules have been approved as ASMs either as add-on therapy or initial monotherapy. New ASMs have been studied in several randomized controlled trials (RCT) and, compared with conventional ASMs, seem to have a better pharmacological profile: linear pharmacokinetics, less drug-drug interactions, different mechanisms of action and better tolerability profiles, which are important advantages for polytherapy (Park et al., 2019). Perampanel has been recently approved as add-on treatment in patients with focal seizures (with or without secondarily generalization) and primary generalized tonic-clonic seizures. It is well tolerated and it has been proved to be effective on idiopathic generalized and focal DRE (French et al., 2015; Krauss et al., 2018; Operto et al., 2020). Brivaracetam has been approved as adjunctive treatment in adults and pediatric patients aged 4 years and older with focal onset seizures. It seems to show a positive response also in patients affected by some encephalopathic epilepsies (Tulli et al., 2021; Verrotti et al., 2021). Another emergent promising ASM is Cannabidiol (CBD). Several trials have proved its effectiveness in DS and LGS patients (Devinsky et al., 2017; Lattanzi et al., 2019; Verrotti and Striano 2021). A highly purified plant-based form of oral CBD formulation was approved by the Food and Drug Administration (FDA) in 2018 and the European Medicines Agency (EMA) in 2019 for the treatment of seizures associated with DS and LGS (Contin et al., 2021). Cenobamate, a novel tetrazole-derived carbamate compound, has been recently approved in the United States for the treatment of partial-onset seizures in adult patients [Keam 2020] (Löscher et al., 2020). Fenfluramine (FFA), first used as an antidepressant and later as an appetite suppressant, was withdrawn from the market because of cardiac side effects. Nowadays, FFA is reintroduced as ASM at a lower dosage (Odi et al., 2021). The use of new ASMs in the pediatric population is often limited by different pharmacokinetics and pharmacodynamics and by ethical and regulatory issues. The aim of this review is to provide a comprehensive analysis of the current literature regarding the FFA pharmacologic profile and the clinical data regarding its safety and efficacy which may justify its use as an ASM, especially in pediatric population.

Literature Search

Electronic databases MEDLINE, EMBASE, and the Clinical Trial Database were systematically searched to identify relevant studies published through November 2021. Papers were searched using the following terms: “fenfuramine”, “pharmacodynamics and fenfuramine”, “pharmacokinetics and fenfuramine”, “Dravet syndrome”, “Lennox Gastaut syndrome”. The abstracts of retrieved references were reviewed and prioritized by relevant content and by the quality of evidence reported. Reference lists of the selected articles were used to search for further relevant papers. Only articles in English were reviewed. Additional information was also obtained from the websites of US and European Union agencies (US Food and Drug Administration and European Medicines Agency).

Fenfluramine: Pharmacodynamics and Pharmacokinetics

FFA is a derivative of amphetamine and its chemical name is 3-trifluoromethyl-N-ethylamphetamine. It is a racemic mixture of dexfenfluramine and levofenfluramine (Odi et al., 2021; Balagura et al., 2020). The D-enantiomer dexfenfluramine promotes serotonin-mediated neurotransmission by inhibiting serotonin (5-HT) reuptake and it has been used as an appetite suppressant to treat obesity (Garattini et al., 1987). The L-enantiomer, which lacks serotoninergic activity,

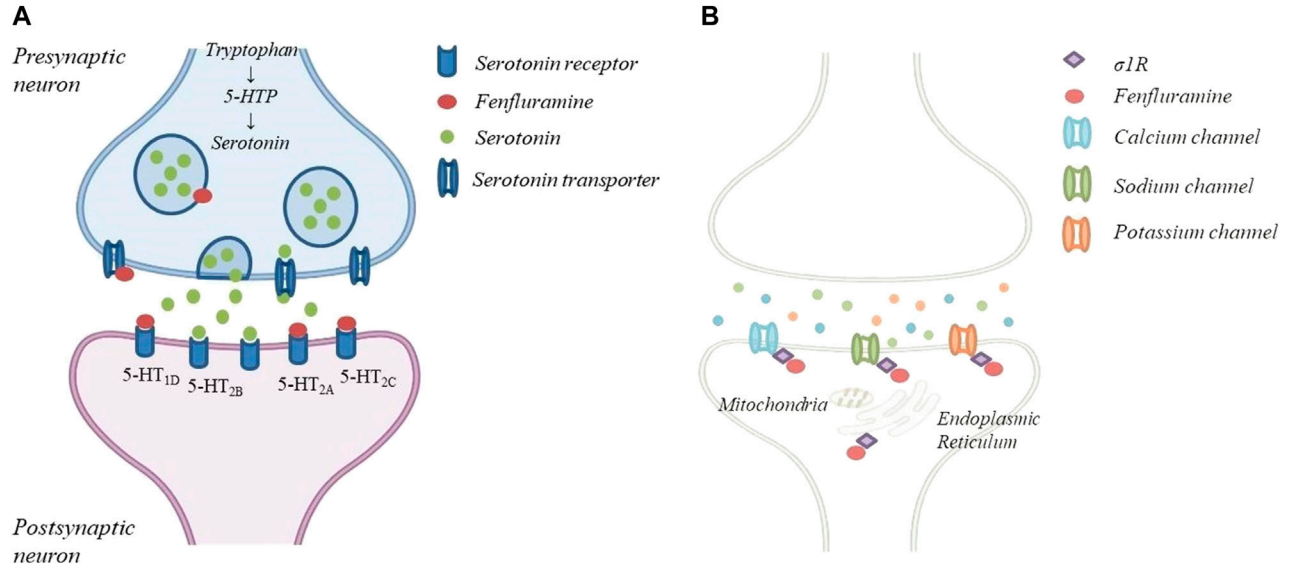

FIGURE 1 | The mechanism of action of FFA: **(A)**FFA causes the release of serotonin by disrupting vescicular storage, reversing serotonin transpoter function and a agonist of specific serotonin receptor; **(B)**FFA can regulate the activity od 1R located in the mitochondria-associated endoplasmic reticulum and several ion channels.

can suppress dopaminergic transmission (Invernizzi et al., 1989; Wurtman and Wurtman 2018). The racemic mixture, now proposed as ASM, acts on serotonin receptors (5HT2R) and on sigma 1 receptors (σ1R), as demonstrated *in vitro* and *in vivo* models of DS (Sourbron et al., 2017; Rodríguez-Muñoz et al., 2018; Martin et al., 2020) (**Figure 1**). In particular, FFA and its metabolite norfenfluramine exert the antiseizure activity as agonist of 5-HT1D and 5-HT2C type receptors, while the 5-HT2B receptor seems not to be involved. The role of the 5-HT2A receptor is not fully understood (Sourbron et al., 2017). In addition, FFA and norfenfluramine can regulate the activity of σ1R, a class of receptor that exert a modulatory effect on neurotransmitters involved in the genesis of seizures. In a mouse model of induced seizures, FFA seems to disrupte the association of the σ1R with NR1 subunits of glutamate N-methyl-D-aspartate receptors (NMDAR), restricting NMDAR activity. Thanks to this mechanism of action FFA seems to evade the negative side effects of direct NMDAR antagonists and may improve the quality of life of patients with DS and LGS (Rodríguez-Muñoz et al., 2018). The antagonism of σ1R by FFA was also confirmed by Soubron et all. in a SCN1a mutant Zebrafish model reproducing DS (Sourbron et al., 2017). On the contrary, Martin et all. demonstrated that FFA shows a positive modulation of σ1R, leading to an improvement in executive function (Balagura et al., 2020; Martin et al., 2020; Martin et al., 2021). However, the exact mechanism underlying the anticonvulsant activity of FFA is not yet completely understood (Gharedaghi et al., 2014; Rodríguez-Muñoz et al., 2018). FFA is a fat-soluble drug, it is administered orally and it is rapidly absorbed from the gastrointestinal tract. It has a good bioavailability, not affected by food intake and peak plasma concentration is observed about 3 h after a single oral dose (Gammaitoni et al., 2018; Balagura et al., 2020; Odi et al., 2021). Steady state is reached after 3–4 days of treatment (Ceulemans et al., 2012). FFA is extensively metabolized to active metabolites d-norfenfluramine and l-norfenfluramine, mostly by cytochromes CYP2D6, CYP1A2 and CYP2B6 (Boyd et al., 2019; Odi et al., 2021) and lesser by CYP2C9, CYP2C19, CYP3A4 (Balagura et al., 2020). Both FFA and norfenfenfluramine are about 50% bound to plasma proteins. The half-life of FFA is 20 h, while the half-life of norfenfluramine is longer (from 24 to 48 h), with a fast urinary excretion rate (Gammaitoni et al., 2018; Boyd et al., 2019). The fraction of the dose excreted in urine as unchanged FFA and norfenfluramine is from 6 to 24% (Balagura et al., 2020). The extensive metabolism involving different CYPs may mitigate the metabolic interactions with other ASMs. However, a moderate interaction is present when FFA, Valproate and Clobazam are used in association with stiripentol. In this case an adjustment of the FFA dosage is needed (Boyd et al., 2019). Pharmacokinetic and tolerability of FFA in children and adolescents were studied in several RCTs of patients with DS or LGS (Lagae et al., 2018; Nabbout et al., 2020). At a dose from 0.2 mg/kg/day to 0.7 mg/kg/day (with a maximum of 26 mg/day) FFA has proven to have a good pharmacological profile, with few and mild adverse events (AEs). The most common AEs were pyrexia, nasopharyngitis, decreased appetite, diarrhea, fatigue, lethargy, somnolence, and decreased weight. No valvular heart disease or pulmonary arterial hypertension were observed (Ceulemans et al., 2012; Schoonjans et al., 2017; Lagae et al., 2018; Lagae et al., 2019; Nabbout et al., 2020). Indeed, cardiac valve toxicity and pulmonary hypertension, which lead to withdrawal of FFA from the market in 1997, were achieved at higher dosages (60–120 mg/day) and they were caused by the stimulation of the 5-HT2B receptor, not involved in FFA antiseizure activity (Fitzgerald et al., 2000; Odi et al., 2021). Neverthless, a follow-

TABLE 1 | Main results from clinical trials for fenfluramine (FFA) use in pharmacoresistant epilepsies.

References (First Author, year)	Sample Size (age)	EE	Number of Concomitant AEDs at Baseline	Treatment Duration	Treatment Arms (Number of patients)	Global Seizure Reduction ≥50% (%)	Most Common Adverse Events
Schoonjans et al. (2017)	9 (1.2–29.8 years)	DS	2–5	Median 1.5 years	FFA 0.25–1.0 mg/kg/d (Kwan and Brodie 2000)	78%	somnolence (55.6%) anorexia (44.4%) fatigue (33.3%)
Nabbout et al. (2020)	87 (2–18 years)	DS	2–5	15 w	FFA 0.4 mg/kg/d (Scheffer and Nabbout 2019) Placebo (Specchio et al., 2020)	54% 5%	decreased appetite (44%) fatigue (26%) pyrexia (26%) diarrhea (23%)
Lagae et al. (2019) (Lagae et al., 2019)	119 (2–18 years)	DS	Mean 2.3 Mean 2.5 Mean 2.4	14 w	FFA 0.2 mg/kg/d (Schoonjans et al., 2017) FFA 0.7 mg/kg/d (Fitzgerald et al., 2000) Placebo (Fitzgerald et al., 2000)	38% 68% 12%	decreased appetite diarrhea, fatigue
Specchio et al. (2020)	45 (2.1–28.6 years)	DS	1–3	Median 9 months	FFA 0.2–0.7 mg/kg/d (Sullivan et al., 2020)	71.1%	decreased appetite (15.5%)
Sullivan et al. (2020) (Sullivan et al., 2020)	232 (2–19 years)	DS	n.a	Median 256 days	FFA 0.2–0.7 mg/kg/d[a] (232)	64.4%	pyrexia (21.6%) nasopharyngitis (19.4%) decreased appetite (15.9%)
Lagae et al. (2018)	13 (3–17 years)	LGS	2–5	20 w (core study) 15 months (extension study)	FFA 0.2–0.8 mg/kg/d (Park et al., 2019)	62% (core study) 67% (extension study)[b]	decreased appetite (31%) decreased alertness (15%)
NCT03355209 (Knupp, 2021a)	263 (2–35 years)	LGS	1–5	14 w	FFA 0.2 mg/kg/d (89) FFA 0.7 mg/kg/d (87) Placebo (87)	28.1% 25.3% 10.3%[c]	decreased appetite, somnolence, fatigue, vomiting, diarrhea
NCT03355209 (Knupp 2021b)	170	LGS	1–7	10–12 months	FFA 0.2–0.7 mg/kg/d	51.2%[c]	Decreased appetite (16.2%) Fatigue (13.4%) Nasopharyngitis (12.6%)
Devinsky et al. (2021)	6 (2–26 years)	CDD	2–5	Mean 5.3 months	FFA 0.2–0.7 mg/kg/d	Median 90% reduction in GTCS	decreased appetite (16.6%) flatus (16.6%) lethargy (16.6%)
NCT04289467 (ClinicalTrial.gov, 2020)	Estimated 10	West syndrome	-	21 days	FFA 0.8 mg/kg/d	-	-
Geenen et al. (2021)	9 (7–24 years)	Sunflower syndrome	1–2	3 months	FFA 0.2–0.7 mg/kg/d	88.8%[d]	fatigue (40%) loss of appetite (30%) rhinorrhea (10%)

Note: a maximum of 0.4 mg/kg/d in patients receiving concomitant stiripentol; b Nine patients entered the extension study; c ≥ 50% reduction in monthly drop seizures; d Responder: ≥30% reduction in seizure activity; Epileptic Encephalopathy: EE.

up echocardiography and weigh monitoring are mandatory while treating with FFA (Balagura et al., 2020).

Fenfluramine in Dravet Syndrome

DS is a rare developmental and epileptic encephalopathy characterized by highly treatment resistant seizures and progressive neuro-cognitive decline (Brigo et al., 2018). Children with DS have normal development in the first year of life. Seizures occur at an average age of 6 months and are usually hemiclonic or generalized tonic-clonic, triggered by fever. Over time, other seizure types appear including myoclonic, atypical absence and focal seizures. Intellectual disability and behavioural disorders also become a serious concern. DS is associated with mutations of the SCN1A gene in 70–80% of patients. SCN1A encodes the alpha1 subunit of the sodium channel and its mutation results in a broad spectrum of clinical phenotypes (Connolly 2016; Scheffer and Nabbout 2019). The increasing number of antiseizure medications in the last decades has led to the development of new successful therapies in DS including FFA (**Table1**). Schoonjan et al. evaluated FFA as adjunctive therapy in 9 DS patients refractory to standard AEDs. FFA yielded significant

improvements, with 78% of patients having a ≥50% reduction in major motor seizure frequency for the whole duration of the treatment (Schoonjans et al., 2017). In an open-label study conducted in 4 Italian centers, FFA was added to conventional therapy in 52 DS patients, all carrying SCN1A genetic variants. In a median follow-up of 9 months 71.1%, out of 45 patients, had a ≥50% reduction in convulsive seizures, 11.1% of patients became seizure-free (Specchio et al., 2020). In a multi-centre double-blind RCT, 87 DS patients receiving a stable, stiripentol-inclusive AED regimen, were randomized to receive fenfluramine or placebo. 54% of patients treated with fenfluramine experienced a ≥50% reduction in monthly convulsive seizure frequency compared to 5% of placebo group (Nabbout et al., 2020). Efficacy and safety of FFA were assessed by Lagae et al. in 119 children and young adults with DS and seizures not completely controlled by their current regimen of AEDs. Patients were randomly assigned to receive FFA 0.2 mg/kg/day, FFA 0.7 mg/kg/day or placebo. A responder rate (≥50% seizure reduction) of 68 and 38% was reported in patients treated with FFA 0.7 mg/kg/day and FFA 0.2 mg/kg/day respectively (Lagae et al., 2019). Patients who completed any of the phase 3 core clinical trials (Lagae et al., 2019; Nabbout et al., 2020) were enrolled in an open-label extension study. A total of 232 DS patients were treated with fenfluramine at a starting dose of 0.2 mg/kg/day and subsequently increased up to a maximum of 0.7 mg/kg/day. Final results confirmed the short-term data with 64.4% of patients showing a ≥50% reduction in convulsive seizure frequency (Sullivan et al., 2020). Based on these data, the main adverse effects related to the use of FFA were decreased appetite, fatigue, diarrhea, and pyrexia. Cardiac monitoring did not reveal clinical or echocardiographic evidence of valvular heart disease or pulmonary arterial hypertension in the cohorts of patients examined.

Fenfluramine in Lennox–Gastaut Syndrome

LGS is a childhood epileptic encephalopathy characterized by multiple seizure types, abnormal electroencephalographic features and cognitive impairment, leading to life-long disability (Cross et al., 2017). LGS can have different underlying etiologies, which are identifiable in 65–75% of the patients (Asadi-Pooya 2018). The most common types of seizures associated with LGS are tonic, atonic or atypical absence seizures, although other seizure types may occur. LGS is one of the most challenging epilepsy: the first-line therapy is represented by valproate, to which lamotrigine and clobazam can be added (Strzelczyk and Schubert-Bast 2021). However, prognosis remains poor and complete seizure control with resolution of neurocognitive disorders are often not achievable (Borrelli and El Tahry 2019). The promising results of fenfluramine in DS encouraged its use in LGS as well (**Table1**). In a phase III, multicenter, double-blind, placebo-controlled study (NCT03355209), a total of 263 patients with LGS were randomly assigned to receive FFA 0.7 mg/kg/day, FFA 0.2 mg/kg/day and placebo. FFA dosage was gradually titrated over 2 weeks and then maintained for an additional 12 weeks at a stable dosage. 25.3 and 28.1% of patients treated respectively with FFA 0.7 mg/kg/day and FFA 0.2 mg/kg/day had a ≥50% reduction in monthly drop seizures, compared with 10.3% of placebo. Overall, FFA therapy was well tolerated. Most frequent adverse events (at least 10%) included decreased appetite, somnolence, fatigue, vomiting, diarrhea and pyrexia. No cardiovascular complications were reported (Knupp 2021a). After completion of the randomized-controlled phase, patients were enrolled in the open-label extension study and received FFA twice daily for up to 1 year. After 10–12 months of treatment, among 170 patients 51.2% achieved a ≥50% reduction in drop seizures (Knupp 2021b). No patients developed valvular heart disease or pulmonary arterial hypertension. In a phase II, open-label study (NCT02655198), 13 LGS patients were administered adjunctive FFA at an initial dose of 0.2 mg/kg/day gradually increased up to 0.8 mg/kg/day in non-responders. In the 20-weeks core study 62% of patients achieved a ≥50% reduction in convulsive seizures frequency, while at 15 months 67% had a ≥50% reduction. In this patients, the most common adverse event was decreased appetite. No patient developed cardiac complications (Lagae et al., 2018).

Fenfluramine in Others Drug-Resistant Epileptic Syndromes

Recent clinical trials conducted in small groups of patients have demonstrated the efficacy of FFA in other drug-resistant epilepsies (**Table1**), including CDKL5 deficiency disorder (CDD). CDD is an X-linked pharmacoresistant disorder characterized by early onset refractory epilepsy, generalized hypotonia, intellectual disability and cortical vision impairment (Jakimiec et al., 2020). In a clinical trial, 6 children with CDD were treated with FFA at 0.4 mg/kg/day or 0.7 mg/kg/day. Five patients with generalized tonic-clonic seizures at baseline achieved a ≥75% of seizure reduction after FFA treatment. Two patients with tonic seizures at baseline achieved a ≥50% of seizure reduction after FFA treatment, while the only patient with myoclonic seizures had a 71.4% of seizure reduction after FFA treatment. Adverse events including decreased appetite were reported in 2 patients, but no one developed valvular heart disease or pulmonary arterial hypertension (Devinsky et al., 2021). A phase II clinical trial of fenfluramine in patients with refractory infantile spasms is currently enrolling patients (NCT04289467). Inclusion criteria provides diagnosis of infantile spasms not responsive to adequate treatment with ACTH and vigabatrin. Enrolled patients are treated with FFA 0.8 mg/kg/day, for an initial duration of 21 days. Patients with favorable response will have an option to continue treatment for up to 6 months (ClinicalTrial.gov 2020). FFA has also been tested on a small group of patients suffering from Sunflower syndrome a rare photosensitive epilepsy. Patients with Sunflower Syndrome have the tendency to seek light sources and present highly stereotyped behaviors defined as hand waving episodes (HWE) (Belcastro et al., 2021). In this open-label study, 10 patients with Sunflower syndrome were treated with FFA at an initial dose of 0.2 mg/kg/day, subsequently increased to a maximum of 0.7 mg/kg/day. Of the 9 patients who completed the 3 months core-study, 6 achieved a ≥70% reduction in seizure frequency. No cardiac complications were observed in any of the treated patients

during the observation period. The most common adverse event were fatigue, loss of appetite, rhinorrhea and diarrhea (Geenen et al., 2021).

CONCLUSION

Despite many years of research, the treatment of DREs still represents a major challenges for clinicians and, of course, for patients and their families. Particularly in pediatric age, the greater impact of ethical issues and adverse effects, makes this condition even more challenging. Hence the need for new drugs that can lead to improvements in the field of pediatric epilepsies. Recently, fenfluramine has been the focus of several studies which evaluated its efficacy and safety for the treatment of DS, LGS and other refractory epilepsies. The pharmacology of fenfluramine is complex and multiple mechanisms involving both serotonergic and sigma-1 activity may work collectively to promote antiseizure activity. Both in randomized controlled trials and open-label studies, fenfluramine has proven to be effective as adjunctive therapy in reducing convulsive seizures associated with DS and to a lesser extent in LGS. This could be attributed to the more heterogeneous pathogenesis of LGS compared to DS. Echocardiographic monitoring is recommended when initiating FFA therapy. However, it is yet to clarify whether the adverse cardiovascular effects observed in adult treated with high doses of FFA (>60 mg/day) can actually translate into a pediatric population treated with lower doses. FFA showed an overall favorable profile of safety and tolerability, with mostly mild side effects, pointing out that significant benefits outweigh potential cardiac risks.

AUTHOR CONTRIBUTIONS

GD, ET and GBD put forward the conception of the review and wrote the manuscript. AV and EM, participated in the proposal of the concept and revised the manuscript. GD and PS proposed suggestions for revision. All authors approved the submitted version.

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Update on Treatment of Infantile Hemangiomas: What's New in the Last Five Years?

Laura Macca[1†], Domenica Altavilla[2†], Luca Di Bartolomeo[1], Natasha Irrera[2], Francesco Borgia[1], Federica Li Pomi[1], Federico Vaccaro[3], Violetta Squadrito[4], Francesco Squadrito[2] and Mario Vaccaro[1]*

[1]Department of Clinical and Experimental Medicine, Dermatology, University of Messina, Messina, Italy, [2]Department of Clinical and Experimental Medicine, Pharmacology, University of Messina, Messina, Italy, [3]Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy, [4]Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi, Pediatry", University of Messina, Messina, Italy

Among benign vascular tumors of infancy, hemangiomas are the commonest, affecting approximately 5–10% of one-year-old children. They are derived from a benign proliferation of vascular endothelial cells (VECs) in the mesoderm and may arise anywhere on the body around 1–2 weeks after birth. Infantile hemangiomas (IHs) are characterized by an early proliferative phase in the first year followed by a spontaneous progressive regression within the following 5 years or longer. IH prevalence is estimated to be 5%–10% in one-year-old children and commonly affects female, Caucasian and low-birth weight infants. Although most of them spontaneously regress, approximately 10% requires treatment to prevent complications due to the site of occurrence such as bleeding, ulceration, cosmetically disfigurement, functional impairment, or life-threatening complications. For over 30 years, steroids have represented the first-line treatment for IHs, but recently topical or systemic β-blockers are increasingly being used and recognized as effective and safe. A search for "Cutaneous infantile hemangioma" [All Fields] AND "Treatment" [All Fields] was performed by using PubMed and EMBASE databases. Treatment of IHs with labeled drugs, such as oral propranolol, but also with off-label drugs, such as topical β-blockers, including topical timolol and carteolol, steroids, itraconazole or sirolimus, with a focus on formulations types and adverse events were described in our review. We also discussed the benefits of pulsed dye laser and the treatment of IHs with involvement of central nervous system, namely the PHACE and LUMBAR syndrome.

Keywords: infantile hemangioma, steroids, propranolol, timolol, carteolol, itraconazole, pulsed dye laser, segmental hemangioma

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***Correspondence:**
Luca Di Bartolomeo
lucadibartolomeo@live.it

†These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Infantile hemangiomas (IHs) are the commonest vascular tumors of the children (Munden et al., 2014; Püttgen, 2014). The lesions arise from the benign proliferation of vascular endothelial cells (VECs) in the mesoderm occurring anywhere on the body, more frequently on the head or face 1–2 weeks after birth (Hoornweg et al., 2012). The course of hemangiomas is characterized by a proliferative phase followed by a plateau and a regression phase. During the first year (proliferative phase), IH grow rapidly and may also

ulcerate, bleed, or become infected. This phase is followed by gradual spontaneous involution (regression phase) over the next 1–5 years or longer (Chang et al., 2008; Yanes et al., 2016).

IHs can be classified by general appearance: 1) superficial, located in the upper dermis 2) deep, extending to subcutaneous fascia, 3) mixed (Hoornweg et al., 2012) and the diagnosis is predominantly clinical. The estimated incidence of IHs is 1.1–2.6% in newborns, while IH prevalence is estimated to be 5%–10% in one-year-old children and commonly affects female, Caucasian and low-birth weight infants (Püttgen, 2014; Wang et al., 2017; Price et al., 2018). The use of drugs in first-degree relatives increase IHs risk (Li J. et al., 2011). Other predisposing factors associated with IHs are represented by old maternal age, placenta previa and pre-eclampsia (Bauland et al., 2010).

MATERIALS AND METHODS

PubMed (https://ncbi.nlm.nih.gov/PubMed) and EMBASE databases were checked by using the string "Cutaneous

TABLE 1 | Papers concerning treatment of cutaneous infantile hemangioma (CIH).

Publication (authors and year)	Type of study	Study population and treatment outcomes
de Castro et al. (2017)	Retrospective cohort study	18 patients (9 female) with vascular lip anomalies underwent a single or double pentagonal-shaped wedgeresection of the involved upper or lower lip
Hutchins et al. (2017)	Case report	a 3-year-old girl presenting diffuse cutaneous, hepatic and pulmonary IHs with progressive pulmonary hypertension treated with oral sirolimus
Kim et al. (2017)	Clinical trial	34 patients (15 boys, 19 girls) randomized to receive either propranolol or steroid treatment (17 in each treatment group). The treatment response rate in the propranolol group was 95.65%, and that of the steroid group was 91.94%. Propranolol was considered noninferior. There was no difference between the groups in safety outcomes
Mashiah et al. (2017)	Retrospective cohort study	63 patients with a total of 75 IHs treated with topical propranolol 4%. Of the total number of IHs, 43 (57.3%) showed a good response to treatment, 19 (25.3%) a partial response, and 13 (17.33%) poor or no response
Yu et al. (2017)	Case report	A 1-month-old female infant with LUMBAR syndrome treated with oral propranolol (2 mg/day)
Wang et al. (2017)	Clinical trial	40 infants treated with 2% propranolol cream followed up for 12 months after 3-month treatment. Poor response was observed in 2 patients, moderate response in 15 patients, good response in 17 patients, and excellent response in 6 patients
Ying et al. (2017)	Clinical trial	21 patients with superficial IH. Each lesion was divided into two regions; one part was treated with 0.5% topical timolol cream four times daily, and the other part was treated monthly with PDL. Both treatments were continued for 2–6 months. Both treatments resulted in significant clinical improvements after 3.39 sessions in the 2-month follow up
Le Sage et al. (2018)	Case series	6 patients of which 1 with an infantile hemangioma on the right forehead without intracranial extension treated with topical sirolimus 0.1% without improvement
Xu et al. (2018)	Clinical trial	Thirty-five children achieved the treatment of Intralesional Compound Betamethasone, 134 children achieved the treatment of oral propranolol, and 16 children achieved the treatment of topical carteolol. In the follow-up, the treatment could be repeated or switched to oral propranolol if the tumor tended to grow again. At the end of follow- up, 89% of the patients' tumors shrinked or involuted completely, 5 patients switched to oral propranolol
Igarashi et al. (2018)	Case report	A case of an extremely low-birth-weight infant with massive cutaneous IH complicated with hypothyroidism, which had improvement of hypothyroidism and regression of cutaneous hemangioma after propranolol therapy
Xu et al. (2020)	Retrospective cohort study	Eighty-one lip infantile hemangiomas patients treated with systemic propranolol (2 mg/kg/die). Lesions showed the same outcomes and prognosis involving the upper lips as the lower lips. Lesions involving the vermillion border had longer treatment lengths, poorer outcomes, and prognosis than lesions confined to one side of the vermilion
Chen et al. (2019)	Research study	The inhibitory effects of itraconazole on IH and its underlying molecular mechanisms were explored usingthe endothelial cells of mouse hemangioendothelioma (EOMA) cell line and infantile primary hemangioma endothelialcell (HemEC)
Tani et al. (2020)	Case series	A3-month-old boy with cheek's hemangioma (mixed type), a 3-month-old boy with forehead and abdomen's hemangioma (mixed type), a 6-month-old girl with head's hemangioma (superficial type), a 4-month-old girl with buttock's hemangioma (superficial type), and a 4-month-old boy with forehead's hemangioma (deep type). All patients treated with oral propranolol (3 mg/kg/die)
Diociaiuti et al. (2020)	Retrospective cohort study	Seven patients with intracranial or intraspinal infantile hemangiomas were selected and treated with oral propranolol, without side-effects
Zeng et al. (2020)	Research study	PRH-CNPs were tested on abdominal skin from Sprague Dawley (SD) rats to evaluate skin permeation. PRH-CNPs cytotoxicity on endothelial cells of mouse hemangioendothelioma (EOMA) was also assessed
Cheng et al. (2020)	Clinical trial	Forty-two children received 12 months of 0.5% TTM solution (24 assigned to the TTM group and 17 to the no-TTM group) The TTM group had fewer complications than the no-TTM group (4.2% versus 29%). Mean IH volume percentage reduction was significantly more for the and no-TTM group at 3, 6 and 12 months

infantile hemangioma" [All Fields] AND "Treatment" [All Fields]. Only papers written in English language, concerning humans and with 5 years' time limits were included. The references retrieved were critically examined to select those pertinent, thus reporting the type of the selected articles (review, retrospective cohort study, clinical trial, case series, research studies, case reports). The reference lists of these papers were also examined to find other relevant articles, which were eventually included if appropriate.

RESULTS

As of 21 October 2021, we found 23 articles of interest. Among these, seven reviews, four retrospective cohort studies, five clinical trials, 2 case series, two research studies and 3 case reports were selected. In **Table 1** are summarized the following features of each article: first author, year of publication, type of study, study population and strength level.

TREATMENT OF IHS

This is the case of a particular rare, self-limited disease featured by multiple cutaneous IHs without markable visceral lesions: benign neonatal hemangiomatosis (BNH). BNH lesions spontaneously subside within 4 months of onset or within the second year of life. In an infant with multiple (>5) cutaneous hemangiomas, it is important to differentiate BNH from diffuse neonatal hemangiomatosis (DNH), which is marked by multiple cutaneous and visceral hemangiomas with mortality ranging from 60% to 81% (Korekawa et al., 2020).

Given that most IHs spontaneously regress, they do not require any treatment, so periodic follow-up is sufficient (Schwartz et al., 2009).

Generally, complications are mild but some IHs can dramatically grow and leave residual cutaneous modifications after the involution phase, including telangiectasia, atrophy, scarring and skin laxity (Bauland et al., 2011; Léauté-Labrèze et al., 2017). Approximately 10% requires treatment to prevent distressing complications due to the site of occurrence such as bleeding, ulceration, visual impairment, eating disorder, airway obstruction, lifelong disfigurement, congestive heart failure, or bowel obstruction (Frieden et al., 2005; Hemangioma Investigator Group et al., 2007; Cheng and Friedlander, 2016; Novoa et al., 2019). In the next paragraphs, we'll report the last 5-years experiences with propranolol, the first line therapy for IHs treatment, and, in particular, with off-label drugs, such as topical β-blockers, including topical timolol and carteolol, steroids, itraconazole or sirolimus.

STEROIDS

For over 30 years, steroids have been used as the first-line therapy for IHs (Zarem and Edgerton, 1967; Folkman, 1984) thanks to their possible antiangiogenic effect. Corticosteroids can be orally, intravenously, intramuscularly, and topically administered. However, their systemic use may lead to various complications including Cushing-like manifestations, adrenal suppression, gastroesophageal reflux and growth disorders, even though such complications are linked with long-term and high dose therapy (George et al., 2004). Topical administration is advantageous, safe with fast response, and is extensively used for small tumors (Yuan et al., 2015). A significant lesion reduction (85.7% response rate) was observed in the study of Xu et al. (2018): 35 out of 185 IHs patients (mean age 3.9 months), presenting small-size hemangiomas which were raised and <3 cm X 3 cm, were treated with intralesional administration of betamethasone (one or two injection of Diprospan 1 ml/ampoule).

ORAL PROPRANOLOL

Oral Propranolol Versus Steroids

Some positive effects of propranolol use for the treatment of IH have been reported. Differently from Zimmermann et al. (2010), the clinical trial lead by Kim et al. (2017) showed non-inferiority of the therapeutic effects of propranolol compared with steroids, with a response rate of 95.65% in the propranolol-treated group and of 91.94% in the steroids-treated one; therefore, no statistically significant difference between the two groups (3.71%) was observed, thus demonstrating that propranolol shows safe outcomes and effectiveness if compared with steroids (Kim et al., 2017).

Propranolol: Mechanism of Action

The therapeutic effect of oral propranolol administration in IHs was incidentally discovered in 2008 (Léauté-Labrèze et al., 2008). Propranolol is a nonselective beta-blocker that acts on both β-1 and β-2 adrenergic receptors. Several studies demonstrated that the natural course of IHs can be shortened by propranolol-based therapy, which also suppress lesion's proliferation (Chen et al., 2013; Tan et al., 2015). As its mechanism of action has not been fully understood, it has been suggested that propranolol suppresses both vascular endothelial growth factor (VEGF) (Tang et al., 2015) and vascular endothelial growth factor receptor-2 (VEGFR-2) expression (Lamy et al., 2010). Tani et al. (2020) measured the serum cytokine concentrations of five patients with IH before and during the treatment with propranolol. The authors observed a significant reduction of the platelet-derived growth factor-BB (PDGF-BB) during treatment. This report suggested that PDGF-BB may be involved in propranolol effects and might be considered as a potential marker of the therapeutic effect.

Propranolol as First Line Treatment

Oral propranolol actually represents the first line approach for the pharmacological treatment of IHs (Hoeger et al., 2015). Oral beta-blocker are typically indicated in case of functional impairment (e.g., periocular IH causing amblyopia, nasal IH causing nose deformity, lip IH leading to feeding difficulties, and auricular IH causing deafness) and to avoid life-threatening

complications due to lesion's locations (e.g., respiratory distress caused by lung IH, airways obstruction caused by subglottic IH, hepatic dysfunction and heart failure subsequent to large cutaneous IH) (Wedgeworth et al., 2016).

In the study conducted by Xu et al. (2018), 134 out of 185 patients received treatment with oral propranolol (1.5 mg/kg/day) and the response rate was 91.7%. The effective dose of oral propranolol is between 1 and 3 mg/kg/day (Léauté-Labrèze et al., 2015).

Oral propranolol may be administered also in cases of IHs and comorbidities, such as hypothyroidism because of the hypotensive effects. Hypothyroidism has been also reported in association with hepatic IHs (Igarashi et al., 2018). Generally, the cells of hepatic IHs lead to thyroid hormone inactivating enzyme type-3 iodothyronine deiodinase (D3) overexpression, causing rapid degradation of thyroid hormones with consequent hypothyroidism (Simsek et al., 2018). Igarashi et al. (2018) reported a case of an extremely low-birth-weight infant with extensive cutaneous IH associated with hypothyroidism. The Authors observed an improvement of hypothyroidism and regression of cutaneous hemangioma following propranolol therapy, thus providing evidence for its effectiveness.

Adverse Events

The potential propranolol-related AEs generally comprise hypoglycemia, bradycardia, hypotension, bronchospasm, and electrolyte disturbance, even if the overall risk of AEs outbreaks is relatively low, in particular when used at low doses (Hoeger et al., 2015; Tan et al., 2015).

TOPICAL PROPRANOLOL

Topical Versus Oral Propranolol

Topical propranolol seems to be less effective but safer than oral administration propranolol and particularly helpful in patients that present small superficial hemangiomas, where the aesthetic or asymptomatic impact did not require oral propranolol treatment (Baselga et al., 2016; Zaher et al., 2013). A systematic review identified 12 studies published between 2012 and 2017 and reported that the administration of topical propranolol was the first-line therapy for IHs in over 600 patients, which did not show any systemic side effect. Propranolol preparations comprehend creams, unguents and gels prepared by galenic formulations with a concentration of propranolol ranging from 0.5% to 5%; treatment duration ranged from 2 weeks to 16.5 months. Overall, the initiation of topical propranolol led to lesion's improvement in 90% of cases, reducing lesion's size of at least 50% in 59% of IHs (Price et al., 2018).

Topical Propranolol Formulations

Casiraghi et al. (2016) showed that hydrophilic preparations, such as cream or gel, guarantees higher levels of propranolol permeation than hydrophobic ointments. Mashiah et al. (2017) Fare clic o toccare qui per immettere il testo. observed that 43 out of 65 patients (57.3%), with a total of 75 IHs, had a good response to the treatment with propranolol 4% gel. They observed minor local side effects, namely irritation, redness, and scaling of the treated area, in only two cases but did not observe systemic adverse effects (Mashiah et al., 2017).

Wang et al. (2017) treated proliferating IHs evaluating the effectiveness and the safety of 2% propranolol cream. In two patients the treatment response was graded scale 1, in 15 patients scale 2, in 17 patients scale 3, and in 6 patients scale 4. The Authors did not observe significant differences in location/size-related outcomes.

Transdermal Propranolol

Several *in vitro* studies have been carried out to improve transdermal delivery of propranolol in order to reach deep IHs. Zeng et al. (2020) tested propranolol hydrochloride-loaded cubic nanoparticles (PRH-CNPs) on the abdominal skin obtained from Sprague Dawley rats and on endothelial cells of mouse hemangioendothelioma (EOMA). Smaller-sized PRH-CNPs demonstrated enhanced skin permeation towards EOMA cells when compared with the PRH solution.

Chopra et al. (2019) described the effects of a preparation composed by amorphous melts of propranolol incorporated into transdermal patches. The amorphous melts of propranolol were prepared using ionic liquids (ILs), which may be used as formulation additives, replacing oil or water, and may enhance transdermal penetration. In addition to a significant improvement in propranolol transdermal permeability from its amorphous melts, Chopra et al. (2019) observed also a reduction of skin irritation.

TOPICAL TIMOLOL

Topical Timolol Versus Oral Propranolol

Recently, timolol, a non-selective topical beta-blocker, was studies as a valid and safe option for the treatment of superficial, localized, small and uncomplicated IHs with less systemic absorption as well as absence of adverse effects (Khan et al., 2017).

According to Novoa et al. (2019), oral propranolol (1.0 mg/kg tablet once a day) and topical timolol maleate (0.5% eye drops twice a day) may equally produce a 50% or greater decrease in hemangioma diameter at 24 weeks (low-quality evidence). Although topical timolol was found as an effective treatment for superficial IHs, Khan et al. (2017) advised against its use when systemic treatment is justified by the anatomical location or size of the hemangioma that could lead to hepatic or cardiac impairment.

Adverse Events

Eczema, ulcers, skin rashes, desquamation, and erythema are frequent adverse effects of topical timolol (Filoni et al., 2021). A large recent study in over 700 superficial IHs reported that 3.9% of patients treated with oral propranolol (2 mg/kg/day) presented systemic adverse events (AEs) while topical timolol treatment (0.5% hydrogel, three times daily) did not cause AEs (Wu et al., 2018).

High-Risk Areas

Timolol is superior to watchful waiting in infants with superficial IH in high-risk areas. Cheng et al. (2020) studied infants of <1-year-old (within 13-month) which presented superficial IHs in high-risk areas. The IHs were smaller than 2 cm. Patients who received timolol showed significantly fewer complications than the control group, in which watchful waiting has been performed (4.2% versus 29%).

Lip represents a high-risk region for ulceration in IHs patients (Cheng and Friedlander, 2016). Xu et al. (2020) demonstrated that topical timolol administration was not effective for lip IHs compared to oral beta-blockers. Although very efficient and safe, therapy with oral propranolol is often not sufficient for IHs of lips and most cases needed additional therapy after systemic propranolol. A retrospective study showed that a pentagonal wedge resection of the segmental lip IHs is also an effective procedure for the treatment of lip IHs as well as for other vascular lip anomalies (de Castro et al., 2017). Other well-known surgical procedures for lip IHs include wedge resections and elliptical excisions (Zide et al., 1997) or rectangular block excision technique (Li W. Y. et al., 2011).

TOPICAL CARTEOLOL

Carteolol is another nonselective beta-blocker, which shared with propranolol similar mechanisms of action; superficial and small IHs were successfully treated with topical carteolol. Xu et al. (2018) demonstrated a reduction of tumors and a response rate of 75% following treatment with carteolol. In addition, patients that showed complications, such as erythema and scarring, recovered without concern. This study suggested that topical carteolol is an effective, safe and noninvasive therapeutic alternative for IHs. Moreover, carteolol showed few complications. Head and neck hemangiomas, such as periorbital or cervical IHs, as well as localized and superficial hemangiomas, are particularly suitable for this therapy.

ITRACONAZOLE

A single study by Chen et al. (2019) tested the effects of itraconazole on endothelial cells of mouse hemangioendothelioma (EOMA) cell line and infantile primary hemangioma endothelial cells (HemEC). Itraconazole blocked cellular proliferation in a dose-dependent manner and caused apoptosis in both cell lines. Moreover, itraconazole reduced vascular endothelial cell angiogenesis of HemEC and inhibited the expression of platelet-derived growth factor D (PDGF-D), thus reducing the PI3K/Akt/mTOR signaling, which is involved in the IHs pathogenesis. Gastrointestinal and liver disorders may appear as intraconazole side effects. Moreover, a possible interaction with any other concurrent medications should be considered (Chen et al., 2019).

SIROLIMUS

Sirolimus is an inhibitor of mammalian target of rapamycin (mTOR), which is involved in the regulation of cell cycle and, therefore, of the vascular endothelial proliferation. Hutchins et al. (2017) observed a 3-year-old girl presenting diffuse cutaneous, hepatic and pulmonary IHs with progressive pulmonary hypertension which was treated with oral sirolimus (0.8 mg/m^2 per dose twice daily). During the treatment, improvement of hepatic lesions and pulmonary hypertension was noted but the child died because of development of unexpected severe hypoglycemia. The authors, advocating the safety of sirolimus, however recommended its use in complicated cases with multi-organ involvement.

Le Sage et al. (2018) observed no improvement in patients with infantile hemangioma treated with sirolimus. The authors claimed that the absence of a lymphatic component in IHs may explain the low effectiveness of topical sirolimus in these lesions: this would explain its useful treatment for cutaneous manifestations of lymphatic malformations.

PULSED DYE LASER

Pulsed dye laser (PDL) is widely used for the treatment of IHs but its use is controversial. Ying et al. (2017) observed that both timolol maleate 0.5% cream and 595-nm pulsed dye laser (PDL) resulted in a significant clinical improvement of superficial IH during the proliferating phase.

The association of PDL with beta-blockers for the treatment of superficial and mixed IHs have appeared superior to PDL in efficacy and cost benefit (Asilian et al., 2015; Reddy et al., 2013). According to Valdebran et al. (2017), lasers are recommended for the treatment of superficial and thin IHs, whereas oral propranolol is mandatory for the therapy of deep IHs affecting the airways or obstructing the visual field. Finally, combined therapies may improve the outcome of mixed IHs and refractory superficial IHs (Valdebran et al., 2017).

INFANTILE HEMANGIOMAS WITH INVOLVEMENT OF CENTRAL NERVOUS SYSTEM: PHACE AND LUMBAR SYNDROME

PHACE syndrome is characterized by a large (>5 cm) facial hemangioma which is associated with several congenital anomalies (Judd et al., 2007).

Instead, in LUMBAR syndrome a segmental IH in the lower body region is associated with urogenital, bony, anorectal, arterial anomalies and mielopathy (Golabi et al., 2014).

The risk of hidden arterial anomalies in LUMBAR syndrome requires a careful investigation before starting treatment with oral propranolol (Johnson and Smidt, 2014; Yu et al., 2017). In a recent European multicenter observational retrospective study, seven infants with large or segmental cutaneous IHs, involving the head, neck, lumbar or sacral area, were screened using MRI for PHACES or LUMBAR syndromes revealing intracranial or intraspinal IH. All patients underwent oral propranolol treatment for 6–14 months. All CNS lesions responded to treatment and five

patients had a complete or almost complete resolution of the cutaneous IHs, thus demonstrating that propranolol can pass the blood-brain barrier. This study suggest that oral propranolol should be considered the first-line approach for intra-CNS IHs to avoid possible complications (Diociaiuti et al., 2020).

CONCLUSION

The treatment of infantile hemangiomas is challenging even today. In our review, we described the last 5-year experiences with propranolol, the first line therapy for IHs treatment, and, in particular, with off-label drugs, such as topical β-blockers, including topical timolol and carteolol, steroids, itraconazole or sirolimus. Currently, oral propranolol is used in the majority of cases, but topical β-blockers can be preferred in superficial and uncomplicated forms. Oral and topical β-blockers have changed the prognosis of IHs, but some parents and physicians are reluctant to systemic therapies because of the risk of adverse events. Some alternative therapies are emerging, but data are not still enough to evaluate their efficacy and safety.

The need to lower as much as possible the risk of adverse events in pediatric population drives the search of other therapies than oral propranolol or steroids. We hope that further studies may confirm the safety of current therapies for IHs and expand the range of alternatives.

AUTHOR CONTRIBUTIONS

ML: Conceptualization, data curation, writing–original draft, first authorship; AD: Methodology, writing–review and editing, first authorship; DL: Conceptualization, writing–review and editing; IN: Validation, writing–review and editing; BF: Methodology, supervision; LF: Investigation, validation; VF: Investigation, validation; SV: Methodology, data curation; SF: Supervision, methodology, validation; VM: Conceptualization, investigation, supervision, writing–review and editing.

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Appropriateness of Antibiotic Prescribing in Hospitalized Children: A Focus on the Real-World Scenario of the Different Paediatric Subspecialties

Chiara Nasso[1†], *Alessandro Scarfone*[1†], *Igor Pirrotta*[1†], *Michelangelo Rottura*[1], *Domenico Antonio Giorgi*[1], *Giovanni Pallio*[1], *Natasha Irrera*[1], *Violetta Squadrito*[2], *Francesco Squadrito*[1]*, *Pierangela Irrera*[1], *Vincenzo Arcoraci*[1] and *Domenica Altavilla*[3]

[1]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy, [2]Department of Human Pathology and Evolutive Age "Gaetano Barresi", University of Messina, Messina, Italy, [3]Department of Biomedical and Dental Sciences and Morphological and Functional Imaging, University of Messina, Messina, Italy

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***Correspondence:**
Francesco Squadrito
fsquadrito@unime.it

[†]These authors have contributed equally to this work and share first authorship

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Background: Antibiotics are prescribed for children both in hospital and community settings, particularly at preschool age. Italy shows a high rate of inappropriate antibiotic prescriptions which may represent a serious problem in the hospital scenario. Thus, the aim of this study was to investigate appropriateness of antibiotic prescribing in the context of different paediatric subspecialties in a hospital setting.

Methods: Antibiotics prescribing was retrospectively analysed in paediatric patients (0–18 years) admitted in the emergency paediatrics, general paediatrics, paediatric nephrology and rheumatology units between January and December 2019. Patients were stratified by age in neonates, infants, toddlers, children and adolescents. Assessments were conducted by trained local assessors and appropriateness was classified as appropriate, inappropriate and not assessable.

Results: Empirical antibiotics were mainly prescribed following a diagnosis of respiratory, gastrointestinal and/or urinary infection. A total of 825 antibiotic prescriptions were recorded in the three subspecialties; 462 antibiotic prescriptions (56%) out of 825 were assessed as inappropriate and 55 prescriptions (6.7%) were not assessable. Inappropriateness considerably varied within subspecialties: the risk of inappropriate antibiotic prescribing was higher in emergency paediatrics and general paediatric than in children, according to age. Ceftriaxone and clarithromycin were the most inappropriate prescribed antibiotics in the emergency paediatrics whereas amoxicillin/clavulanic acid represented the most inappropriate antibiotic prescribed in general paediatrics.

Conclusion: The present data may be useful in order to reduce inappropriate antibiotic prescribing in the paediatric setting; antibiotic stewardship and clinical improvement programs in hospital paediatric care are strongly recommended.

Keywords: inappropriate antibiotic use, hospitalized children, active pharmacovigilance, cost, real-word scenario

INTRODUCTION

Antibiotics are frequently prescribed for children both in hospital and community settings (Principi and Esposito, 2016), particularly at preschool age (Clavenna and Bonati, 2009). Antimicrobial stewardship programs (ASPs) are different in children and adults in terms of outcome measurement: antibiotic dosage in children is based on body weight or body surface area, and therefore, the defined daily dose (DDD) as a preferred measurement of antibiotic consumption is not applicable. An alternative for measuring antimicrobial use is therapy days (i.e., per 1000 patient-days), but unfortunately, such data cannot be compared with adult DDDs (Araujo da Silva et al., 2018).

A quantitative and qualitative variability was observed between countries in antibiotic prescriptions, both in hospitalised and outpatient children and in particular increased antibiotic prevalence rates (number of patients treated with at least 1 antibiotic/100 patients) were detected among non-European paediatric patients (43.8%, range 32.2–65.1%) (Versporten et al., 2013). The Antibiotic Resistance and Prescribing in European Children Point Prevalence Survey (ARPEC-PPS) identified variations among hospitalized children; Africa, Australia, Western Europe and Northern Europe showed a high rate of older narrow-spectrum antibiotics, such as benzylpenicillin, sulfamethoxazole/trimethoprim, amoxicillin and gentamicin. On the contrary, children in Eastern Europe, Southern Europe, Asia, North America and Latin America were mainly treated with broad-spectrum antibiotics, especially third generation cephalosporins, cefepime and meropenem. In addition, a reduction of antibiotic treatment was reported in European neonates (22.8%) than non-European ones (39.4%) (Clavenna, 2015). However, Italy is one of the European countries with the higher rate of inappropriate antibiotic prescriptions (Agenzia Italiana del Farmaco, 2019): antimicrobial drugs were prescribed in 37–61% of hospitalized infants and children (Versporten et al., 2016) and the high rate (20–50%) of these prescriptions are unnecessary or inappropriate (Hecker et al., 2003) (Hulscher et al., 2010). Moreover, different studies demonstrated that many children received broad-spectrum antibiotics to treat viral respiratory infection, thus increasing the risk of antibiotic resistance appearance (Hersh et al., 2011) (McCaig et al., 2003). Additionally, some antibiotics are administered to children for a period considerably longer than that needed or with an incorrect total daily dosage (Levy et al., 2012) (Esposito et al., 2001). However, this abuse and misuse of antimicrobials have numerous negative consequences: an increase of adverse drug reaction (ADR) incidence (Cosgrove, 2006) and risk of toxicity (Donà et al., 2020) were observed. As previously mentioned, the high number of inappropriate antibiotic prescriptions contributes to the antimicrobial resistance (AMR) (Hagedoorn et al., 2020); AMR is described as the capacity of a microorganism (bacterium, parasite, virus or fungus) to avoid an antimicrobial effect against itself (World Health Organization, 2021), therefore it is considered a quickly increasing global public health emergency (World Health Organization, 2021) which has to be managed for epidemiological and economic reasons. In fact, AMR might lead to longer hospital stay, increased risk of mortality, health care costs and treatment failures (Maragakis et al., 2008). Thus, the health and economic consequences of antibiotic resistance are severe. Today, drug-resistant infections lead to approximately 700,000 deaths per year globally. This is projected to increase to 10 million by 2050, with associated costs as high as US $100 trillion worldwide. Each year, in the European Union (EU) alone, 25,000 patients die due to infections caused by multiresistant bacteria, costing society approximately €1.5 billion annually. By 2050, expected cumulative losses due to multiresistance will reach 2.9 trillion USD per year (Machowska and Lundborg, 2019). Previous reports also demonstrated that AMR may be related to a growing incidence of *Clostridium difficile* infection (Baur et al., 2017) as well as negative impact of microbiota (Vangay et al., 2015). For these reasons, antimicrobial stewardship (AS) is a key approach to reduce AMR incidence in hospital settings and may point at a rationale and effective use of drugs in children (World Health Organization, 2021). However, no report has previously analysed the appropriateness of antibiotic prescribing in relation to the different paediatric subspecialties in a hospital setting. Thus, the aim of the present study was to investigate this issue in the real word scenario of an Italian hospital characterized by the presence of several paediatric Units.

MATERIALS AND METHODS

In a retrospective observational study, antibiotics prescribing was analysed in paediatric patients (0–18 years) admitted to the University Hospital "G. Martino" of Messina, (Sicily, Italy) for the period between January and December 2019. Antibiotics were classified according to the Anatomical Therapeutic Chemical Classification (ATC) and in particular the prescription of drugs in 2nd level ATC = J01 was evaluated. Emergency paediatrics, general paediatrics, paediatric nephrology and rheumatology were involved in this study and its protocol was approved by the Ethics Committee of the A.O.U. "G. Martino" of Messina (project identification code N°283–20 Bis data of approval 11/11/2020) according to the legal requirements concerning observational studies; patient's consent to participate was not requested for this kind of study. Data were anonymously recorded by clinical doctors and pharmacists during hospitalization, including patient demographic features, indication for antibiotics, route of antibiotic administration and type of antibiotics as norm of clinical practice. All paediatric patients were stratified by age group: neonates (<28 days), infants (28 days to <1 year), toddlers (1year to <3 years), children (3 years to <12 years), adolescents (12 years to <18 years). Data about consumption and cost of antibiotics prescriptions were extracted from the pharmacy administrative database; the used drugs by ATC code, the consumption in unit dose and the cost in euro (€) were extracted for each unit.

Antibiotic Appropriateness

The appropriateness of antibiotic prescribing was investigated by consulting the patient's clinical records of the emergency

TABLE 1 | Demographic characteristics of patients and antibiotic prescriptions.

Demographic	Number of Patients (%) total = 626	Number of Antibiotic Prescriptions (%) total = 825
Sex male	358 (57.8)	453 (54.9)
Age		
neonates (<28 days)	2 (0.3)	2 (0.2)
infants (28 days to <1 year)	170 (27.2)	226 (27.4)
toddlers (1 year to <3 years)	148 (23.6)	199 (24.1)
children (3 years to <12 years)	234 (37.4)	300 (36.4)
adolescents (12 years to <18 years)	72 (11.5)	98 (11.9)
Paediatric Subspecialties		
Emergency Paediatrics	325 (51.9)	414 (50.2)
General Paediatrics	191 (30.5)	273 (33.1)
Paediatric Nephrology and Rheumatology	110 (17.6)	138 (16.7)

paediatrics, paediatrics, paediatrics nephrology and rheumatology wards. Patients with at least one antibiotic administration were selected. All clinical information (reason for hospitalization, infections, administered drugs) and socio-demographic (age and sex) features of each patient were collected. The appropriateness of antibiotic prescribing was assessed by the World Health Organization (WHO) AWaRe classification guidelines and by the regional guidelines for appropriate antibiotic prescribing in children (Oteri et al., 2013). Assessments were conducted by trained local assessors (pharmacology trainers, specialist of pharmacology and pharmacist) and appropriateness was classified as follows: appropriate (the best therapeutic approach for the treatment of each diagnosis conducted by clinician), inappropriate (antibiotics not recommended for each diagnosis conducted by clinician) and not assessable (antibiotics not present in the guidelines for the use in a specific diagnosis conducted by clinicians).

Statistical Analysis

The results were expressed as absolute and relative frequencies of the categorical variables, with 95% confidence intervals (CIs) and as medians and interquartile range (Q1—Q3), respectively. The Mann—Whitney *U* test for independent sample and two—tailed Pearson chi—squared test was performed to compare continuous variables and categorical variables. Odds ratios (ORs) with 95% CIs were estimated for each covariate of interest in univariate (Crude OR) and multivariate (adjusted OR) regression models. A *p-value* < 0.05 was assessed as statistically significant. Statistical analysis was carried out using the SPSS version 23.0 (IBM Corp. SPSS Statistics).

RESULTS

Characteristics of Patients

Between January and December 2019, 626 patients were hospitalized in the units of emergency paediatrics, general paediatrics, paediatric nephrology and rheumatology (**Table 1**). Patients were stratified by age as neonates, infants, toddlers, children and adolescents; the unit of emergency paediatrics admitted the highest number of patients (*N* = 325) and mostly were male (57.8%) and children (37.4%).

Antibiotic Prescribing

During the hospital stay, 825 prescriptions of antibiotics were recorded (**Table 1**); more specifically, children received more antibiotics (36.4%) followed by infants (27.4%), toddlers (24.1%), adolescents (11.9%) and neonates (0.2%). The unit of emergency paediatrics showed a greater number of antibiotic prescriptions than the other paediatric subspecialties (**Table 1**). All the antibiotics were empirically prescribed based on the clinical diagnosis (dermatological diseases, respiratory infections, abscesses, gastrointestinal diseases, central nervous system infection, urinary infections, traumatic wounds). The main hospitalization diagnoses in the paediatrics units were respiratory infections 318 (50.9%), urinary infections 88 (14.0%) and gastrointestinal diseases 83 (13.2%). In addition, among patients with respiratory diseases, 54 (8.6%) patients had upper respiratory tract infections. **Figure 1** reports the main prescribed antibiotics in relation to the hospitalization diagnosis. Amoxicillin was the most widely prescribed antibiotic for respiratory infections treatment followed by clarithromycin, amoxicillin/clavulanic acid and ceftriaxone (**Figure 1A**). Amoxicillin/clavulanic acid was the main antibiotic prescribed for urinary infections treatment, followed by ceftriaxone, cefixime and amoxicillin (**Figure 1B**). Metronidazole was mostly prescribed for gastrointestinal diseases (**Figure 1C**) followed by ceftriaxone, amoxicillin/clavulanic acid and amoxicillin.

Appropriateness

The appropriateness of antibiotic prescriptions was evaluated by the means of the WHO AWaRe classification and by using the regional guidelines for the antibiotic treatment in the paediatric population. A total of 825 antibiotic prescriptions was recorded in the three subspecialties. 462 antibiotic prescriptions (56%) out of 825 were assessed as inappropriate and 55 (6.7%) were not assessable (**Figure 2**). Inappropriateness also varied considerably by subspecialty. Emergency paediatric showed the greatest number of inappropriate prescriptions (*n* = 246; 53.24%) followed by general paediatrics (*N* = 159; 34.42%) and paediatric nephrology and rheumatology (*N* = 57; 12.34%) (**Figure 2**).

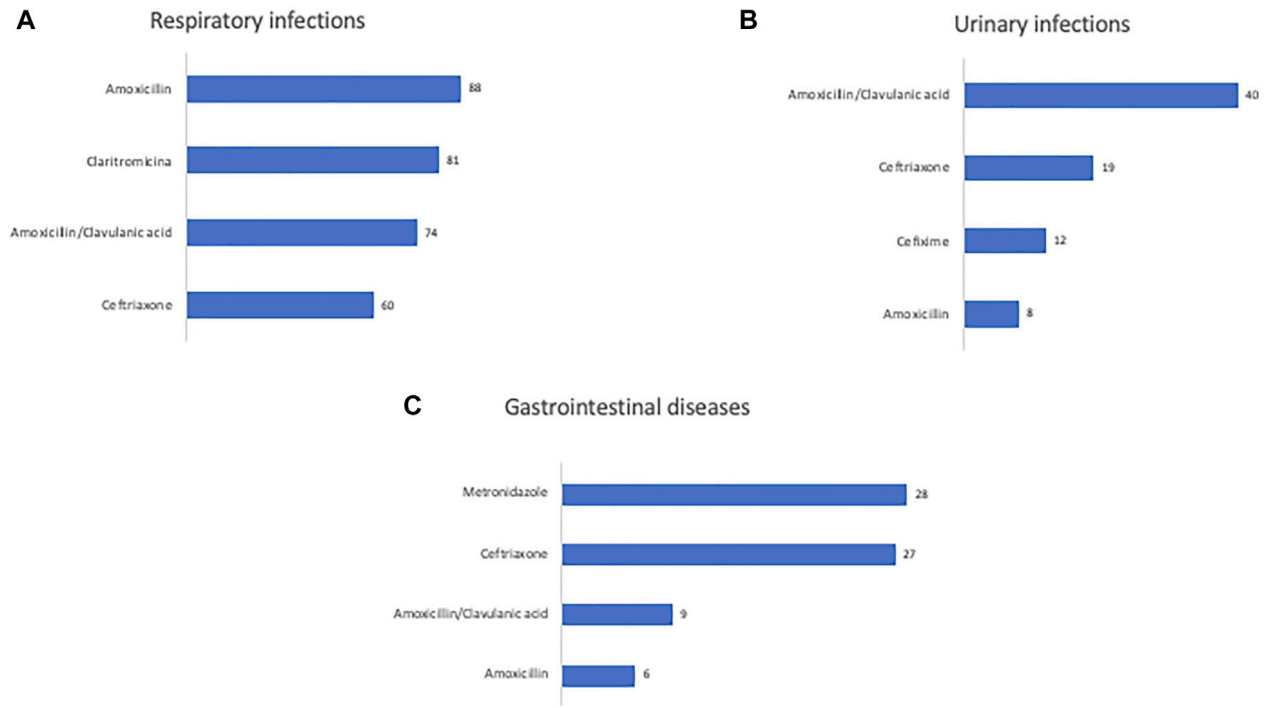

FIGURE 1 | Number of antibiotics prescriptions used for the main diagnoses of patients admitted to pediatric units.

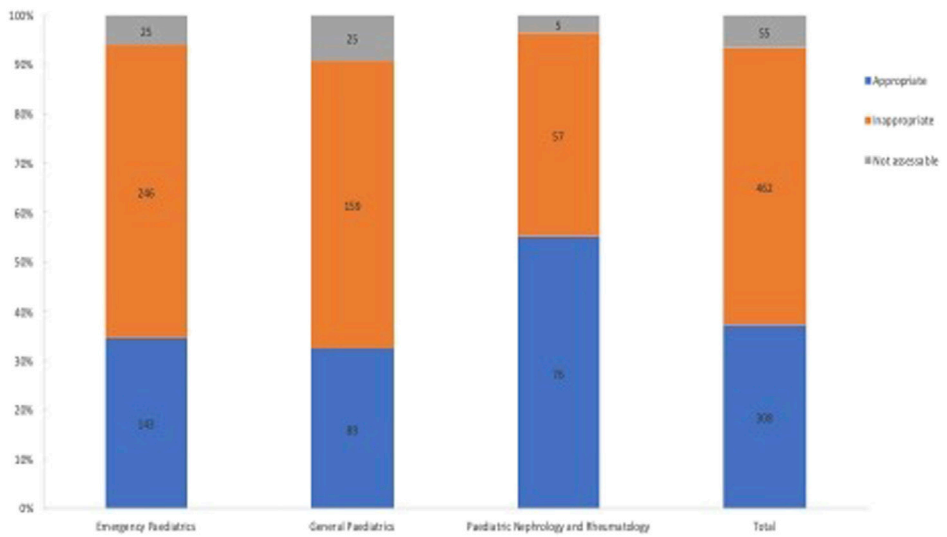

FIGURE 2 | Distribution of appropriateness stratified by paediatric subspecialties.

Figure 3 shows the inappropriate use of the single antibiotic molecule stratified in the several paediatric units. Ceftriaxone and clarithromycin were the most inappropriate antibiotics in the emergency paediatrics; ceftriaxone and amoxicillin were the most inappropriate antibiotics in paediatric nephrology and rheumatology units while amoxicillin/clavulanic acid represented the most inappropriate antibiotic prescribed in general paediatrics (**Figure 3**). A regression analysis was performed in order to explore the risk factors associated with the occurrence of inappropriate antibiotic use. In this analysis, inappropriate antibiotic prescribing was significantly higher in emergency paediatrics and general paediatric. Conversely, gender, age and hospitalization diagnosis did not significantly influence an inappropriate antibiotic prescribing (**Table 2**).

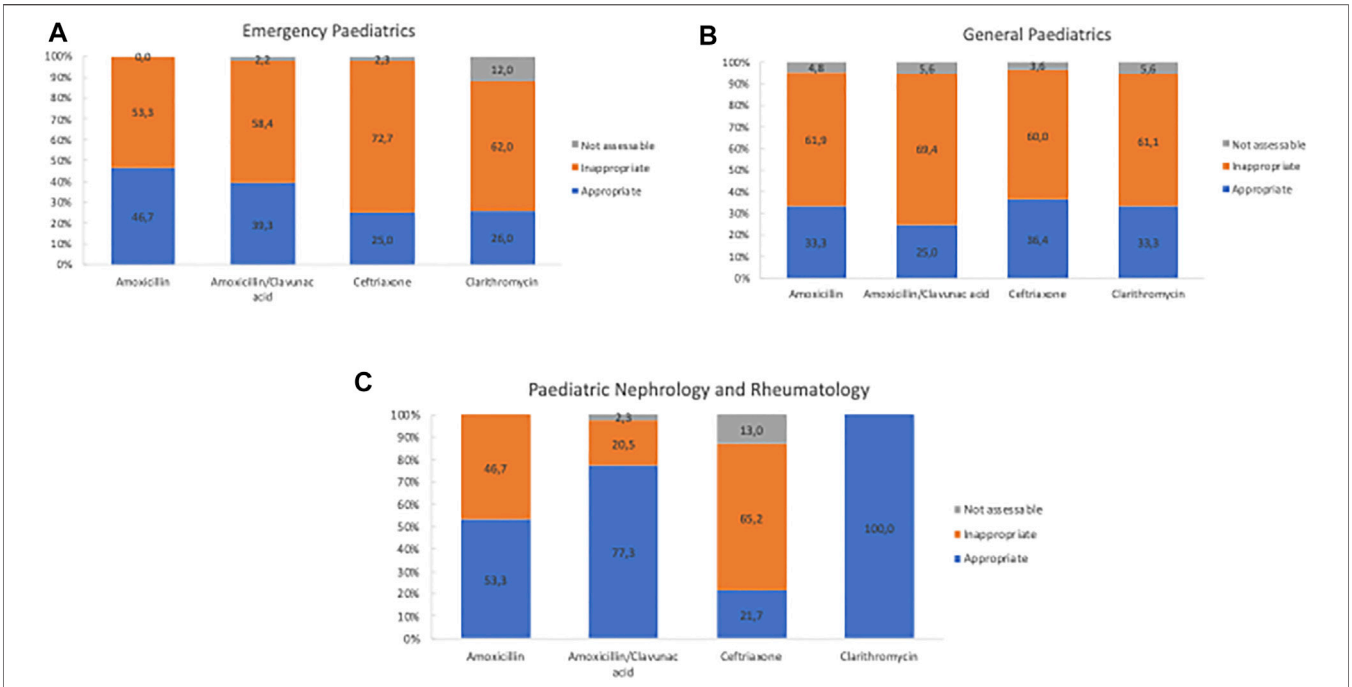

FIGURE 3 | Distribution of appropriateness of the main antibiotic prescriptions stratified by subspecialties.

TABLE 2 | Risk factors for inappropriate antibiotic prescribing.

	Crude OR (IC 95%)	*p* Value	Adjusted OR (IC 95%)	*p* Value
Gender, (M)	1.10 (0.81–1.45)	0.595	1.09 (0.80–1.47)	0.588
Hospital Units				
Paediatric Nephrology and Rheumatology	Ref	—	Ref	—
Emergency Paediatrics	2.30 (1.54–3.42)	<0.001	1.81 (1.09–3.01)	0.022
General Paediatrics	2.38 (1.55–3.66)	<0.001	1.85 (1.08–3.17)	0.026
Age				
Neonates and infants	Ref	—	Ref	—
Toddlers	0.68 (0.45–1.02)	0.062	0.67 (0.44–1.03)	0.069
Children	0.70 (0.48–1.00)	0.050	0.70 (0.48–1.04)	0.078
Adolescents	0.73 (0.44–1.21)	0.220	0.82 (0.47–1.42)	0.475
Hospitalization diagnoses				
Urinary infections	Ref	—	Ref	—
Gastrointestinal diseases	1.83 (1.02–3.29)	0.042	1.13 (0.56–2.23)	0.728
Respiratory diseases	2.63 (1.66–4.17)	<0.001	1.69 (0.94–3.05)	0.079
Other diagnosis	2.66 (1.64–4.32)	<0.001	1.76 (0.98–3.19)	0.060

Utilization and Expenditure

In our study, drug utilization and expenditure were also evaluated in the paediatric units throughout the observation period. The 18.8% of the 26.617 consumed drugs in all paediatric units was antibiotics and more than half of the antibiotic utilization (54.9%) was observed in the emergency paediatric unit (**Table 3**). Moreover, a total of 53.318,06 euros was spent for drugs in all paediatrics units and the highest cost (60,7%) was related to general paediatrics unit with an amount of 32354,11 euros. When only the expenditure for antibiotics (2nd level ATC = J01) was considered the total cost was 8.003,06 euros and emergency paediatrics unit showed the greatest cost (61,9%) with an amount of 4956,36 euros.

DISCUSSION

This retrospective study provides an analysis of appropriateness of antibiotic prescribing in hospitalized

TABLE 3 | Utilization and expenditure of the total amount of drugs and antibiotics (2nd level ATC = J01) for each single paediatrics units.

	Utilization (unit Dose)		Cost (€)	
Paediatrics units	Total N = 26.617 (100%)	J01 N = 4.996 (100%)	Total € 53.318,06 (100%)	J01 € 8.003,06 (100%)
Emergency Paediatrics	14702 (55,2%)	2743 (54,9%)	14897,44 (27,9%)	4956,36 (61,9%)
General Paediatrics	6435,88 (24,2%)	1752 (35,1%)	32354,11 (60,7%)	2374 (29,7%)
Paediatric Nephrology and Rheumatology	5480 (20,6%)	501 (10,0%)	6066,51 (11,4%)	336,35 (4,2%)

paediatric patients allocated in different subspecialties in agreement with their medical needs. As far as we know, no previous study has focused attention on the antibiotic prescribing related to the different paediatric subspecialties. This issue was investigated taking advantage of the availability of a real word scenario: a hospital characterized by the presence of several paediatric units. Our results showed the occurrence of a high rate of antibiotic inappropriate prescription; more than half of the prescribed antibiotics was inappropriate. Therefore, the co-presence of several paediatric subspecialties units does not allow a better management of antibiotic prescribing, even if they are empirically prescribed based on the clinical diagnosis. Furthermore, we tried to investigate the "clinical scenario" more "at risk" of antibiotic inappropriateness. As expected, the emergency paediatrics unit showed the higher rate of inappropriate antibiotic prescribing. This is not surprisingly if the "characteristics of emergency" of this clinical setting are taking into account. Indeed, previous data have suggested that antibiotics represent the most commonly drugs prescribed with inappropriateness in the general emergency department either in adults and children, in turn causing unwanted adverse events and contributing to the development of therapeutic failure and antimicrobial resistance (Denny et al., 2019; Denny et al., 2020). However, the same disaggregated information for emergency department exclusively dedicated to the paediatric population is scarce. The present data confirm that the availability of "a paediatric emergency unit" does not cause any improvement in the appropriateness of antibiotic therapy and in the selection of the right antibiotic, likely as a consequence of the need to treat patients for a fast clinical evaluation without any in-depth further analysis of the clinical status. Furthermore, our results showed that ceftriaxone and clarithromycin were the most inappropriate molecules. By contrast, the paediatric nephrology and rheumatology units showed a tendency towards a more rationale and appropriate use of the antibiotics. The reason of this finding may be ascribed to the availability of a closer clinical evaluation of patients admitted to these yards that allows a better understanding of the underling infectious disease. Paediatric population was also stratified by age, but any significant correlation was observed between age and inappropriate antibiotic prescribing. This result is, at least in part, in disagreement with a large Australian nationwide survey assessing over 6000 prescriptions for 4000 hospitalized paediatric patients (McMullan et al., 2020) In fact, this Australian survey showed that older age was significantly associated with inappropriate prescribing (McMullan et al., 2020). Indeed, information on the antibiotic prescribing in hospitalized paediatric population is scarce in Italy and the most relevant data are available for primary care patients: more specifically, it has been shown that during 2019, in Italy, 40.9% of children under the age of 13 received at least one antibiotic prescription, with an average of 2.6 prescriptions for child and with a high rate of inappropriateness (Agenzia Italiana del Farmaco, 2019). Our results confirm, in a paediatric hospital setting, the poor attitude to an appropriate antibiotic prescribing. As already known, paediatric population shows a high frequency of exposure to antibiotics (Piovani et al., 2013). For this reason, an adequate correlation of the qualitative-quantitative profile of antibiotic administration could limit the risk of adverse events appearance and reduce the paediatric expenditure for drugs of the National Health System (NHS). According to a recent study by the ARPEC (Antibiotic Resistance and Prescribing in European Children project group) carried out on hospitalized paediatric patients, ceftriaxone was the active ingredient with the highest prevalence of use (9.8%), followed by amoxicillin and clavulanic acid (7.6%) (Versporten et al., 2016). In our study amoxicillin was the most widely used antibiotic drug (44.4%) for the respiratory infection treatment followed by clarithromycin, amoxicillin/clavulanic acid and ceftriaxone. These results were in accordance with the regional guidelines for the antibiotic treatment in the paediatric population that indicates amoxicillin use for all the respiratory infections (Oteri et al., 2013). By contrast, amoxicillin/clavulanic acid was the main antibiotic prescribed for the urinary infections management, followed by ceftriaxone, cefixime and amoxicillin. Indeed, the different pattern of antibiotic prescription may be characteristic of the hospital setting because of polytherapy of hospitalized patients. Furthermore, drug use and expenditure were also explored in the several paediatric subspecialties throughout the study. The 18.8% of the 26.617 consumed drugs in all paediatric units was antibiotics and more than half of the antibiotic use (54.9%) was observed in the emergency paediatric unit. This leads us to speculate that an inappropriate antibiotic prescription leads to an exaggerated cost which represents a burden for the national health system as confirmed by the results of Agenzia Italiana del Farmaco 2019 report which showed that antibiotic use represents the 3.6% of Italian public health expenditure with an amount of 692.000.000 Euros (Agenzia Italiana del Farmaco, 2019).

LIMITATION

The present study has some limitations. Firstly, this is a single-centre study; secondly, enrolled patients were only hospitalized, and our survey does not capture data on community or primary care, which represent the bulk of antibiotic prescribing. Moreover, antibiogram was not considered during the assessment of the suitability of antibiotics. Also, we did not analyse precise diagnoses in accordance with International Statistical Classification of Diseases, Injuries and Causes of Death (ICD) but only groups of diagnosis.

CONCLUSION

Our study suggests the need to implement measures of a close monitoring of antibiotic prescriptions in the paediatric population. Furthermore, our results on antibiotic prescribing in the different paediatric units can be useful for future interventions directed towards reducing inappropriate antibiotic use. More specifically, we have clearly shown that emergency paediatrics unit deserves a close monitoring and additional attention. We suggest that antibiotic stewardship and clinical improvement programs in hospital paediatric care should be strongly recommended. Finally, our study confirms the need to create professional's positions of clinical pharmacologists with the aim to cooperate and synergize with paediatric clinicians to reduce the exposure of hospitalized patients to an inappropriate antibiotic treatment.

DATA AVAILABILITY STATEMENT

The dataset generated for this study will not be made publicly available. Further inquires can be directed to the author FS, fsquadrito@unime.it.

AUTHOR CONTRIBUTIONS

DA, FS, and VA: conceptualization. CN, AS, PI, and IP: data curation. MR and AS: formal analysis. MR, DG, NI, PI, and GP: methodology. FS: project administration. NI, GP, and VA: supervision. DA and VA: validation. CN, AS, and MR: writing—original draft preparation. NI, DA, FS, and VA: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Antibiotic-Induced Neutropenia in Pediatric Patients: New Insights From Pharmacoepidemiological Analyses and a Systematic Review

Vera Battini[1†], Alessandra Mari[2†], Michele Gringeri[1], Francesca Casini[2], Francesco Bergamaschi[2], Giulia Mosini[1], Greta Guarnieri[1], Marco Pozzi[3], Maria Nobile[3], Gianvincenzo Zuccotti[2], Emilio Clementi[1,3], Sonia Radice[1], Valentina Fabiano[2†] and Carla Carnovale[1†]*

[1]Unit of Clinical Pharmacology, Department of Biomedical and Clinical Sciences, "Luigi Sacco" University Hospital, Università degli Studi di Milano, Milan, Italy, [2]Unit of Pediatrics, Department of Biomedical and Clinical Sciences, "Vittore Buzzi" Children's University Hospital, Università degli Studi di Milano, Milan, Italy, [3]Scientific Institute IRCCS E. Medea, Bosisio Parini, Lecco, Italy

Aim: to characterize pediatric cases of antibiotic-associated neutropenia through a multidisciplinary approach, focusing on the temporal association between the wide spectrum of treatment options and the occurrence of this relatively uncommon but potentially clinically relevant adverse event.

Methods: we carried out a pharmacoepidemiological analysis based on the FDA Adverse Event Reporting System (FAERS) database, a retrospective chart review and a systematic review of the literature, focusing on the time to onset (TTO) of this side effect, in the pediatric clinical setting.

Results: A total of 281 antibiotic-related neutropenia events, involving 11 categories of antibiotics, were included in the time to onset analysis. The median TTO ranged from 4 to 60 days after the start of the therapy. A shorter median TTO was found from the retrospective chart review [16 patients: median days (25th-75th percentiles) = 4 (3–5)], compared to 15 (9–18) vs. 10 (6–18) for literature (224 patients) and FAERS (41 cases), respectively. The Anatomical Therapeutic Chemical classes, J01X, J01F, J01E and J04A, and the median TTOs retrieved from more than one source revealed high accordance ($p > 0.05$), with J01X causing neutropenia in less than a week and J01F/J01E/J04A in more than 10 days. Antibiotics were discontinued in nearly 34% of cases. In FDA Adverse Event Reporting System reports, half of the patients experiencing neutropenia were hospitalized.

Conclusion: Whereas antibiotic associated neutropenia is benign in the majority of cases, yet it should not be neglected as, even if rarely, it may put children at higher risk of clinical consequences. Clinicians' awareness of antibiotic-associated neutropenia and its mode of presentation contributes to the continuous process of monitoring safety of antibiotics.

Keywords: neutropenia, antibiotics, pediatrics, pharmacovigilance, FAERS

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Lana Nezic, University of Banja Luka, Bosnia and Herzegovina
Dmitry A. Sychev, Ministry of Health, Russia
Chengwen Teng, University of South Carolina, United States

***Correspondence:**
Sonia Radice
sonia.radice@unimi.it

[†]These authors have contributed equally to this work

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1 INTRODUCTION

Drug-induced neutropenia is a relatively rare but potentially fatal disorder (with a significant impact on health care costs); it occurs in susceptible individuals, with an incidence of 2.4–15.4 cases per million population (Strom et al., 1992). It also raises serious concern during the development of new drugs (Uetrecht, 2007) because it can be missed in clinical trials due to its low incidence. This issue is of paramount importance since, in severe cases, it can even lead to withdrawal of a drug from the market (Thompson et al., 2016).

Neutrophils play a crucial role in the defence and control of infections, especially bacterial ones; the normal range for the absolute neutrophil count (ANC) varies with age; the lower limit of normal is 5,000/ml (5.0 × 109/L) for the first week of life, then falls to 1,000/ml (1.0 × 109/L) between 2 weeks and 1 year of age (Bhatt and Saleem, 2004; Hsieh et al., 2007; Segel and Halterman, 2008; Newburger and Dale, 2013; Barg et al., 2015; Dale, 2017). According to the ANC, neutropenia can be graded as mild (1,000–1,500 cells/μL), moderate (1,000–1,500 cells/μL) and severe (<500 cells/μL) (Bhatt and Saleem, 2004).

Over the last 20 years, a number of medications have been strongly implicated as potential causes of idiosyncratic neutropenia, including antithyroid agents, psychotropic drugs, anticonvulsants, and antibiotics (Andersohn et al., 2007a; Andrès et al., 2011; Johnston and Uetrecht, 2015; Andrès et al., 2017).

Antibiotics are the most commonly prescribed therapy to children (Nicolini et al., 2014) and, especially for preterm infants or children in neurological rehabilitation, who are more prone to the risk of bacterial infections (Bhutta and Black, 2013; Sankar et al., 2016; Shane et al., 2017), antibiotics are an essential lifesaving treatment. The use of unlicensed or off-label antibiotics is indeed a common practice in neonatal intensive care units (Kumar et al., 2008).

Up to 30% of children exposed to long-term antibiotic therapy (>14 days) is expected to develop adverse effects, with an incidence of neutropenia that varies dramatically in publications (Gomez et al., 2001; Maraqa et al., 2002; Andersohn et al., 2007b; Olson et al., 2015; Tribble et al., 2020).

Drug-induced neutropenia is the second commonest cause of acquired acute neutropenia in children, after post-infectious neutropenia (Barg et al., 2015; Knight, 2015). The true incidence of this phenomenon is not known, as most reports focus on the rare and more severe agranulocytosis, which has an incidence of 1–10 cases per million per year.

Clinicians who prescribe to and treat patients with antibiotics regularly (Andersohn et al., 2007a; Andrès et al., 2011, 2017) and are aware of antibiotic side effects, may not detect neutropenia since patients developing this rare side effect are either asymptomatic or experience non-specific symptoms such as fever and skin rash (Andersohn et al., 2007a; Andrès et al., 2011, 2017). Moreover, in the context of septicemia, severe sepsis or viral infections, the role of antibiotics as causative drugs of neutropenia is often difficult to define (Andrès et al., 2017).

Affected patients experience severe neutropenia within several weeks to several months after first exposure to a drug; the temporal association between the wide spectrum of treatment options and the occurrence of neutropenia has not been clarified yet, negatively impacting on its early identification and eventual management.

The pathogenesis of antibiotic-induced neutropenia is complex, not fully understood yet and potentially multifactorial (Murphy et al., 1985; Olaison et al., 1999; Andrès et al., 2017). Some studies have suggested an immune-mediated etiology due to production of anti-neutrophil antibodies, similarly to the phenomenon of penicillin-induced hemolytic anemia (Kirkwood et al., 1983; Murphy et al., 1983, 1985; Salama et al., 1989).

Other reports have described a myelosuppressive effect of antibiotics, by demonstrating a lack of differentiated myeloid elements in bone marrow aspirates of subjects with antibiotic-induced neutropenia (Neftel et al., 1985). Recent studies have also suggested that the fecal microbiota regulate the number of circulating neutrophils, and that microbiota changes observed in course of antibiotic therapy may be linked to antibiotic-induced neutropenia (Balmer et al., 2014; Zhang et al., 2015; Josefsdottir et al., 2017).

Although the issue of antibiotic-induced neutropenia in the general population has been previously addressed (Holz et al., 2021), a unique focus on the pediatric population is missing; it would provide a more defined and comprehensive framework on the topic, underlining specific problems and details, and contributing to fill the gap in knowledge, thus helping physicians to face it. To this end we decided to proceed through the gathering of data by using an integrated approach based on pharmacoepidemiological analyses and a systematic review of all the currently available pediatric evidence; this strategy allows to define specifically aspects not retrievable otherwise, namely time to onset (TTO), distribution of agent groups, duration and treatment options, contributing to increase awareness about this potentially dangerous side effect among clinicians, towards improving of its management.

Despite the intrinsic limitations, we used the spontaneous reporting systems FDA Adverse Event Reporting System (FAERS), as it still represent a valuable source of real-world data about the safety/efficacy profile of specific drugs; it also allows to compare therapeutic options, gain relevant insights on potential mechanisms of adverse drug reactions (ADRs) (Carnovale et al., 2018, 2019a, 2019b; Mazhar et al., 2019), and estimate the time to onset of ADRs (Mazhar et al., 2021), thus contributing to prevent ADRs and improve the pharmacological management of iatrogenic disorders. The results we obtained provide new insights towards improving the diagnosis of antibiotic-induced neutropenia, in the pediatric clinical setting.

2 PATIENTS AND METHODS

2.1 Pharmacovigilance Study

Data were obtained from the FAERS, one of the largest and most comprehensive spontaneous reporting system databases. It receives millions of reports of adverse events per year from

healthcare practitioners, consumers, companies, and other sources, concerning drugs. Adverse events are recorded in the FAERS using the Medical Dictionary for Regulatory Activities (MedDRA®) preferred terms. The database is largely used to detect novel drug-related safety events, to identify possible mechanisms of adverse events, to explore potential drug-drug interactions (Carnovale et al., 2019b; Mazhar et al., 2019). Adverse events recorded in the FAERS were downloaded from the Food and Drug Administration (FDA) website (FDA Adverse Event Reporting System, 2019 (FAERS) Quarterly Data Extract Files). The database consists of seven datasets, namely patient demographic and administrative information (file descriptor DEMO), drug and biologic information (DRUG), adverse events (REAC), patient outcomes (OUTC), report sources (RPSR), start and end dates of drug therapy (THER), and indications for use/diagnosis (INDI). These seven datasets were joined by unique identification numbers for each FAERS report and a relational database was built. Data extraction was restricted to reports without missing values for age and gender. Duplicate records were detected and deleted accordingly as previously described (Carnovale et al., 2018).

The cohort was retrieved from the FAERS database in the period covering the first quarter of 2010 to the second quarter of 2021 and consisted of all adverse events (AEs) occurred in paediatric patients (<18 years.o.). Since neutropenia must be carefully diagnosed in patients under the age of 18, we limit data extraction to those Individual Case Safety Reports (ICSRs) reported by physicians. A custom list of neutropenia-related event terms was then *ad hoc* created, combining different Preferred Terms that contain a range of Lowest Level Terms (LLTs) reflecting the same medical concept expressed by synonyms and lexical variants (Fescharek et al., 2004). After a review of all LLTs in MedDRA, 11 terms were selected: band neutrophil count decreased, band neutrophil percentage decreased, granulocyte count decreased, granulocyte percentage decreased, granulocytes maturation arrest, granulocytopenia, idiopathic neutropenia, neutropenia, neutrophil count decreased, neutrophil count abnormal, neutrophil percentage decreased. We filtered for ICSRs reporting at least one of the LLT above mentioned. At the same time, we excluded those ICSRs where the terms "sepsis" and "myelosuppression" were reported concomitantly with neutropenia. Finally, we included only those cases involving at least one antibiotic [Anatomical Therapeutic Chemical (ATC) code: J01] reported as "suspect drug" and clearly specifying in the report the start date of the therapy. If a patient had been treated with more than one antibiotic and the information was available for all the antibiotics, the single ICSR was split in more cases.

2.2 Case Series

We retrospectively reviewed medical records of children treated with oral (PO) or intravenous (IV) antibiotic therapy for bacterial infections, who presented neutropenia in the course of treatment. We considered patients who received inpatient antibiotic therapy in our Pediatric Department, Vittore Buzzi University Children's Hospital, Milan, from 1 January 2020, until 30 June 2021.

To avoid confounding factors regarding neutropenia's etiology, we included in the study only patients who presented neutropenia after the introduction of the antibiotic therapy, considering the pivotal role of the temporal relationship to assess causality. We have excluded patients with other possible underlying causes of neutropenia, including oncologic diseases, concomitant viral infections, bacterial sepsis, personal history of autoimmune disease, cyclic neutropenia or any pre-existent neutropenia. Neonates were not included in our analysis.

Furthermore, we assessed improvement of the neutrophil count over time in all patients.

Patients were included if complete blood count (CBC) had been performed at time of diagnosis (T0), before starting antibiotic therapy, and if at least one other CBC analysis was made for clinical necessity after starting therapy.

For the selected patients, we collected data on neutropenia's TTO (i.e., the number of days between the beginning of antibiotic therapy and the detection of neutropenia) analyzing changes in CBC. Neutropenia was defined as an absolute neutrophil count (ANC) lower than 1,500/mmc (Bhatt and Saleem, 2004; Segel and Halterman, 2008).

2.3 Systematic Review

We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). We searched PubMed/MEDLINE, Embase, and the ClinicalTrials.gov database up to 21 January 2021 for the evidence of neutropenia following antibiotic treatment in a paediatric setting. The complete PubMed search string is described in the **Supplementary Material S1**. The search strategy was adapted as needed for each database. Essentially, we used the following terms combined with the Boolean operator "AND": antibiotic therapy, neutropenia and adverse drug reaction, in order to obtain a wide selection of articles to be subsequently screened individually.

2.3.1 Eligibility Criteria

Inclusion criteria were the following: any clinical trial, cohort or case-control study, case-report or case-series that reported a decrease in the neutrophil count that was ascribed to the treatment with one or a combination of antibiotics in at least one paediatric patient (neonates were excluded because of rapidly changing normal values references in the first weeks of life and consequently variable definitions of neutropenia at this age). We did not contact authors for unpublished data.

The formal assessment of causal relationship between neutropenia and antibacterial drugs was not included in the list of inclusion criteria. However, in order to avoid confounding factors, studies were also excluded if neutropenia occurred in patients affected by conditions that could trigger it, such as some specific infections (e.g., *salmonella typhi*, beta-haemolytic *streptococcus*, brucellosis, aspergillosis, malaria) and some concomitant diseases (e.g., oncologic and hematologic diseases). Studies reporting patients under concomitant therapy (e.g., chemotherapy, post-transplant anti-rejection

medication) that could concur in the development of neutropenia were also discarded.

Reviews, systematic reviews, meta-analyses, guidelines, book chapters, unpublished theses, and *in vitro*/animal study were excluded, as well as articles written in languages other than English, French or Spanish. Moreover, any study referred to adult patients were excluded, as well as those where data concerning the paediatric population was not discernible from the adult one and studies in which antibiotics administration did not imply a systemic distribution (i.e., topic administration).

2.3.2 Study Selection

After duplicate removal, our search results were screened by title and abstract and those potentially relevant were retrieved in full text and assessed for eligibility based on our prespecified inclusion criteria. The entire search process was performed by two independent researchers and disagreements about eligibility were solved upon reaching a consensus with a third investigator.

2.3.3 Outcome Measures

Primary outcome was change (from baseline) in neutrophil count (N/mmc) associated with antibiotic use. Studies that reported only general leukopenia, or pancytopenia, were disregarded (and categorized as "no outcome of interest"), as were studies that did not specify the administered antibiotic, generically referring to "antibiotic therapy" (categorized as "no drug of interest").

2.3.4 Data Extraction

For each included study, we extracted the following information: study design (study type, study duration, sample size), patient characteristics (age, sex, and number exposed to antibiotics), therapy (drug name, dose, route of administration, reason for use, duration of treatment, and concomitant medication) and outcomes (number of cases out of exposed, TTO, neutropenia laboratory values, symptoms and complications, antibiotic discontinuation, days to resolution).

In view of the nature of our aim (i.e., collecting ADRs as they were reported in the eligible studies) we did not perform a formal assess of the quality of studies. However, to properly discuss findings we took into account study design and methodological aspects of eligible studies included in the systematic review.

2.4 Time-to-Onset Analysis

In the observational cohort, TTO was first calculated for each patient from the start of the therapy to the date of the laboratory data confirming neutropenia.

As for the systematic review, TTO was measured using the start date of the treatment and the date of neutropenia occurrence, by retrieving specific information provided by authors. Whether not explicitly reported, it was estimated using the median duration of treatment.

From the FAERS database, TTO was calculated from the time of the patient's start date of the treatment (as reported in the ICSRs) to the occurrence of the neutropenia.

Drugs were merged following the Anatomical Therapeutic Chemical (ATC) codes and the median TTO was then calculated.

2.5 Statistical Analyses

Descriptive analysis was performed in terms of age, sex and the use of concomitant medications. Reduced levels in WBC and neutrophils were tested with one-tailed Wilcoxon test. The median (25th–75th percentiles) duration was used to evaluate the TTO. In order to compare our results concerning TTO, the two-tailed Mann-Whtiney test was used between two groups and two-tailed ANOVA Kruskall-Wallis among three groups. Concerning all tests, significance was set at a p-value of 0.05. Data reading, filtering, processing and statistical analysis were performed through RStudio (R Core Team, 2019).

3 RESULTS

3.1 Pharmacovigilance Study

From the 63,084 pediatric ICSRs sent by physicians to the FAERS, 1,210 (1.91%) reported neutropenia as ADR; of these, 26 (2.14%) reported antibiotics as "suspect drugs" involved in the occurrence of neutropenia and provided data both on start therapy and AE dates. Details of all the retrieved cases related to the occurrence of Antibiotic-associated neutropenia from the FAERS are reported in the **Supplementary Table S1**. The mean age of the patients was 10 ± 6 years (min-max: 0–17) and 54% (n = 14) were females. Indications were largely heterogenous, but all related to serious infection like meningitis (n = 2), pyelonephritis (n = 1), osteomyelitis (n = 2). Concomitant medications were used in 25 (89%) patients and 24 (86%) used more than one antibiotic, mostly intravenous administered. 13 (50%) ICSRs required hospitalization; however, the remaining reports were related to other serious events, too (unfortunately, the reporter did not specify what type of other events). Only three ICSRs reported "neutropenia" as unique AE, while the other cases described the AE by using several MedDRA terms especially related to a systemic reaction against the suspect drugs. After splitting the ICSRs, 41 cases were available for the TTO analysis.

3.2 Case Series

Sixteen patients (9 females, 7 males) treated with intravenous (IV) or oral (PO) antibiotic therapy who developed neutropenia in course of treatment and did not present the above-mentioned exclusion criteria were included in our analysis. Patients' age ranged from 1 to 51 months [median (25th–75th percentiles) = 5.5 (2.5–21)]; they were treated for pyelonephritis (n = 12, 75%), osteomyelitis (n = 2, 12.5%) and soft tissues infections (n = 2, 12.5%). All patients were previously healthy and did not receive any medications other than the prescribed antibiotics, except for paracetamol or ibuprofen for fever and pain relief.

IV ceftriaxone was the most frequently used antibiotic (n = 6), followed by PO amoxicillin/clavulanic acid (n = 4) and IV ampicillin/sulbactam (n = 2). The remaining four patients received respectively IV meropenem, IV oxacillin, PO ceftibuten and IV ceftriaxone + metronidazole. Three patients presented severe neutropenia, with an ANC <500/mmc. **Figure 1** shows the median of the pre-and post-

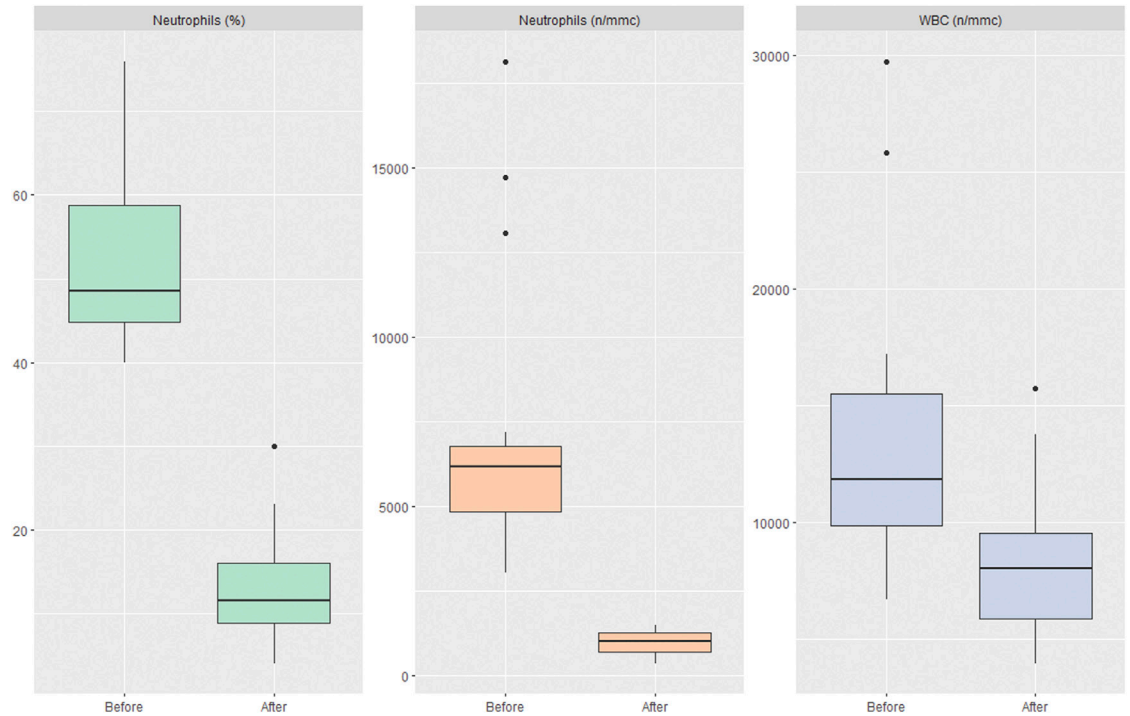

FIGURE 1 | The median of the pre-and post-therapy neutrophils (%, n/mmc) and WBC levels (n/mmc).

TABLE 1 | Anagraphic characteristics of the included patients, details on antibiotic therapy, in terms of drug and indication use, and variations into ANC and white blood cells (WBC) count.

Case	Age (months)	Sex	Diagnosis	Antibiotic (Route of administration)	WBC T0 (n/mmc)	Neutrophils T0 (ANC, %)	Time to onset (days)	WBC TX (n/mmc)	Neutrophils TX (ANC, %)	Symptoms	Therapy discontinued
1	25	F	Osteomyelitis	Oxacillin (IV)	14660	7138 (49%)	27	4370	1000 (23%)	No	No
2	5	M	UTI	Ceftriaxone (IV)	17180	13056(76%)	3	6220	1030 (16%)	No	No
3	22	F	UTI	Amoxicillin/ Clavulanic Acid (PO)	7380	3025(41%)	3	4410	350 (8%)	No	No
4	2	M	UTI	Ceftriaxone (IV)	10200	4896(48%)	6	9880	1230 (12%)	No	No
5	12	F	UTI	Ceftibuten (PO)	6670	3068(46%)	4	13740	960 (7%)	No	No
6	2	F	UTI	Amoxicillin/ Clavulanic Acid (PO)	10170	4983(49%)	2	6100	980 (16%)	No	No
7	3	M	UTI	Ceftriaxone (IV)	29740	18141(61%)	3	5040	520 (10%)	No	No
8	3	M	UTI	Amoxicillin/ Clavulanic Acid	16530	6612(40%)	4	15740	1430 (9%)	No	No
9	3	M	Soft tissues infection	Ceftriaxone (IV)	15170	6068(40%)	4	9400	410 (4%)	No	No
10	6	F	UTI	Amoxicillin/ Clavulanic Acid (PO)	7380	4649(63%)	5	7960	420 (5%)	No	No
11	2	F	UTI	Ceftriaxone (IV)	13790	6619(48%)	3	10480	1150 (11%)	No	No
12	8	F	UTI	Ceftriaxone (IV)	25800	14706(57%)	5	8110	1370 (17%)	No	No
13	51	M	Osteomyelitis	Ampicillin/ Sulbactam (IV)	10260	6258(61%)	14	3950	1190 (30%)	No	No
14	24	F	UTI	Meropenem (IV)	9820	4050(41%)	5	8950	1280 (14%)	No	No
15	18	F	Soft tissues infection	Ceftriaxone (IV) + Metronidazole (IV)	13390	4050(48%)	6	8990	1490 (16%)	No	No
16	1	M	UTI	Ampicillin Sulbactam (IV)	9880	5640(58%)	2	6330	730 (11%)	No	No

ANC, Absolute Neutrophil Count; F,Female; IV, Intravenous; M,Male; PO, Per Os (oral); UTI, Urinary Tract Infection; T0, Time of diagnosis; TX, Detection time of neutropenia; WBC, White Blood Cells.

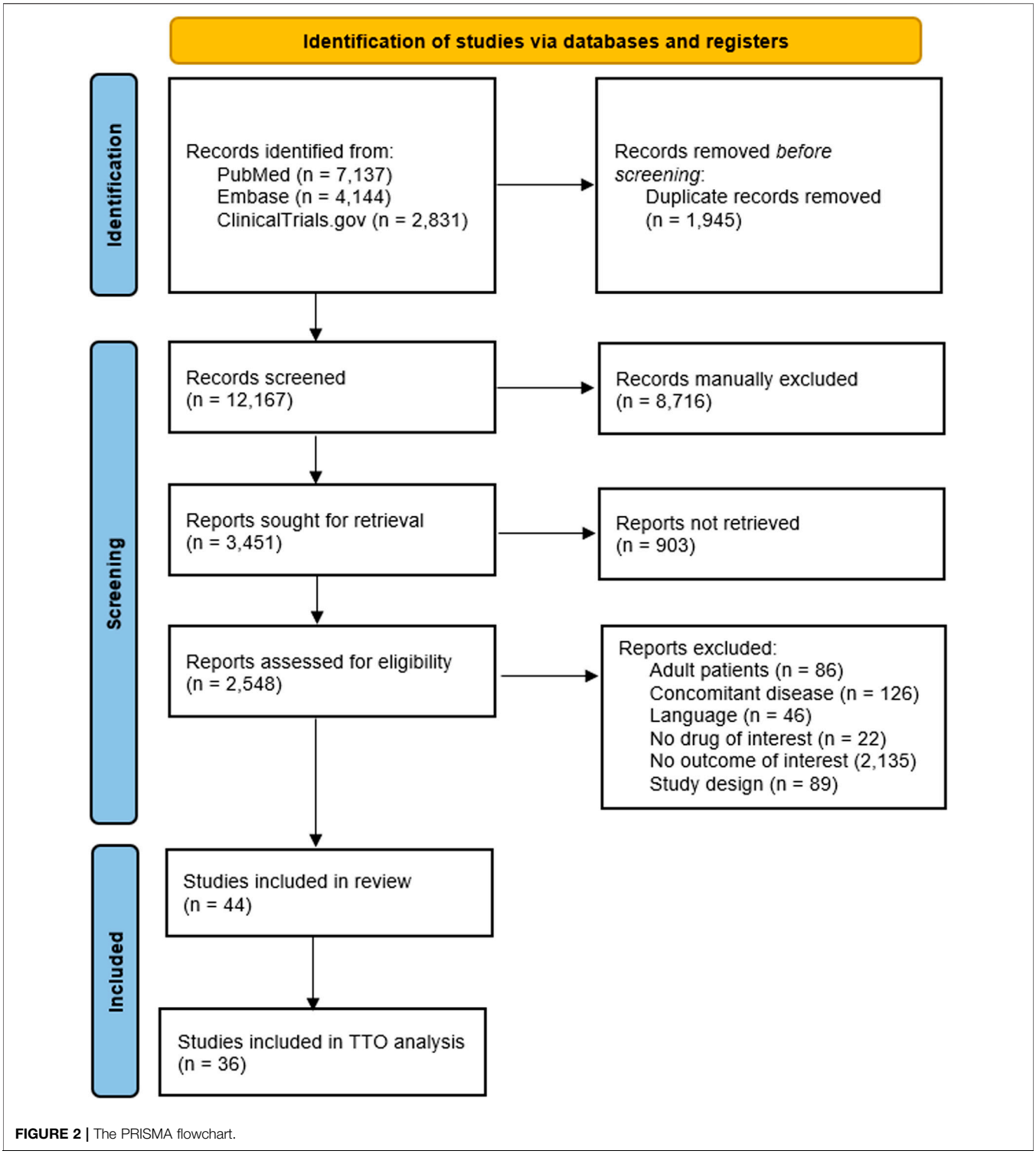

FIGURE 2 | The PRISMA flowchart.

therapy neutrophils (%, n/mmc) and WBC levels (n/mmc). Neither leukopenia, thrombocytopenia nor anemia were observed in the treated patients. None of the patients presented symptoms associated to neutropenia, nor clinical reasons to discontinue antibiotic therapy. Data on resolution of neutropenia are not available, as patients were discharged before full normalization of ANC. **Table 1** provides demographic characteristics of the included patients, details on antibiotic therapy, in terms of drug and indication for use and variations into ANC and WBC count. Characteristics of

TABLE 2 | Details of neutropenia occurrence in studies included in TTO analysis.

Study	Main author (year)	Antibiotic [ATC Code]	n patients with neutropenia (% out of total patients)	n neutrophils/ mmc	TTO (days)	Antibiotic withdrawal	Resolution (days)	Symptoms or complications	Other associated ADR
1	Jarkowski TL and Martmer EE (1962)	Sulfadimethoxine [J01ED01]	1 (100)	914	5	Y	NA	Toxic epidermal necrosis	Death
2	Leventhal JM and Silken AB (1976)	Oxacillin [J01CF04]	1 (100)	0	19	Y	2	NA	NA
		Oxacillin [J01CF04]	1 (100)	297	19	Y	4	N	NA
		Oxacillin [J01CF04]	1 (100)	560	19	Y	4	N	NA
3	Chu JY, et al. (1977)	Oxacillin, ampicillin [J01CF04, J01CA01]	1 (100)	0	17	Y	5	N	N
4	Greene GR and Cohen E (1978)	Nafcillin [J01CF06]	1 (100)	600	24	Y	6	N	NA
		Nafcillin [J01CF06]	1 (100)	690	4	Y	3	N	NA
5	Ardati KO, et al. (1979)	Trimethoprim, sulfamethoxazole [J01EE01]	9 (50)	480 (1 pt); 560 (1 pt); 1,150-1,420 (7 pt)	4 (3 pt); 7 (1 pt); 11 (1 pt); 12 (1 pt); 23 (1 pt); NA (2 pt)	Y (2 pt)	NA	N	Eosinophilia, thrombocytopenia, transient elevation of liver enzymes
6	Feldman WE, et al. (1980)	Cefoxitin [J01DC01]	2 (11)	<1,000	9 (1 pt); R: 3-21 (1 pt)	Y (1 pt)	2 (1 pt)	N	Eosinophilia
7	Asmar BI, et al. (1981)	Trimethoprim/ Sulfamethoxazole [J01EE01]	17 (34)	344 (1 pt); <750 (7 pt); <1,200 (7 pt)	M: 5.8	Y (1 pt)	M: 8.9; R: 3-23	N	Eosinophilia, thrombocytopenia, anemia
		Amoxicillin J01CA04	1 (5)	1,309	10	N	NA	N	Eosinophilia
8	Dutro MP, et al. (1981)	Nafcillin [J01CF06]	1 (100)	54	22	Y	4	N	N
		Nafcillin [J01CF06]	1 (100)	252	9	Y	3	N	N
9	Kumar K and Kumar A (1981)	Ampicillin [J01CA01]	1 (100)	156	10	N	16	Fever	NA
		Chloramphenicol, ampicillin [J01BA01, J01CA01]	1 (100)	0	14	Y	25	NA	NA
10	St John MA and Prober CG (1981)	Cloxacillin [J01CF02]	2 (3)	<500	2 (1 pt); 10 (1 pt)	N	NA	N	Eosinophilia, elevated liver enzymes
11	Tuomanen EI, et al. (1981)	Chloramphenicol [J01BA01]	11 (25)	<1,000	M: 8.2, R: 7-10 (5 pt); 45 (4 pt); 120 (1 pt); 330 (1 pt)	Y	1-2 (5 pt); 7 (4 pt); 14 (1 pt); 21 (1 pt)	N	N
12	Kaplan SL, et al. (1983)	Moxalactam [J01DD06]	20 (53)	<500 (1 pt); <1,000 (10 pt); <1,500 (8 pt)	R: 1-21; 5 (11 pt); 21 (9 pt)	N	NA	N	Eosinophilia, thrombocytopenia
13	Chonmaitree T, et al. (1984)	Ceftriaxone [J01DD04]	2 (4)	390 (1 pt); 616 (1 pt)	R: 5-14; 5 (1 pt); 14 (1 pt)	Y	R: 2-7	N	Thrombocytosis, eosinophilia
14	Dubs MMA (1985)	Amoxicillin/ clavulanic acid, ticarcillin/clavulanic acid [J01CR02, J01CR03]	1 (100)	212	23	Y	5	N	N
15	Feldman S, et al. (1985)	Trimethoprim/ sulfamethoxazole [J01EE01]	28 (57)	<1,500	M: 18.4 ± 4.3; R: 10-23	NA	R: 23-37	N	Thrombocytopenia, hemoglobin decrease
		Amoxicillin [J01CA04]	22 (54)	<1,500	M: 17.8 ± 4.4	NA	R: 23-86	N	Thrombocytopenia, hemoglobin decrease

(Continued on following page)

TABLE 2 | (*Continued*) Details of neutropenia occurrence in studies included in TTO analysis.

Study	Main author (year)	Antibiotic [ATC Code]	n patients with neutropenia (% out of total patients)	n neutrophils/ mmc	TTO (days)	Antibiotic withdrawal	Resolution (days)	Symptoms or complications	Other associated ADR
16	Higham M, et al. (1985)	Ceftriaxone [J01DD04]	2 (6)	150 (1 pt); 700 (1 pt)	6 (1 pt); 3 (1 pt)	Y	7 (1 pt); NA (1 pt)	N	NA
17	Schaad UB, et al. (1987)	Amoxycillin/ clavulanic acid [J01CR02]	1 (1)	248	6	N	21	N	Elevated liver enzymes
18	Ahonkhai VI, et al. (1989)	Imipenem/cilastatin [J01DH51]	4 (2)	<1,000	M: 6.3; N: 5; R: 1-26	N	NA	N	NA
19	Al-Fadley F (1992)	Ampicillin, cloxacillin [J01CA01, J01CF02]	1 (100)	950	15	Y	8	Rash, fever	N
		Ampicillin, cloxacillin [J01CA01, J01CF02]	1 (100)	340	15	Y	95	Rash, fever	N
		Ampicillin, cloxacillin [J01CA01, J01CF02]	1 (100)	620	14	Y	38	Rash	Eosinophilia
		Cloxacillin [J01CF02]	1 (100)	230	23	Y	25	Rash, fever	Eosinophilia
		Cloxacillin, piperacillin [J01CF02, J01CA12]	1 (100)	1,400	20	Y	41	Rash, fever	Eosinophilia
20	Dagan R, et al. (1994)	Ceftriaxone, cefetamet pivoxil [J01DD04, J01DA26]	1 (2)	NA	7	N	NA	N	N
21	Shinohara YT and Colbert J (1994)	Vancomycin [J01XA01]	1 (100)	990	15	Y	NA	N	NA
22	Bégué P and Astruc J (1995)	Roxithromycin [J01FA06]	5 (1)	500-1,000	M: 9 (4 pt); 2 (1 pt)	Y (2 pt)	1 (1 pt); 4 (1 pt)	NA	NA
23	Arguedas A, et al. (1996)	Amoxicillin/ clavulanic acid, [J01CR02]	3 (7)	<1,500	10	N	NA	N	N
24	Arguedas A, et al. (1997)	Azithromycin [J01FA10]	2 (6)	<1,500	3	N	NA	NA	NA
		Clarithromycin [J01FA09]	3 (6)	<1,500	10	N	NA	NA	NA
25	Hori C, et al. (1997)	Trimethoprim/ sulfamethoxazole [J01EE01]	2 (6)	<1,000	365	N	NA	N	N
26	Losurdo G, et al. (1998)	Rifabutin, clarithromycin [J04AB04, J01FA09]	3 (43)	NA	M: 21; R: 14-28	N (dose reduction)	5	N	NA
27	Kaplan SL, et al. (2001)	Linezolid [J01XX08]	5 (8)	58 (1 pt); 1,020 (1 pt); 1,150 (1 pt); 1,370 (1 pt); 1,470 (1 pt)	3 (3 pt); M: 12.2, R: 6-41 (2 pt)	Y	11	N	NA
28	Wee IY and Oh HM (2001)	Teicoplanin [J01XA02]	1 (100)	706	14	Y	7	Rash, fever	Elevated liver enzymes, rash
29	Arguedas A, et al. (2003)	Azithromycin [J01FA10]	19 (14)	<1,500	14	N	NA	NA	NA
		Ceftriaxone [J01DD04]	8 (13)	<1,500	14	N	NA	NA	NA
30	Jacobs RF, et al. (2005)	Azithromycin [J01FA10]	2 (6)	1,400 (1 pt); 1,500 (1 pt)	3	N	36	N	N

(Continued on following page)

TABLE 2 | (*Continued*) Details of neutropenia occurrence in studies included in TTO analysis.

Study	Main author (year)	Antibiotic [ATC Code]	n patients with neutropenia (% out of total patients)	n neutrophils/ mmc	TTO (days)	Antibiotic withdrawal	Resolution (days)	Symptoms or complications	Other associated ADR
31	Pietroni M (2005)	Cloxacillin [J01CF02]	1 (100)	140	34	Y	NA	Fever	Sepsis, death
32	Van Den Boom J, et al. (2005)	Flucloxacillin [J01CF05]	8 (100)	M: 710; R: 190-1,250	15 (1pt); 20 (3 pt); 25 (1 pt); 27 (1 pt); 36 (1 pt); 58 (1 pt)	Y	5.6	Rash, fever	NA
33	Hettmer S and Heeney MM (2008)	Cefepime [J01DE01]	1 (100)	20	19	Y	5	NA	NA
34	Yusef D, et al. (2017)	Piperacillin/ Tazobactam [J01CR05]	10 (26)	<1,500	Median: 18	Y	NA	NA	Fever, elevated liver enzymes, elevated CRP, abdominal pain
35	Patel S, et al. (2018)	Ceftriaxone [J01DD04]	1 (1)	NA	21	Y	NA	Rash, fever	NA
36	Fernando M, et al. (2019)	Dapsone [J04BA02]	1 (100)	0	60	Y	5	Skin sepsis	Leukopenia, bite cells, blister cells, agranulocytosis

ADR, Adverse Drug Reaction; ATC, Anatomical Therapeutic Chemical; M, mean; N, no; n, number; NA, not available; pt, patients; R, range; TTO, Time To Onset; Y, yes. The detailed list of references is reported in ***Supplementary Table S3***

antibiotic courses and neutropenia's TTO are reported in the **Supplementary Table S2**.

3.3 Systematic Review

The study selection and screening process is presented in the PRISMA flowchart (**Figure 2**). Out of the 12,167 unique titles retrieved (7,137 articles from PubMed, 4,144 from Embase, and 2,831 from Clinical.Trials.gov), 2,548 full-text articles were found and assessed for eligibility. Ultimately, 44 studies fulfilled our inclusion criteria. **Supplementary Table S3** summarizes the characteristics of all the studies included in the systematic review (including the related references).

The retrieved studies comprise case reports or case series (n = 13), prospective observational studies (n = 11), randomized controlled studies (n = 10), retrospective observational studies (n = 5), and non-randomized clinical trials (n = 5), published between 1962 and 2020, and reporting on a total of 2,602 pediatric patients treated with antibiotics.

Administered antibiotics were mainly penicillins alone (n = 35 studies; amoxicillin, ampicillin, cloxacillin, flucloxacillin, methicillin, nafcillin, oxacillin, piperacillin) or associated with β-lactamase inhibitors (n = 7; amoxicillin/clavulanic acid, piperacillin/tazobactam, ticarcillin/clavulanic acid), followed by cephalosporins (n = 12; cefaclor, cefepime, cefetamet pivoxil, cefixime, cefoxitin, ceftriaxone), macrolides (n = 7; azithromycin, clarithromycin, roxithromycin), sulphonamides (n = 7; sulfadimethoxine, trimethoprim/sulfamethoxazole), glycopeptides (n = 2; teicoplanin, vancomycin), and others (chloramphenicol, n = 2; moxalactam, n = 2; dapsone, n = 1; daptomycin, n = 1; imipenem/cilastatin, n = 1; linezolid, n = 1; rifabutin, n = 1).

These antibiotics were mostly used to treat otitis (n = 16), osteomyelitis (n = 9), pneumonia (n = 8), cellulitis (n = 7), bone/joint infections (n = 7), and bacterial meningitis (n = 7).

In all studies, treatment lasted less than 2 months, apart from an observational study (Losurdo et al., 1998) that reported 6 months of rifabutin and clarithromycin administration, and a clinical trial that investigated trimethoprim/sulfamethoxazole use for UTI prophylaxis, for which the drug administration ranged from 6 to 50 months (Hori et al., 1997).

A total of 228 pediatric patients experienced neutropenia following antibiotic administration: 77 patients required antibiotic withdrawal following neutropenia occurrence (and one dose reduction), while in all others cases it resolved spontaneously. All cases resolved within 2 months, apart from one patient reported by Al-Fadley et al., who was treated with ampicillin/cloxacillin for 26 days and required 95 days after withdrawal to reach a neutrophils level of 1,500 cells/mmc; and one patient treated with amoxicillin for 10 days took 86 days for his neutropenia to resolve (Feldman et al., 1985).

Neutropenia was rarely accompanied by complications, such as rash and fever. Associated adverse events observed in patients experiencing neutropenia were mainly eosinophilia, thrombocytopenia and elevation of liver enzymes.

TTO was measured for 215 patients (extracted from 36 studies); details concerning studies included in the TTO analysis are reported in **Table 2**.

TABLE 3 | Comparisons of TTO analyses among findings from pharmacovigilance study, clinical observation and literature.

ATC Class (WHO)	Retrospective chart review		Systematic review		FAERS		p^a
	n	TTO (days)	n	TTO (days)	*n*	TTO (days)	
J01X: Other antibacterials	-	-	7	12 (3–13)	4	4 (3–8)	>0.05
J01G: Aminoglycoside antibacterials	-	-	-	-	3	7 (6–92)	-
J01D: Other beta-lactam antibacterials	9	4 (3–5)	42	11 (5–18)	7	9 (6-15)	<0.05
J01M: Quinolone antibacterials	-	-	-	-	5	9 (8–14)	-
J01F: Macrolides, lincosamides and streptogramins	-	-	34	14 (9–14)	3	10 (10 -10)	>0.05
J01E: Sulfonamides and trimethoprim	-	-	55	18 (6–18)	3	10 (10–20)	>0.05
J01C: Beta-lactam antibacterials, penicillins	7	4 (3-10)	70	18 (16–19)	8	14 (6–16)	<0.05
J04A: Drugs for treatment of tuberculosis	-	-	3	21 (21-21)	7	16 (9–32)	>0.05
J02A: Antimycotics for systemic use	-	-	-	-	1	22	-
J01B: Amphenicols	-	-	12	30 (10–45)	-	-	-
J04B: Drugs for treatment of lepra	-	-	1	60	-	-	-
Total	16	4 (3–5)	224	15 (9–18)	41	10 (6–18)	

n, number of cases of neutropenia related with an antibiotic of that class; TTO, time to onset, median (25th-75th percentiles).
[a]Wilcoxon-Mann-Whtiney for comparison between two groups and ANOVA Kruskall-Wallis for three groups.

Fifty-seven patients (8 studies) were excluded from the TTO analysis because a precise date of neutropenia occurrence was not provided. Details of neutropenia occurrence reported in the studies excluded from TTO analysis are reported in **Supplementary Table S4**.

3.4 Time-to-Onset Analysis

We analysed (a total of) 281 antibiotic-related neutropenia events and 11 categories of antibiotics (**Table 3**). In general, the retrospective chart review (n = 16) detected neutropenia during the first days of treatment [median days (25th–75th percentiles) = 4 (3–5)], while data from the literature (n = 224) and FAERS (n = 41) show longer times [median days (25th–75th percentiles), 15 (9–18) vs. 10 (6–18) for literature and FAERS, respectively]. Median TTO ranged from 4 to 60 days after the start of the therapy. For ATC class J01X, J01F, J01E and J04A median TTOs retrieved from more than one source revealed high accordance ($p > 0.05$) with J01X causing neutropenia in less than a week and J01F/J01E/J04A in more than 10 days. On reverse, J01D and J01C were discordant among resources ($p < 0.05$), with a reduced median TTO (less than 7 days) in patients included in the retrospective chart review.

Unfortunately, some ATC classes were only available from one resource: a median TTO of 7 days was reported for J01G (n = 3), 9 days for J01M (n = 5), 22 days for J02A (n = 1), 30 days for J01B (n = 12), 60 days for J04B (n = 1).

4 DISCUSSION

Antibiotic-associated neutropenia is a relatively uncommon AE with a largely variable prevalence in the pediatric age (Gomez et al., 2001; Maraqa et al., 2002; Olson et al., 2015). Clinicians' awareness about common and uncommon antibiotic-related AEs, including neutropenia, is of great importance to improve quality of healthcare for children. The integrated approach based on pharmacoepidemiological analyses and a systematic review of all the currently available pediatric evidence used for this review allow us to provide detailed characterization in terms of distribution of agent groups, duration and treatment options, seriousness, among which the temporal association between the wide spectrum of antibiotic options and this rare but potentially clinically relevant adverse event. The first important finding is on TTO as data available on this matter in pediatric population are still scarce, especially regarding shorter and oral antibiotic therapy. In fact, most of the evidence available in pediatric age derives from studies on complications of outpatient, long-term, parenteral antibiotic therapy, as the those conducted by Olson et al. and Gomez et al., which found, respectively, a TTO of 21 and 20 days since the beginning of therapy (Gomez et al., 2001; Olson et al., 2015).

Our analysis with an integrated approach, reveals that the median TTO of antibiotic-induced neutropenia in the pediatric population is in fact shorter than previously reported (Gomez et al., 2001; Olson et al., 2015; Holz et al., 2021). The most discordant datum was found in the retrospective chart review, where median TTO we found to be 4 days. Different aspects should be taken into account for explaining this observation. All patients included in the case series analysis were infants or young children (median age 5 months), and all were hospitalized for acute and relatively severe infections. In these patients, a stricter follow-up through repeated assessments of laboratory values has allowed an earlier detection of neutropenia. Also in the pharmacovigilance study, median TTO was 10 days, that was, again, a much shorter period respect to findings of previous reports. As FAERS data extraction was limited to ICSRs reported by physicians, an easier access to laboratory assessments and a generally greater attention to ADRs may also in this case explain this datum. We cannot exclude that the reports of AEs may have been carried out by clinicians with greater awareness of potential ADRs and are more prone to strictly monitoring them. Indeed, in the systematic review analysis, neutropenia median TTO was 15 days, in line with the only previous study (Gomez et al., 2001; Olson et al., 2015; Holz et al., 2021); this may be also due to the type of included studies (mainly retrospective and case series), hence the heterogeneity of patients (different age range), settings

(inpatient and outpatient) and severity of treated infections. As for the antimicrobial agent groups, the shortest TTOs were seen in patients treated with ß-lactam antibacterials, other than penicillins (ATC code J01D) in all the analyses we carried out (case series, pharmacovigilance study and systematic review).

However, we found a significantly shorter TTO in the chart review respect to FARES reports and systematic review (4 vs. 9 vs. 11 days). On the contrary, both in systematic review and FAERS analysis, longer TTO were detected in patients treated with antituberculars (21 and 16 days, respectively).

As for the distribution of antimicrobial agent groups, we found that, penicillins and other ß-lactams, followed by sulphonamides and macrolides, resulted to be more frequently associated to neutropenia; similarly, in the case series, patients who developed neutropenia were mostly treated with cephalosporins and ß-lactams. Penicillins and other ß-lactams are the most widely prescribed antibiotics in pediatric age, either orally or intravenously. Consequently, AEs in general, and neutropenia specifically, may be more frequently detected with the use of these antimicrobial drug classes. This finding is in contrast to what Olson et al. (2015) observed in their systematic review, in which they did not find an association between neutropenia and ß-lactams antimicrobials, even if the majority of detected AEs occurred in patients treated with cephalosporines. The 41 neutropenia cases reported to FAERS resulted to be much more distributed among the different antimicrobial classes; once again, this observation may be result of a greater attention by the clinicians who are more familiar with AEs reporting. Recent evidence in adult population (Holz et al., 2021) found neutropenia to be mostly observed in patients treated with vancomycin and ceftaroline; however, these drugs are not so frequently prescribed in children.

It is well-known that drug-drug interactions (DDIs) may exacerbate ADRs, including antibiotic-associated neutropenia. In the retrospective chart review, we excluded patients treated with drugs other than antibiotics and all children, apart one, received an antibiotic monotherapy. On the contrary, in 88.46% of cases, patients included in FAERS analysis received more than one antibiotic as well as other concomitant treatments; only in two cases pharmacokinetic DDIs might have increased the risk for neutropenia (**Supplementary Table S2**) (Drug Interaction, 2022).

The consequences of antibiotic-associated neutropenia may vary in severity (Bhatt and Saleem, 2004; Segel and Halterman, 2008; Holz et al., 2021); in the pediatric age most cases are asymptomatic and resolve spontaneously without any treatment (Gomez et al., 2001; Barg et al., 2015; Celkan and Koç, 2015). In our case series, all neutropenia cases were asymptomatic and none of them required leukopoiesis stimulants, withdrawal of therapy, dose reduction or prolongation of hospitalization, differently to what we have observed in the systematic review, where antibiotics were discontinued in nearly 34% of cases (without requiring any treatment).

In FAERS reports, half of the patients experiencing neutropenia were hospitalized. However, only three presented neutropenia alone; in all other cases, systemic symptoms and other laboratory abnormalities were also present and may have contributed to the need of hospitalization. Moreover, FAERS reports likely include more severe ADRs. Although benign in the majority of cases (Segel and Halterman, 2008), antibiotic associated neutropenia should not be neglected as, even if rarely, the AE may put children at higher risk of severe infections and, in few serious cases, may also be fatal. Finally, development of antibiotic-associated neutropenia may result in discontinuation or modification of the ongoing and causative treatment with a potentially negative impact on the overall efficacy of therapy.

4.1 Strengths and Limitations

This is the first study aimed at specifically investigating the occurrence of neutropenia following antibiotic treatment that may be used to improve of antibiotic-induced neutropenia recognition in the pediatric clinical setting.

The use of a spontaneous reporting system database has some important implicit limitations because reporting is influenced by factors such as notoriety bias [media attention and recent publication of an adverse drug reaction in the literature might stimulate the reporting trend activity (Teng and Frei, 2022)], selection bias and under-reporting (Faillie, 2019).

Moreover, the quality of information, including the grade of completeness, may be suboptimal leading to misclassification bias. The certain causal correlation and incidence rates cannot be determined from the FAERS since the primary goal of spontaneous reporting systems is to signal the existence of a possible relationship between a drug or drug class and an adverse event, without proving any causality or providing the denominator (drug exposure). With regard to the case series, it is worth mentioning that the period of observation (one and a half year) is not long enough to detect the events of low incidence. The attempts to minimize these potential biases, we included high-quality reports (providing all relevant information such as sex, age, drug name, indication use, concomitant therapy, start and event date) sent by physicians only. Furthermore, when selecting the patient cohort from the three sources, we took into account the same inclusion/exclusion criteria to avoid confounding factors regarding neutropenia's etiology.

5 CONCLUSION

Our analysis extends previous evidence on the occurrence of neutropenia in the pediatric clinical setting providing precise information on the temporal association between the wide spectrum of antibiotic options and this uncommon adverse event, thus supporting its early identification and eventual management.

The integrated approach based on pharmacoepidemiological analyses and a systematic review of all the currently available pediatric evidence suggest that a stricter follow-up through repeated assessments of laboratory values is of crucial importance for an earlier detection of neutropenia (median TTO: 4 days). The

shortest TTOs were seen in patients treated with other than penicillins ß-lactams in either the case series, pharmacovigilance study and systematic review (range: 4–11 days); in contrast, longer TTO were detected in patients treated with antituberculars (16 > days).

More importantly, antibiotics were discontinued in nearly 34% of pediatric cases detected in the literature; in FAERS reports, half of the patients experiencing neutropenia (along with systemic symptoms and other laboratory abnormalities) were then hospitalized. Given the potential clinical consequences of this rare but potentially life-threatening event, continuous attention for this side effect with an appropriate monitoring are warranted in pediatric patients receiving antibiotic-based therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VB and AM conceptualized and designed the study, interpreted the data drafted the manuscript, revised and approved the final manuscript as submitted. MG, FC, FB, GM, and GG participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. MP, MN, GZ, EC, and SR participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted. CC and VF conceptualized and designed the study, interpreted the data, coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.877932/full#supplementary-material

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Clinical Application Value of Pharmacokinetic Parameters of Vancomycin in Children Treated in the Pediatric Intensive Care Unit

Bo Zhou[1,2,3,4†], Wenyi Xiong[5†], Ke Bai[2,3,4,6], Hongxing Dang[2,3,4,6], Jing Li[2,3,4,6], Feng Xu[2,3,4,6], Yue-qiang Fu[2,3,4,6†] and Chengjun Liu[2,3,4,6*†]*

[1] Department of Pharmacy, Children's Hospital of Chongqing Medical University, Chongqing, China, [2] National Clinical Research Center for Child Health and Disorders, Chongqing, China, [3] Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing, China, [4] Chongqing Key Laboratory of Pediatrics, Chongqing, China, [5] Department of Pediatrics, Chengdu Seventh People's Hospital, Chengdu Tumor Hospital, Chengdu, China, [6] Department of Critical Care Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China

Objective: To explore the efficacy and safety of vancomycin as measured by pharmacokinetic/pharmacodynamic parameters in children with severe infection in the Pediatric Intensive Care Unit (PICU) and to determine the appropriate threshold for avoiding nephrotoxicity.

Methods: The medical records of hospitalized children with severe infection treated with vancomycin in the PICU of a tertiary pediatric hospital from September 2018 to January 2021 were retrospectively collected. Univariate analysis was used to assess the correlation between vancomycin pharmacokinetic/pharmacodynamic parameters and therapeutic efficacy or vancomycin-related nephrotoxicity. Binary logistic regression was used to analyze the risk factors for vancomycin-related nephrotoxicity. The vancomycin area under the concentration-time curve over 24 h (AUC_{0-24}) threshold was determined by receiver operating characteristic (ROC) curve analysis.

Results: One hundred and 10 patients were included in this study. Seventy-six patients (69.1%) exhibited clinically effective response, while the rest exhibited clinically ineffective response. There were no significant differences in APACHE II score, steady-state trough concentration, peak concentration or AUC_{0-24} of vancomycin between the effective and ineffective groups. Among the 110 patients, vancomycin-related nephrotoxicity occurred in 15 patients (13.6%). Multivariate analysis showed that vancomycin treatment duration, trough concentration, and AUC_{0-24} were risk factors for vancomycin-related nephrotoxicity. The ROC curve indicated that $AUC_{0-24} < 537.18$ mg.h/L was a suitable cutoff point for predicting vancomycin-related nephrotoxicity.

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***Correspondence:**
Yue-qiang Fu
480887@hospital.cqmu.edu.cn
Chengjun Liu
liucwd@163.com

† These authors have contributed equally to this work

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Conclusion: No significant correlations were found between the trough concentration or AUC_{0-24} of vancomycin and therapeutic efficacy when the daily dose of vancomycin was approximately 40 mg/kg d, while the trough concentration and AUC_{0-24} were both closely related to vancomycin-related nephrotoxicity. The combination of AUC_{0-24} and trough concentration for therapeutic drug monitoring may reduce the risk of nephrotoxicity.

Keywords: nephrotoxicity, children, therapeutic drug monitoring, pharmacokinetics/pharmacodynamics, vancomycin

INTRODUCTION

Gram-positive bacteria (GPB) have become the primary cause of severe infection in children, especially the emergence of multiple drug-resistant bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), which brings great challenges to the choice of antibacterial drugs. Vancomycin is the primary drug of choice for the treatment of severe Gram-positive bacterial infections, such as MRSA; however, improper application will lead to poor clinical treatment effects and may lead to adverse drug reactions, including rash (1), ototoxicity (2, 3), and nephrotoxicity (4–7). At present, studies and guidelines on the therapy, therapeutic drug monitoring (TDM) and side effects of vancomycin mostly focus on adults, while there are relatively few studies in pediatric populations.

Children are constantly growing, and visceral function is not yet mature, which leads to the metabolism of the drug in the body not being the same as that of adults and making them more likely to induce adverse drug reactions. The relationship between PK/PD parameters of vancomycin and clinical efficacy is not entirely clear in pediatric populations, and some viewpoints of studies regarding vancomycin are conflicting. Some studies have shown that a higher initial trough concentration achieves better outcomes with vancomycin treatment in pediatric patients (8, 9). However, Yoo et al. (10) indicated that the initial trough concentration was not associated with 30-day.

In addition to clinical efficacy, the relationship between vancomycin and renal injury is another focus of pediatricians. Higher serum trough levels of vancomycin have been associated with AKI in pediatric patients (7, 11, 12). However, Moffett et al. (13) found that serum vancomycin concentrations did not predict vancomycin-associated AKI in the pediatric population. Although vancomycin has been used in severe pediatric infections for a long time, an analysis of vancomycin TDM is warranted.

TDM based on PK/PD parameters may improve clinical efficacy and reduce adverse reactions, which is of great significance for the rational use of vancomycin in children with severe infection (14). In the present study, we analyzed the relationship between PK/PD (the trough concentration and the area under the 24-h drug concentration-time curve (AUC_{0-24}) of vancomycin) and efficacy and nephrotoxicity. We hypothesized that PK/PD parameters of vancomycin could predict nephrotoxicity.

MATERIALS AND METHODS

Patient Population

Children with severe infection who received intravenous vancomycin treatment and underwent serum concentration (trough concentration and peak concentration) monitoring in the Pediatric Intensive Care Unit (PICU) at Children's Hospital of Chongqing Medical University from September 2018 to January 2021 were retrospectively identified and included in this study. The Institutional Review Board of Children's Hospital, Chongqing Medical University approved the study with a waiver of informed consent given that the data were analyzed anonymously, because there were no interventions performed as part of this retrospective study.

The inclusion criteria were as follows: (1) patients aged from 1 month to 18 years old; (2) a usage time of vancomycin $\geq$ 72 h with serum vancomycin (trough concentration and peak concentration) measurements available; and (3) renal function was normal before vancomycin treatment (normal range of serum creatinine in the Clinical Laboratory of Children's Hospital of Chongqing Medical University: 15.4–90.4 μmol/L using dry chemistry method; 14–60 μmol/L using enzymatic method).

The exclusion criteria were as follows: (1) patients were treated with other antibacterial drugs within 72 h before the use of vancomycin, such as teicoplanin, linezolid and rifampin; (2) vancomycin was not intravenously administered; (3) blood purification was conducted during vancomycin treatment; (4) there was concomitant use of other nephrotoxic drugs, such as amphotericin B, methotrexate, cyclophosphamide, cyclosporine and tacrolimus; and/or (5) clinical data were missing multiple variables.

Data Collection

Basic information including age, sex, height, weight, infection site, and primary diagnosis were collected, as well as clinical manifestations, symptoms and signs, blood laboratory tests and imaging examinations before and after vancomycin administration. Meanwhile, data on vancomycin administration (including initial dose, adjustment of administration plan, intravenous infusion time, treatment drug monitoring results),

treatment course, duration of mechanical ventilation, length of stay (LOS) in the PICU, LOS in the hospital and mortality.

Methods of Vancomycin Trough/Peak Concentration Determination

All children included in this study were administered intravenous vancomycin (trade name: Vancocin, 500 mg/bottle, Lilly Suzhou Pharmaceutical Co., Ltd.) for at least 60 min at each administration. According to the Experts' Consensus on Monitoring Therapeutic Drugs for Children (14), within 30 min to 1 h after the fourth vancomycin infusion, 2 ml venous blood samples were collected and labeled as peak concentration blood samples, and another of 2 ml venous blood sample was collected 30 min before the fifth vancomycin infusion and marked as a valley concentration blood sample. Patients who underwent adjustments to the dosing regimen were required to monitor the serum drug concentration again after the fourth treatment with the adjusted dose. The serum concentration of vancomycin was assessed using chemiluminescent enzyme immunoassay.

Calculation Method of AUC_{0-24}

A method based on the primary rate cancelation equation was used to calculate AUC_{0-24} (**Figure 1**, Equation 1) (15):

$$K_e = \frac{Ln\left(C_1/C_2\right)}{\Delta t} \quad C_{mac} = \frac{C_1}{e^{-Ke\cdot tl}} \quad C_{min} = C_2 \cdot e^{-Ke\times t2}$$

$$AUC_{0-24} = \left(\frac{t_{inf} \times (C_{max} + C_{min})}{2} + \frac{(C_{max} - C_{min})}{K_e}\right) \times \text{Number of dose/day} \quad (1)$$

C_1 is the measured peak concentration, C_2 is a measurement of trough concentration, Δt is the time period (in hours) between the two serum concentrations, t_{inf} represents the infusion time, t_1 is the time between the end of infusion and collection of C_1, and t_2 is the time between C_2 acquisition and the next infusion. The true peak concentration (C_{max}) and trough concentration (C_{min}) were extrapolated in the reverse and forward directions by K_e, respectively. AUC_{0-24} was calculated using the modified trapezoidal rule.

Efficacy Definition

The Acute Physiology and Chronic Health Evaluation II (APACHE II) score was used to quantify the severity of disease (calculated by the worst physiological parameters within 24 h after entering the PICU). The clinical efficacy of treatment in patients was primarily evaluated through the clinical manifestations and laboratory and imaging findings. Clinical efficacy was divided into three levels: cure, improved and ineffective. Patients whose temperature, inflammatory indicators (such as white blood cells, neutrophil percentage, and procalcitonin) and imaging examinations returned to normal and whose bacterial culture turned negative after vancomycin treatment were defined as cured. Compared to before treatment, if the above evaluation indices improved but did not return to normal levels, and the bacterial culture turned negative, this was regarded as improvement. Both cure and improved are considered clinically effective. If after treatment, all evaluation indices showed no significant improvement or even aggravation that needed additional antibacterial drugs, such as linezolid and teicoplanin, or/and the bacterial culture was still positive, this was defined as ineffective.

Definition of Renal Toxicity

Vancomycin-related nephrotoxicity was defined as at least two consecutive renal function tests suggesting an increase in serum creatinine concentration [absolute increase greater than 0.5 mg/dl (44.2 μmol/l) or more than 50% of the baseline level] in a patient after vancomycin treatment and cannot be explained by other reasons (16).

Statistical Analysis

SPSS 26.0 software (SPSS Inc., Chicago, IL, United States) was used for statistical processing, and all variables were summarized using descriptive statistics. The mean ± standard deviation ($\bar{x} \pm s$) was adopted for normal distribution of continuous variables, and the median (25% percentile and 75% percentile) was adopted for skewed distribution [*M*(P25,P75)]. Counts and percentages (%) were used to describe categorical variables. For univariate analysis, the *t*-test was used for normal distribution, and Mann-Whitney *U*-test was used for skewed distribution of continuous variables. Comparison of categorical variables was performed by the chi-square test. Multivariate analysis was performed using binary logistic regression. All statistical tests were performed bilaterally, and the difference was considered statistically significant if $P < 0.05$.

RESULTS

Clinical Data

A total of 110 patients were retrospectively enrolled, and their clinical characteristics are shown in **Table 1**. Eighty-four cases (76.4%) among the 110 children were discharged from the hospital, and 76 cases (69.1%) were considered effective after treatment with vancomycin according to the definition provided. With no ototoxicity occurring, the most common adverse reactions among all patients were nephrotoxicity (13.6%) and rash (6.4%). Median steady-state daily dose of vancomycin was 40.00 mg/kg d (IQR: 40.00, 58.71). The steady-state daily dose of 74.5% (82/110) children ranged from 40 to 60 mg/kg.d. Good outcome refers to recovery from the disease and discharge from the hospital, and poor outcome refers to treatment failure and subsequent death in the hospital.

Analysis of the Clinical Efficacy of Vancomycin

According to the efficacy criteria, 110 patients were divided into effective and ineffective groups. There were no significant differences in APACHE II scores, steady-state daily dose, steady-state trough/peak concentrations, AUC_{0-24}, length of vancomycin therapy, LOS in the hospital, LOS in the ICU, or mechanical ventilation time between the effective and ineffective groups (**Table 2**).

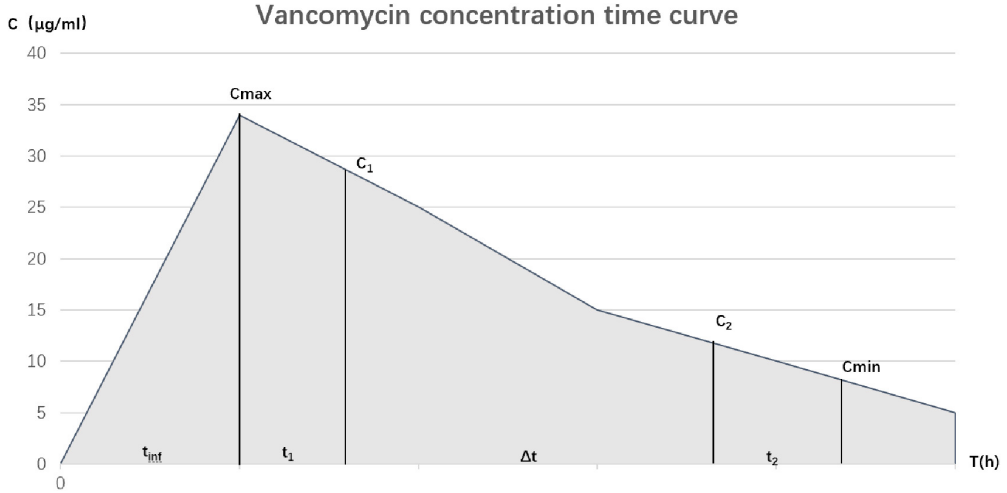

FIGURE 1 | Time curve of vancomycin concentration.

Gram-positive bacteria were detected in 66 of 110 children 83 times in total. The 66 patients with detected pathogens were divided into two groups according to whether the pathogen was cleared after vancomycin treatment. The drug sensitivity results showed that the MICs of vancomycin for Gram-positive bacteria detection were 0.5 mg/L (51/83, 61.4%), 1 mg/L (26/83, 31.3%), and 2 mg/L (6/83, 7.2%). The APACHE II score, steady-state daily dose, steady-state trough/peak concentration, AUC_{0-24}/MIC, and medication course were also compared between the two groups, and the results are shown in **Table 3**. There were no statistically significant differences in the disease severity, medication, therapeutic drug monitoring, or treatment outcome between the two groups.

TABLE 1 | Demographic and clinical characteristics of the patients.

Characteristic	All patients (*n* = 110)
Gender	
Male, *n* (%)	57 (51.8%)
Female, *n* (%)	53 (48.2%)
Age (years)	
< 1, *n* (%)	35 (31.8%)
1–3, *n* (%)	28 (25.5%)
4–7, *n* (%)	23 (20.9%)
8–12, *n* (%)	17 (15.5%)
> 12, *n* (%)	7 (6.4%)
Weight (kg), IQR	12.0 (8.0, 22.1)
Serum creatinine (μmol/L), IQR	28.6 (20.8, 42.9)
Steady-state daily dose (mg/kg d), IQR	40.00 (40.00, 58.71)
Treatment outcome	
Good outcome, *n* (%)	84 (76.4%)
Poor outcome, *n* (%)	26 (23.6%)
Clinical efficacy of vancomycin	
Effective, *n* (%)	76 (69.1%)
Ineffective, *n* (%)	34 (30.9%)
Adverse reactions	
Nephrotoxicity, *n* (%)	15 (13.6%)
Rash, *n* (%)	7 (6.4%)

IQR, interquartile range.

Analysis of Vancomycin Nephrotoxicity

According to the renal toxicity criteria, 110 children were divided into nephrotoxic and non-nephrotoxic groups. The clinical characteristics of the two groups are compared in **Table 4**. The differences in APACHE II scores, single dose and daily dose at steady state, trough/peak concentrations at steady state and AUC_{0-24} between the two groups were all statistically significant.

Variables such as age, sex, weight, and the above clinical characteristics were included in the binary logistic regression. Due to the multiple collinearity relationships between the daily dose and the steady-state single dose, AUC_{0-24} and the steady-state trough/peak concentration, the steady-state trough/peak concentration or AUC_{0-24} and age, sex, weight, Apache II score, steady-state daily dose and vancomycin treatment time were selected for the binary logistic regression analysis, which was shown in **Table 5**. Multivariate logistic analysis revealed that vancomycin treatment time, trough concentration and AUC_{0-24} were independent risk factors for nephrotoxicity.

Curves of prediction probability and receiver operating characteristic (ROC) of renal toxicity of the two prediction models where AUC_{0-24} and trough concentration were drawn (**Figure 2**), showing that both models effectively predicted renal toxicity. The area under the ROC curve of the AUC_{0-24} model was larger than that of the trough concentration model, but there was no statistically significant difference between them.

According to the ROC curve of AUC_{0-24} and nephrotoxicity (**Figure 3**), vancomycin AUC_{0-24} was demonstrated to be a tool for predicting the risk of vancomycin nephrotoxicity, and 537.18 mg.h/L might be a suitable threshold. The sensitivity and specificity for predicting vancomycin-related nephrotoxicity were 73 and 68%, respectively, and if the AUC_{0-24} exceeded 537.18 mg.h/L, the risk of nephrotoxicity was significantly increased.

TABLE 2 | Univariate analysis results between the effective and ineffective groups.

Variable	Effective group	Ineffective group	*t*/*Z*-value	*P*-value
n (%)	76 (69.1%)	34 (30.9%)		
APACHE II score (± *s*)	30 ± 7	29 ± 8	0.419	0.676
Steady-state daily dose (mg/kg d), IQR	40.00 (40.00, 57.06)	40.61 (40.00, 60.00)	1.923	0.054
Steady-state trough concentration (mg/L), IQR	9.56 (6.15, 13.70)	10.71 (7.18, 16.51)	1.410	0.159
Steady-state peak concentration (mg/L), IQR	25.16 (20.33, 33.26)	25.43 (20.03, 34.29)	0.158	0.874
AUC_{0-24} (mg.h/L), IQR	503.53 (386.02, 609.17)	480.74 (386.48, 779.11)	0.647	0.518
Length of vancomycin therapy (days), IQR	10 (7, 15)	10 (6, 18)	0.230	0.818
LOS of hospital (days), IQR	29 (20, 47)	27 (16, 38)	1.598	0.110
LOS of ICU (days), IQR	11 (7, 18)	12 (8, 19)	0.081	0.935
Mechanical ventilation time (hours), IQR	169 (90, 314)	172 (84, 478)	0.010	0.992

Data shown are the mean ± standard deviation or median (interquartile range), ICU, intensive care unit; IQR, interquartile range; LOS, length of stay.

TABLE 3 | Univariate analysis results between the pathogen-cleared and pathogen-uncleared groups.

Variable	Pathogen-cleared group	Pathogen-uncleared group	*t*/*Z*-value	*P*-value
n (%)	49 (74.2%)	17 (25.8%)		
APACHE II score ($\bar{x}$± s)	29 ± 5	29 ± 5	0.341	0.735
Steady-state daily dose (mg/kg d), IQR	42.11 (40.00, 59.23)	40.00 (40.00, 60.00)	0.023	0.982
Steady-state trough concentration (mg/L), IQR	11.12 (7.12, 13.83)	7.70 (5.75, 10.71)	1.628	0.104
Steady-state peak concentration (mg/L), IQR	26.37 (20.19, 32.34)	24.30 (19.28, 29.87)	0.784	0.433
AUC_{0-24}/MIC (mg.h/L), IQR	833.02 (562.65, 1058.96)	622.47 (433.71, 1032.42)	1.063	0.288
Length of vancomycin therapy (days), IQR	10 (7, 17)	13 (5, 22)	0.874	0.382

Data shown are the mean ± standard deviation or median (interquartile range), IQR, interquartile range.

TABLE 4 | Univariate analysis results between the nephrotoxic and non-nephrotoxic groups.

Variable	Non-nephrotoxic group	Nephrotoxic group	*t*/*Z*-value	*P*-value
No. (%)	95 (86.4%)	15 (13.6%)		
APACHE II score ($\bar{x}$± s)	29 ± 7	36 ± 7	3.791	0.001
Steady-state single dose (mg/kg)	10.00 (10.00, 14.91)	10.00 (7.14, 10.00)	3.274	0.001
Steady-state daily dose (mg/kg•d)	40.00 (40.00, 59.64)	40.00 (28.57, 40.00)	3.209	0.001
Steady-state trough concentration (mg/L), IQR	9.33 (6.09, 12.94)	16.41 (11.59, 34.24)	4.277	<0.001
Steady-state peak concentration (mg/L), IQR	25.32 (20.06, 30.93)	33.6 (23.23, 45.86)	2.604	0.009
AUC_{0-24}/MIC (mg•h/L), IQR	478.80 (374.34, 579.55)	763.24 (464.81, 1297.89)	3.401	0.001
Length of vancomycin therapy (days), IQR	10 (7, 15)	10 (7, 14)	0.161	0.872

IQR, interquartile range.

TABLE 5 | Multivariate logistic analysis of nephrotoxicity in the AUC_{0-24} and trough concentration models.

Variable	AUC_{0-24} model		Trough concentration model	
	OR (95% CI)	*P*-value	*OR* (95% CI)	*P*-value
Daily dose (mg/kg d)	0.851 (0.784–0.925)	<0.001	0.872 (0.805–0.944)	0.001
Length of vancomycin therapy (days)	1.099 (0.925–1.202)	0.040	1.102 (1.007–1.206)	0.035
AUC_{0-24}	1.005 (1.003–1.008)	<0.001	–	–
Steady-state trough concentration (mg/L)	–	–	1.176 (1.087–1.272)	<0.001

CI- confidence interval; OR- odds ratio.

Correlation Between Trough Concentration and AUC_{0-24}

In 2015, pediatric experts in China reached a consensus on therapeutic drug monitoring (14) that the suggested monitoring range of vancomycin trough concentration should be 5–10 mg/L for children and 10–20 mg/L for severely infected children. When the trough concentration was above 20 mg/L, nephrotoxicity was more likely to occur (14). Nevertheless, the vancomycin monitoring guidelines of the American Association of Infectious Diseases in 2020 and the evidence-based guidelines

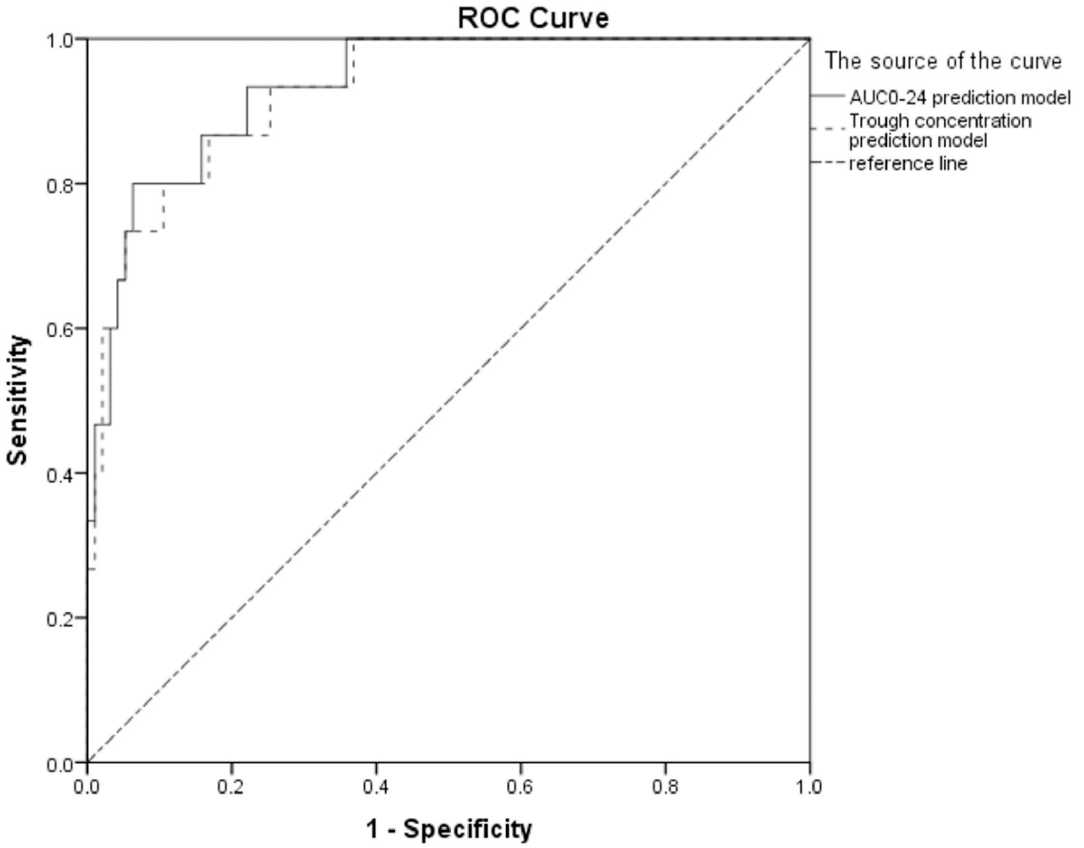

FIGURE 2 | ROC curves of prediction probability and vancomycin nephrotoxicity of two models (the area under the ROC curve of the AUC_{0-24} model was 0.935, 95% CI: 0.878–0.992, $P < 0.001$; the area under the ROC curve of the trough concentration model was 0.928, 95% CI: 0.868–0.989, $P < 0.001$).

for therapeutic drug monitoring of vancomycin (2020 Update by the Division of Therapeutic Drug Monitoring, Chinese Pharmacological Society) recommended keeping AUC_{0-24}/MIC in the range of 400–600, which means AUC_{0-24} should be in the range of 400–600 (mg•h/L), assuming that vancomycin MIC was 1 mg/L (17, 18). The ranges of AUC_{0-24} corresponding to three steady-state trough concentration ranges (<10 mg/L, 10–20 mg/L, > 20 mg/L) were analyzed (**Table 6**). Only 31 of 110 children failed to reach the range of AUC_{0-24} > 400 mg.h/L (**Table 6**). In addition, it can be seen from the re-analysis of the data in **Table 6**. That 51.0% (25/49) of children with AUC_{0-24} ranging from 400 to 600 mg.h/L exhibited trough concentrations < 10 mg/L; 37.5% (15/40) of children with trough concentrations ranging from 10 to 20 mg/L had AUC_{0-24} ranging from > 600 mg.h/L.

DISCUSSION

Vancomycin is widely used in critically ill children suspected of Gram-positive bacterial infection. Due to the characteristics of vancomycin, it is important to monitor its use according to vancomycin PK/PD parameters, and the trough concentration remains the primary indicator that is valued and monitored in the clinic. The consensus of Chinese experts on the clinical application of vancomycin in 2011 (19) and the guidelines for the treatment of MRSA infection conducted by the American Society of Infectious Diseases (20) both recommended maintaining the trough concentration at 15–20 mg/L in children with severe infection. However, it is worth noting that most evidence for these recommendations is derived from adult data.

In recent years, pediatric studies have found that the vancomycin trough concentrations needed to achieve the target AUC/MIC were different than the adult goal troughs (21–25). One qualitative systematic review indicated that trough concentrations of 6–10 mg/l were likely sufficient to achieve AUC/MIC ≥ 400 in pediatric patients (26). Targeting trough concentrations > 15 μg/ml would lead to overshooting the target $AUC_{0-24\ h}$ above 400 and increased the risk of nephrotoxicity and other adverse events (22, 23). Furthermore, the correlation between the vancomycin dose given and the ideal trough concentration achieved was poor (27). In the nephrotoxic group in our study, trough concentrations were 16.41 (11.59, 34.24) mg/L at a median dose of 40 mg/kg d, while the AUC_{0-24} was as high as 763.24 (464.81, 1297.89). This suggested that the significant increase in trough concentration and AUC would lead to nephrotoxicity even at normal daily doses.

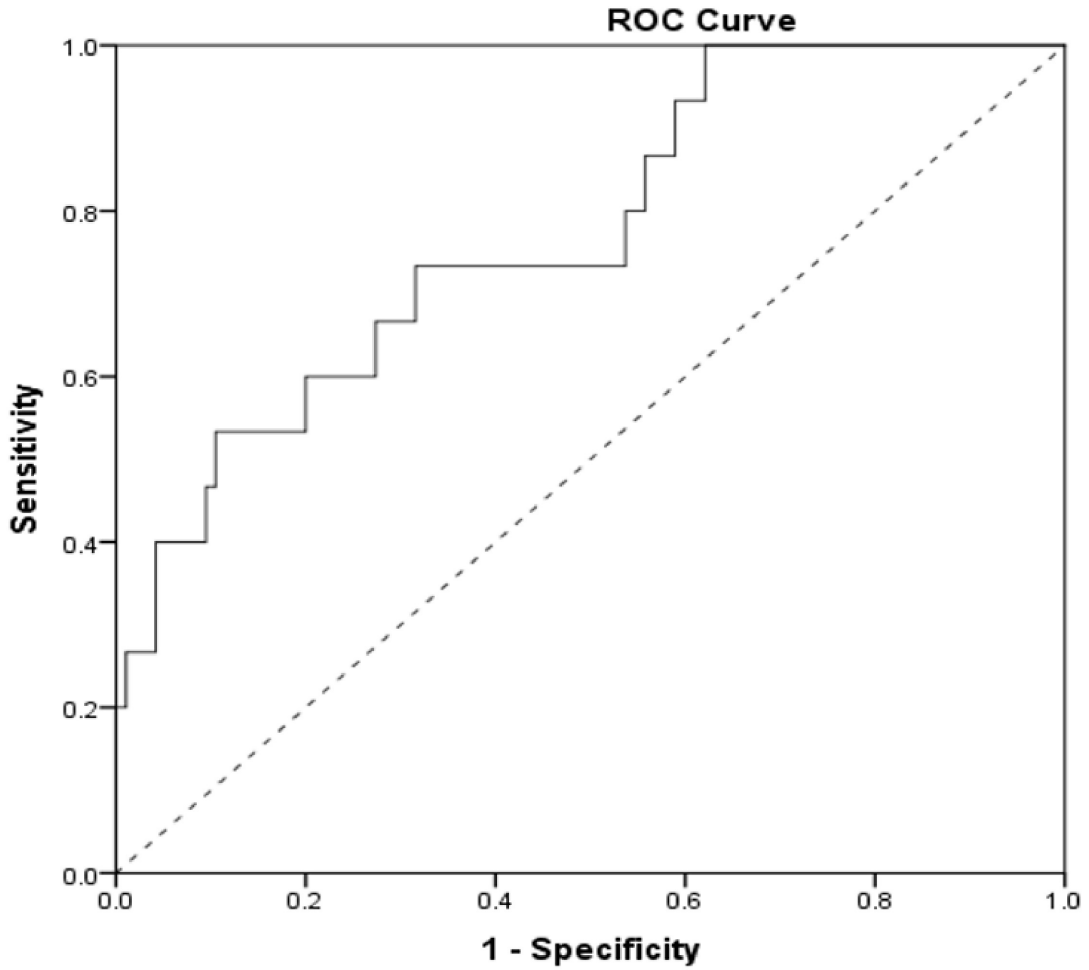

FIGURE 3 | ROC curve of the AUC_{0-24} prediction level of vancomycin-related nephrotoxicity (the area under ROC curve = 0.774, 95% CI: 0.648–0.900, *P* = 0.001).

TABLE 6 | Proportion of AUC_{0-24} in different trough concentration ranges.

No. (%)	AUC_{0-24} range (mg.h/L)			Total
	<400	400–600	>600	
Trough concentration < 10 mg/L, *n* (%)	30 (27.3%)	25 (22.7%)	3 (2.7%)	58 (52.7%)
10 mg/L ≤ trough concentration ≤ 20 mg/L, *n* (%)	1 (0.9%)	24 (21.8%)	15 (13.6%)	40 (36.4%)
Trough concentration > 20 mg/L, *n* (%)	0 (0)	0 (0)	12 (10.9%)	12 (10.9%)
Total	31 (28.2%)	49 (44.5%)	30 (27.3)	110 (100%)

The 2020 guidelines for monitoring the efficacy of vancomycin for the treatment of severe MRSA infection (17) proposed that the AUC_{0-24}/MIC of patients with severe MRSA infection should be kept in the range of 400–600 rather than monitoring trough concentration only. For pediatric patients with non-invasive MRSA or other infections, no sufficient evidence has been suggested regarding whether trough concentration or AUC_{0-24} is more suitable for vancomycin monitoring.

In this study, the calculation of AUC_{0-24} was based on the first-order rate elimination equation (15), which was one of the most recommended methods at present and can be applied in follow-up due to its accuracy and operability in clinical practice. The clinical results were analyzed for both curative effect and bacterial clearance, making the determination of clinical efficiency reliable. Multiple previous cohort studies (28–31) have revealed that TDM with AUC_{0-24} in adults reduced nephrotoxicity compared to monitoring trough concentrations, but there was no significant difference in clinical efficacy. One prospective multicenter observational study demonstrated no significant correlation between trough concentration and curative effect, but there was a correlation between trough concentration and nephrotoxicity in adults (32). However,

the association between trough concentration or AUC_{0-24} and clinical results in children remains unclear (33). Hahn et al. (34) showed that the relationship between vancomycin AUC_{0-24}/MIC and treatment failure or success could not be established in children with MRSA bacteremia due to limitations in the number of their study samples. Our study revealed that steady-state trough concentration, steady-state peak concentration and AUC_{0-24} shared no significant correlation with vancomycin treatment efficacy or bacterial clearance effect in children with severe infection at a median daily dose 40 mg/kg d, while higher steady-state trough concentrations and AUC_{0-24} were closely correlated with nephrotoxicity.

Children hospitalized in the PICU exhibit different pathophysiological characteristics than children not in the PICU. They exhibited more severe infection and reduced immune function and were more prone to organ failure. In addition, the combined use of multiple drugs increased the possibility of acute renal damage. AUCs measured during the first or second 24 h and lower than approximately 650 mg × hour/L may result in a decreased risk of AKI in adults (35). To date, few studies have focused on the AUC_{0-24} threshold in children. In 2015, a retrospective cohort study conducted by Le et al. (36) showed that vancomycin $AUC_{0-24} \geq 800$ mg.h/L was independently associated with an increase in nephrotoxicity risk > 2.5-fold in children. Our study found that predicting nephrotoxicity using a binary logistic regression model of AUC_{0-24} may have higher efficiency than the trough concentration model. Therefore, regular TDM and monitoring of renal function during vancomycin administration are important for reducing renal impairment.

The 2020 IDSA guidelines (17) recommended setting a goal for children suspected or diagnosed with severe MRSA infection in which an AUC_{0-24}/MIC ratio of 400–600 mg.h/L should be achieved, which was determined by analogy with previous studies conducted in adults. The AUC_{0-24} threshold in our study was far less than 800 mg.h/L and close to the threshold of 600 mg.h/L for adults. Our study demonstrated that AUC_{0-24} can be used for TDM to reduce the risk of nephrotoxicity. In addition, $AUC_{0-24} < 537.18$ mg.h/L may be a TDM strategy to be considered on the premise of ensuring the treatment effect in the clinic and avoiding nephrotoxicity.

Binary logistic regression analysis of nephrotoxicity showed that a longer treatment course of vancomycin, a higher trough concentration and a higher AUC_{0-24} were independent risk factors for nephrotoxicity. The daily dose in the nephrotoxicity group was lower than other groups in our study, which might be related to the corresponding adjustment according to the degrees of renal function damage and the clinical trough concentration. When renal function damage occurs clinically, a higher trough concentration or AUC_{0-24} range can be reached with a lower daily dose, so the dose of vancomycin needs to be flexibly adjusted. TDM (trough concentration or AUC_{0-24}) should be performed to reduce the risk of nephrotoxicity when using vancomycin in the clinic. At the same time, physicians should closely monitor longer treatment courses and reduce the medication time to ensure the effect while reducing the risk of nephrotoxicity.

We compared the proportion of different trough concentrations corresponding to reaching the target AUC_{0-24}. When the trough concentrations were between 10 and 20 mg/L, the AUC_{0-24} values of most children (39/40) were greater than 400 mg.h/L. When the trough concentrations were less than 10 mg/L, 48.3% (28/58) of children still reached the AUC_{0-24} level of 400–600 mg.h/L. Blindly increasing the drug dose to reach the trough concentration target value may increase the risk of nephrotoxicity. Consequently, the trough concentration needs to be monitored in combination with AUC_{0-24} to reduce the occurrence of nephrotoxicity.

One retrospective study showed that vancomycin trough concentration was not associated with treatment success or failure in pediatric patients with suspected Gram-positive infection, which was similar to our findings (37). The median APACHE II score of included pediatric patients was 17 in their study (37), which was lower than that of patients treated in PICU in our study. The patients was seriously ill, and the proportion of treatment failure and subsequent death in the hospital was 23.6% in present study. However, one systematic review and meta-analysis indicated that vancomycin trough concentrations of 10–15 mg/L was associated with significantly lower mortality in pediatric patients infected with Gram-positive pathogens (38). This finding seemed to be inspiring, while the high trough concentrations may incur serious adverse effects. Further research is needed to ensure the therapeutic effect of vancomycin and avoid adverse reactions in patients treated in the PICU.

There are several limitations to this study. First, this study was limited by factors that are inherent to the retrospective analysis and interpretation of data. Second, since our study is a single-center and retrospective study with small scales, the power of our results are restricted. Third, not every child had cultural evidence of Gram positive bacterial infection, so the AUC_{0-24}/MIC value could not be calculated for each patient. In the future, multicenter prospective studies are needed to explore differences in the clinical efficacy and safety of vancomycin in the treatment of children with severe infection under the guidance of PK/PD parameters such as trough concentration and AUC_{0-24} and to further clarify the most appropriate AUC_{0-24} range of vancomycin for the treatment of children with severe infection. Finally, the mechanism of vancomycin metabolism differences in patients of different ages, weight and diseases is still not completely clear. Furthermore, the individual difference in the concentration of vancomycin and AUC at similar doses *in vivo* is also a problem perplexing clinicians. This study also can't fully clarify the PK/PD of vancomycin in different children with severe infection in the PICU. The specific mechanism may need further studies.

CONCLUSION

The steady-state trough concentration and AUC_{0-24} of vancomycin were not significantly correlated with the therapeutic efficacy or bacterial clearance effect when the daily dose of vancomycin was approximately 40 mg/kg d in critically ill children. However, the steady-state trough

concentration and AUC_{0-24} were both closely correlated with nephrotoxicity. Therefore, the combination of AUC_{0-24} and trough concentration for TDM may reduce the risk of nephrotoxicity in pediatric populations.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Research Ethics Committee of Children's Hospital Affiliated to Chongqing Medical University. Approval Document No. (2021) ethical review (study) No. (7). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CL and YF conceived the study, coordination and finalized the manuscript, and took responsibility for the article as a whole. BZ and WX participated in the design, data acquisition, database management, statistical analysis, and manuscript draft. KB, HD, JL, and FX participated in statistical analysis, database management, and manuscript draft. All authors contributed to the article and approved the submitted version.

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EDITED BY
Yolanda Gilaberte,
Hospital Universitario Miguel Servet, Spain

REVIEWED BY
Francesca Prignano,
Università di Firenze, Italy
Rony Shreberk-Hassidim,
Hadassah Medical Center, Israel

*CORRESPONDENCE
Luca Di Bartolomeo,
lucadibartolomeo@live.it

†These authors equally contributed to this work and share the first authorship

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Photodynamic therapy in pediatric age: Current applications and future trends

Luca Di Bartolomeo[1*†], Domenica Altavilla[2†], Mario Vaccaro[1], Federico Vaccaro[3], Violetta Squadrito[4], Francesco Squadrito[2] and Francesco Borgia[1]

[1]Department of Clinical and Experimental Medicine, Dermatology, University of Messina, Messina, Italy, [2]Department of Clinical and Experimental Medicine, Pharmacology, University of Messina, Messina, Italy, [3]Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy, [4]Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi, Pediatry", University of Messina, Messina, Italy

Photodynamic therapy (PDT) is a photochemotherapy based on local application of a photosensitive compound and subsequent exposure to a light source of adequate wavelength. It is a non-invasive therapeutic procedure widely used in oncodermatology for treatment of numerous skin cancers, but in the last years its use has been gradually extended to an increasing list of skin diseases of both infectious and inflammatory nature. Although PDT is proven as a safe and effective therapeutic option in adults, its use is not well standardized in the pediatric population. In this review, we will focus on clinical applications, mechanisms of action, protocols, and adverse events in children and adolescents. Most of pediatric experiences concerned treatment of skin cancers in Gorlin syndrome and xeroderma pigmentosum, acne vulgaris, and viral warts, but other applications emerged, such as cutaneous lymphoma and pseudo-lymphomas, necrobiosis lipoidica, hidradenitis suppurativa, dissecting cellulitis, leishmaniasis, angiofibromas, verrucous epidermal nevus, and linear porokeratosis. In these pediatric diseases, PDT appeared as an effective therapeutic alternative. The results on vitiligo were limited and not fully encouraging. Although highly versatile, PDT is not a therapy for all skin diseases, and a deeper knowledge of its mechanisms of action is required to better define its spectrum of action and safety in pediatric patients.

KEYWORDS

photodynamic therapy, child, pediatric dermatology, acne vulgaris, viral warts, Gorlin syndrome, necrobiosis lipoidica, hidradenitis suppurativa

Introduction

Photodynamic therapy (PDT) is an attractive, non-invasive therapeutic procedure widely used in adult patients for treatment of tumoral, inflammatory, and infectious skin diseases. PDT is a photochemotherapy based on local application of a photosensitive compound and subsequent exposure to a light source of adequate wavelength. The most employed photosensitizers commonly used in dermatology are the 5-aminolaevulinic acid (ALA, an intermediate of the heme biosynthetic pathway) and its methyl ester 5-

aminolevulinate (MAL), which are converted inside the target cells to photo-active protoporphyrin IX (PpIX). After an incubation period (generally 3 h), PpIX is activated by an artificial light source (conventional PDT) or by sunlight (daylight PDT), thus leading to the production of reactive oxygen species (ROS), triggering both apoptosis and necrosis of target cells as well as stimulation of an immune modulating response. Different light sources with varying wavelengths can be used in PDT. The absorption spectrum of protoporphyrin IX shows maximal absorption peaks at approximately 410 nm, namely, at the wavelength of blue light, but it also shows smaller absorption peaks at 506, 532, 580, and 630 nm as well, namely, within the red light wavelength (Prieto et al., 2005). Nevertheless, the effect of red light appears to be stronger than that observed with blue light because of the greater depth of penetration of red light into dermis, thus explaining its diffuse use worldwide with respect to blue light. DL-PDT is a novel procedure in which the activation of the topical photosensitizer is induced by exposure to natural daylight, without requiring preliminary occlusion and dedicated instrumental equipment. With respect to the conventional one, DL-PDT has a more superficial depth of penetration, so its use is reserved to thin lesions (Borgia et al., 2020a).

The heterogeneous mechanisms of action and the multiple targets hit by PDT have allowed to progressively extend its use from the treatment of non-melanoma skin cancer to an always increasing list of skin diseases of both infectious and inflammatory nature. PDT displays several major strengths: it is a non-invasive, easily repeatable, outpatient treatment that can be applied to wide areas of affected skin with an overall good profile of safety. PDT can be used in fragile patients, that is, elderly subjects in whom surgery is contraindicated, in immuno-depressed subjects, or to treat large or multiple lesions localized in poor healing areas. Moreover, PDT shows superior cosmetic outcome compared with more invasive therapeutic approaches such as surgery and cryotherapy, with no scarring and pigmentary changes. Although PDT is proven as a safe and effective therapeutic option in several dermatologic diseases in adults, its use is not well standardized in pediatric population. For this reason, we performed a review about the employment of PDT in the pediatric age to provide an overview of the current state of art and to explore new potential fields of use of this technique.

Methods

We checked the PubMed (https://ncbi.nlm.nih.gov/PubMed) database using the string "photodynamic therapy" [All Fields] AND "skin" [All Fields].

Only the research works written in English language, concerning humans and child (birth to 18 years), and with no time limits were included. A systematic literature search was led according to the PRISMA flowchart, also reviewing the abstracts of the articles whose title suggested this association. The references retrieved were critically examined by two experts in the field of dermatology to select those pertinent to our research, namely, clinical trials, retrospective studies, case series, and case reports. Reviews were excluded, but their reference lists were also examined to find other relevant articles, which were eventually revised and included if appropriate.

Figure 1 summarizes the publication screening scheme used according to PRISMA guidelines.

We included only studies on patients treated with topical ALA-PDT or MAL-PDT, the photosensitizers widely available and therefore most commonly used by dermatologists. As of 6 January 2022, 44 articles were identified. Studies exclusively focusing on pediatric patients were 33. Parameters of these studies, including patients' features, type of topical photosensitizers and light sources used, conditions of treatment, number of treatments, and outcomes and adverse events, are summarized in Tables 1, 2. Studies including both adults and children with no specific data on pediatric patients were 11. They were also included in results and summarized in Tables 3, 4. For convenience, the results have been categorized into three main topics (oncologic, anti-inflammatory, and antimicrobial), with a fourth paragraph including a miscellanea mainly dealing with disorders of keratinization.

PDT and pediatric skin cancers

The onset of skin cancers in pediatric age is a rare event. Nevertheless, some genetic syndromes, such as Gorlin syndrome or xeroderma pigmentosum, may predispose to the development of skin tumors since childhood.

Gorlin syndrome

Gorlin syndrome, or nevoid basal cell carcinoma syndrome (NBCCS), is an autosomal dominant syndrome caused by mutations in the PTCH1 (Patched 1) gene, characterized by multiple basal cell carcinomas (BCCs) occurring from puberty, in addition to various dental, osseous, ophthalmic, neurological, and sex organ abnormalities (Jawa et al., 2009).

A total of three Gorlin patients <18 years old (6, 10, and 17 years) with diffused BCCs were treated by means of ALA-PDT, achieving 85 to 98% complete response (Oseroff et al., 2005).

Oseroff et al. (2005) performed several sessions of 10% ALA-PDT on the children with a red light laser for areas with lower diameter (from 2 to 7 cm) and a lamp for those with larger diameter (up to a 16 cm diameter). The patients presented BCCs on 12–25% of their body surface, and four to seven sessions were

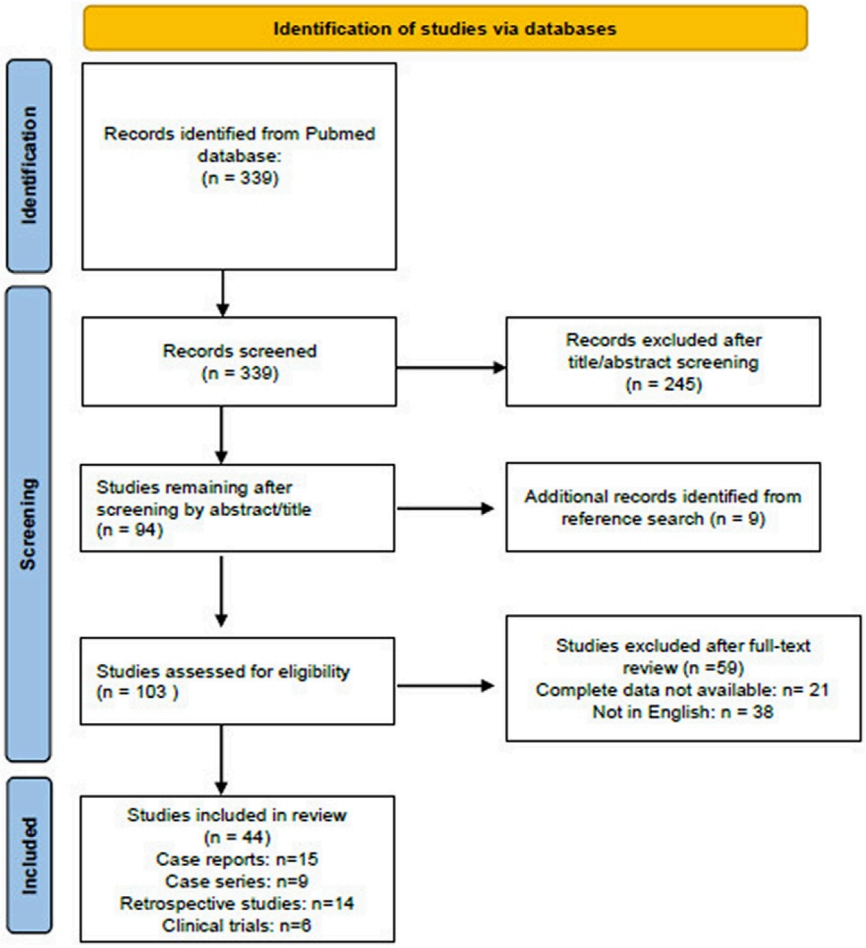

FIGURE 1
Flow diagram of the literature screened using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The figure is adapted from http://prisma-statement.org.

needed for each patient but individual areas received one to three treatments. The patients reported excellent cosmetic outcomes with no evidence of new BCCs in the treated areas up to 6 years of follow-up.

Loncaster et al. (2009) treated 33 Gorlin patients of all ages (range 9–79 years) with ALA-PDT and MAL-PDT, obtaining different control rates depending on the thickness of lesions. They performed ultrasound investigation to assess lesion thickness and used topical PDT to treat only superficial lesions (<2 mm thick). Thicker lesions were treated with a systemic photosensitizer. At 12 months, local control rates were 73.0% for lesions <1 mm, 40.8% for lesions measuring between 1 and 2 mm, and 59.3% for lesions >2 mm.

Gorlin patients are highly susceptible to DNA damage from therapies such as ionizing radiation. However, Oseroff et al. (2005) found no evidence of ALA-PDT inducing or promoting BCCs in pediatric patients. Gorlin patients have increased risk of medulloblastoma and may develop multiple to thousands BCCs in the site of radiotherapy, namely, on the anterior surface of abdomen and on the back. In these cases, the carcinomas are so numerous that surgical excision is impractical. There are reports of pediatric Gorlin patients who benefited from PDT for the treatment of radiotherapy-induced BCCs (Walter et al., 1997; Oseroff et al., 2005). In adult patients, MAL-PDT or nanoemulsion ALA-PDT are considered for non-aggressive, low-risk BCC, that is, small superficial and nodular types not exceeding 2 mm tumor thickness, where surgery is impractical or contraindicated, or avoidance of scarring is a priority (Rhodes et al., 2004; Peris et al., 2019). Ultrasound may be useful to assess BCC thickness prior to treatment and assign Gorlin patients to a PDT treatment, as demonstrated by Loncaster et al. (2009). An international experts consensus established recommendations for the use of MAL-PDT in patients with Gorlin syndrome (Basset-Seguin et al., 2014). Although MAL-PDT is not

TABLE 1 Studies with specific data on pediatric patients. Patients' age, clinical features, and PDT clinical outcomes.

DX	Publication (first author, year)	Site	Previous treatment	No ped pt	Age (y)	Clinical outcome	Adverse event
BCCs	Oseroff et al. (2005)	Anywhere	Surg, laser, topical and systemic retinoids, and ALA-PDT	3/3	6,10,17	85–98% CR	LSRs, pigm, and hair loss
AKs	Larson and Cunningham (2012)	Face	5-FU and imiq	1/1	16	Improvement	Not
BCCs and AKs	Fernández-Guarino et al. (2020)	Face	Imiq and surg	12/13	9.3, range 2–18	Improvement	LSRs
BD	Hyun et al. (2016)	Hand	—	1/1	12	CR	No
LBC	Takeda et al. (2005)	Face	Topical CCS	2/2	16	CR	Pigm
PgR	Mendese et al. (2012)	Foot	—	1/1	10	CR	Pain and pigm
MF	Heng et al. (2014)	Thigh	UVA1, topical PUVA, imiq, LEBT, CCS, and tazarotene	1/46	<16	CR	—
LyP	Snider et al. (2020)	Forearm	Topical and intralesional CCS, doxycycline, and nB-UVB	1/1	13	CR	—
Ang (TS)	Weinberger et al. (2009)	Face	—	2/6	10, 18	PR	LSRs
VEN	Zheng et al. (2018)	Face	Cryo	1/1	17	CR	—
LP	García-Navarro et al. (2009)	Lower leg	Topical calcipotriol	1/1	13	SR	LSRs
	Curkova et al. (2014)	Arm	Emollients	1/1	16	SR	LSRs
	Garrido-Colmenero et al. (2015)	Breast	Adapalene and topical tacrolimus 0.1%	1/1	11	PR	LSRs
Porokeratosis	Gracia-Cazaña et al. (2015)	Neck and popliteal fossa	Calcipotriol, 5-FU, and imiq	1/1	12	CR	—
Acne	Ma et al. (2015)	Face	—	21/21	16.17 ± 1.43	95.23% CR after 8 weeks	Pain, LSRs, and pigm
	Theresia et al. (2017)	Face	Topical and oral retinoids and antibiotics	1/4	17	Improvement	No
	Borgia et al. (2018a)	Buttocks	Oral minocycline and topical and oral retinoids	1/1	16	Improvement	Pain and LSRS
	Li et al. (2018)	Face	Oral retinoids and antibiotics	2/2	18	Improvement	Pain and LSRs
	Itoh et al. (2001)	Face	Yes	2/13	18	Improvement	LSRs and pigm
	Hörfelt et al. (2007)	Face and back	Tetracyclines	4/15	16.5	Improvement	Pain and pigm
NL	Berking et al. (2009)	Lower legs	—	3/18	16.6, range 16–17	1/3 PR and 2/3 NR	Pain
HS	Bu et al. (2017)	Armpit and head and neck	—	2/7	15, 16	Improvement DLQI	Pain
DC	Feng et al. (2019)	Scalp	Oral and topical antibiotics	1/8	15	Improvement	Pain and LSRs
PCAS	Cui et al. (2020)	Scalp	Incision, drainage, and systemic antibiotics	1/9	17	SR	—
Vg	Zhang et al. (2018)	Abdomen and forehead	—	2/2	17, 4	NR (pt A), improvement (pt B)	—
CVW	Borgia et al. (2020a)	Foot	Cryo	1/1	6	CR	LSRs
	Huang et al. (2019)	Plantar regions	Cryo, laser, and 5-FU	1/1	18	CR	Pain
	Wu et al. (2019)	Hand	Cryo, laser, imiq, and intralesional 5-FU	10/23	14.5, range 5–18	90% CR	Pain, LSRs, and onychodystrophy
FVW	Borgia et al. (2019)	Face	Topical retinoid and cryo	1/1	8	CR	Pain and pigm
	Borgia et al. (2020a)	Face	Conventional therapies	30/30	9.67 (gp A), 9.13 (gp B)	73.3% CR (C-PDT),80% CR (DL-PDT)	
GVW	Xu et al. (2018)		Imiq and cryo	8/8		CR	Pain

(Continued on following page)

TABLE 1 (*Continued*) Studies with specific data on pediatric patients. Patients' age, clinical features, and PDT clinical outcomes.

DX	Publication (first author, year)	Site	Previous treatment	No ped pt	Age (y)	Clinical outcome	Adverse event
		Perianal and intra-anal			1.8, range 1–4		
	Macca et al. (2022)	Perineal		1/1	5	CR	LSRs and pigm
CL	Johansen et al. (2019)	Lower leg	MA and topical and oral antimycotics	1/1	15	CR	Pain

approved for children, three of the seven expert panel members had pediatric experience and all agreed that MAL-PDT might also be considered in the pediatric age (Basset-Seguin et al., 2014).

A recent study in adult patients observed higher recurrence rates in BCCs of neck and head treated with MAL-PDT (Condorelli et al., 2022). Vinciullo et al. highlighted that lesions located in the H-zone, whether large or not, had unfavorable CCR following MAL-PDT (Vinciullo et al., 2005). For these reasons, according to the most recent European guidelines for treatment of BCC, less common histologic variants of BCC, such as morphoeic, pigmented, and micronodular types, as well as areas with higher risk of tumor survival and deep penetration (facial 'H'-zone) should not be treated with PDT (Peris et al., 2019). Usually, PDT for pigmented BCC treatment is not performed because of the lower penetration of the light, possibly due to the melanin content of these variants (Salvio et al., 2021). During the treatment of their pediatric patients with Gorlin syndrome, Oseroff et al. (2005) noted that a patient presented a lower response rate probably due to the varying pigmentation of his BCCs, which reduced the effective light dose. A prior debulking of pigmented BCC with removal of pigmented component may be an option for the treatment of these subtypes. Its suitability should also be investigated in pediatric population. In a group of adult patients, Salvio et al. (2021) performed debulking of 30 pigmented BCC before MAL-PDT and obtained complete response in 100% of cases, with no recurrence at mean 24-month follow-up.

Xeroderma pigmentosum and Bowen's disease

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder of defective UV-radiation–induced damage repair that is characterized by photosensitivity and higher risk for developing skin cancer at an early age (Black, 2016). Reports of PDT in XP are scarce because of the fear of developing skin cancers following illumination (Fernández-Guarino et al., 2020). Larson and Cunningham successfully treated facial actinic keratosis of a 16-year-old girl with type C XP, by means of three sessions of ALA-PDT with blue light. Before starting treatment, Larson and Cunningham tested skin photosensitivity of their patient performing a 3 cm^2 test treatment on her left arm, not noticing adverse cutaneous reactions of the exposed area. The treatment was repeated for 1 year and did not cause any adverse events (Larson and Cunningham, 2012). Fernández-Guarino et al. successfully treated 13 young African XP patients, 12 of whom were younger than 18, affected by facial AKs and BCCs, by using one session of DL-PDT. They used indoor DL-PDT because the window blocked UVB radiation, responsible for DNA damage, thus limiting treatment-related skin cancer induction. After two days of treatment, the patients presented with crusting and scaling, but a week later the cutaneous reaction resolved. No adverse events were noted at 3-month follow-up (Fernández-Guarino et al., 2020). Despite these encouraging results, the use of PDT in pediatric patients with XP is still limited and its safety should be confirmed by further studies considering long-term follow-up. Keratinocyte-derived tumors are rare in the pediatric age with a single case of periungueal Bowen's disease treated with PDT in a 12-year-old boy. Hyun et al. treated the child with two sessions of MAL-PDT at an interval of 3 weeks. The authors did not observe any sign of recurrence 9 months after treatment, and no adverse events were reported (Hyun et al., 2016).

Cutaneous lymphomas

Cutaneous lymphomas have a heterogeneous clinical presentation, ranging from pink, red, or violaceous solitary papules or nodules to widespread infiltrative lesions. There is an emerging interest about the antitumor properties of PDT applied to cutaneous lymphomas, particularly T-cell lymphomas, although only a few cases in adults, and even less in children, are described in literature.

Overall, five pediatric patients with various cutaneous lymphomas have been treated with MAL- or ALA-PDT, achieving a complete response in all cases (Takeda et al., 2005; Mendese et al., 2012; Heng et al., 2014; Snider et al., 2020).

Takeda et al. observed complete resolution of periocular lymphadenosis benigna cutis (also called lymphocytoma cutis) in two 16-year-old girls after five sessions of ALA-PDT. They

TABLE 2 PDT protocols.

DX	Publication (name first author and year)	PS	PS incub (h)	Light source	Light dose (J/cm^2)	Mean no. of treat	Associat therapy	Follow-up (w, m, y)
BCCs	Oseroff et al. (2005)	20% ALA	18–24	633 ± 2 nm (laser) and 590–700 nm (lamp)	60–240	1–3	—	6 years
AKs	Larson and Cunningham (2012)	ALA	40 min	417–432 nm (lamp)	—	3	—	1 year
BCCs and AKs	Fernández-Guarino et al. (2020)	ALA and MAL	—	Light indoor	—	1	—	3 m
BD	Hyun et al. (2016)	MAL	3	Red light	—	2	—	9 m
LBC	Takeda et al. (2005)	20% ALA	4–6	630 and 700 nm visible light (lamp)	120	5	—	6 m
PgR	Mendese et al. (2012)	20% ALA	2	417 ± 5 nm (lamp) and 595 nm (PDL)	10–11.5	9	—	46 m
MF	Heng et al. (2014)	MAL	—	—	—	30	—	35 m
LyP	Snider et al. (2020)	20% ALA	1	Blue light (lamp)	10	3	—	2 m
Ang (TS)	Weinberger et al. (2009)	20% ALA	1	417 nm (lamp)	10	3–4	PDL	
VEN	Zheng et al. (2018)	20% ALA	4	635 nm (semiconductor laser)	120	4	Fmp-RF	24 w
LP	García-Navarro et al. (2009)	16% MAL	3	630 nm (LED)	37	2	Shaving	11 m
	Curkova et al. (2014)	16% MAL	3	630 nm (LED)	37	3	Curett	1 year
	Garrido-Colmenero et al. (2015)	16% MAL	2	630 nm (LED)	37	2	—	4 m
Porokeratosis	Gracia-Cazaña et al. (2015)	MAL	3	630 nm (LED)	37	3	—	5 years
Acne	Ma et al. (2015)	5% ALA	1	633 ± 10 nm (LED)	90–96	3	—	8 w
	Theresia et al. (2017)	5–10% ALA	2–3	633 ± 10 nm (LED)	96–180	4	—	6 m
	Borgia et al. (2018b)	10% ALA	3	630 nm (diode)	75	6	—	6 m
	Li et al. (2018)	3% ALA	3	633 ± 6 nm (LED)	50	3	—	2 years
	Itoh et al. (2001)	20% ALA	4	600 ± 700 nm (lamp)	13	1	—	6 m
	Hörfelt et al. (2007)	20% ALA	3	635 nm (lamp)	30–70	1	—	10 w
NL	Berking et al. (2009)	16% MAL	3	630 nm (LED)	37	2–6	—	4–8 w
HS	Bu et al. (2017)	20% ALA	3	635 nm (laser)	—	4	—	6–12 m
DC	Feng et al. (2019)	10% ALA	3	633 ± 10 nm (LED)	96–180	3	p-bn	
PCAS	Cui et al. (2020)	20% ALA	2	635 nm (laser)	80	1	Reverse flap	6 m
Vg	Zhang et al. (2018)	1.5% ALA	3	633 ± 10 nm (semiconductor laser)	96	23	—	2 years
CVW	Borgia et al. (2020b)	10% ALA	3	630 nm (LED)	75	2	—	1 year
	Huang et al. (2019)	10% ALA	4	635 nm (semiconductor laser)	—	3	Shaving	16 m
	Wu et al. (2019)	10% ALA	3	633 nm red light source	—	3	Shaving	12 m
FVW	Borgia et al. (2019)	10% ALA	0.5	Sunlight	—	2	No	1 year
	Borgia et al. (2020b)	10% ALA	3 h (C-PDT), 0.5 h (DL-PDT)	630 nm (diode) for C-PDT and sunlight for DL-PDT	75 (C-PDT group)	3	No	24 w
GVW	Xu et al. (2018)	20% ALA	4	635 nm (semiconductor laser)	—	6	Microwave ablation	6 m
	Macca et al. (2022)	10% ALA	3	630 nm (LED)	75	3		6 m
CL	Johansen et al. (2019)	10% ALA	—	—	—	24		1 m

noted only a transient hyperpigmentation and no recurrences at 6-months follow-up (Takeda et al., 2005). Mendese et al. treated a 10-year-old boy with pagetoid reticulosis, a rare variant of mycosis fungoides (MF), on his right foot by nine sessions of PDT over 13 months. They applied ALA topical solution with subsequent illumination with blue light or pulsed day laser. On

TABLE 3 Studies including both adults and children with no specific data on pediatric patients. Patients' age, clinical features, and PDT clinical outcomes.

DX	Publication (name first author and year)	Site	Previous treatment	No pt	Age (y)	Clinical outcome	Adverse event
BCCs	Loncaster et al. (2009)	All body	surgery, cryo, and 5-FU	33	40, range 9–79	Control rates of 73.0% for lesions <1 mm and 40.8% for lesions between 1 and 2 mm	—
Acne	Hörfelt et al. (2006)	Face	—	30	18, range 15–28	54% mean reduction (inflamed lesions)	Pain and LSRs
	Wang et al. (2010)	Face	Topical and systemic antibiotics, oral retinoids, and sytemic steroids	78	22.9, range 16–37	90% clearance in 90% of patients	Pain, LSRs, and pigm
	Barolet and Boucher (2010)	Face and back	—	10	26.2, range 13–54	73% median reduction (inflamed lesions)	Pain, LSRs, and acneiform folliculitis
	Xu et al. (2017)	Face	—	95	24, range 15–35	74.4% mean reduction of inflamed lesions and 61.7% of non-inflamed lesions	Pain and LSRs
	Rojanamatin et al. (2006)	Face	—	14	16–27	8.7% decrease in lesion counts	Pain, LSRs, and pigm
	Orringer et al. (2010)	Face	—	44	25, range 15–50	30% (inflamed lesions) and 7% (non-inflamed lesions) improvement	LSRs and pigm
NL	Kaae et al. (2018)	Lower legs	Topical corticosteroids and cryotherapy	65	35.5, range 12–65	80% CR (DL-PDT) and 64% CR (MAL-PDT)	Pain
CVW	Schroeter et al. (2005)	Plantar region	Surg, cryo, sa, silver nitrate, and 5-FU	31	29, range 6–74	88% CR	Pain, LSRs, and pigm
	Caccavale et al. (2019)	Hand and plantar region	cryo, etc	13	28.8, range 18–52	84.6% CR	Pain, LSRs, and pigm
GVW	Shan et al. (2016)	Male urethra	—	76	32.6, range 16–65	93.4% CR	Pain and LSRs

three occasions, ALA was injected intralesionally to ensure adequate penetration. The patients reported treatment-related moderate pain and post-inflammatory pigmentation. At the end of the treatment period, he was clinically disease-free. At a follow-up visit 15 months after the last PDT session, two punch biopsies confirmed the absence of atypical lymphocytes in the treated area. Clearance was maintained at 46 months follow-up (Mendese et al., 2012). Heng et al. also reported a case of successful treatment of solitary mycosis fungoides after 30 sessions of MAL-PDT. After two months of stopping PDT, the patient showed no evidence of MF. The patient was disease-free at 35-months follow-up (Heng et al., 2014). Snider et al. used PDT in combination with narrow-band UVB to treat a 13-year-old boy with multiple lesions of lymphomatoid papulosis (LyP) over his elbows, forearms, proximal thighs, and right hip. They used 20% ALA topical solution and LED illumination to treat right forearm nodules resistant to nb-UVB treatment. He achieved the clearance of all lesions on his right arm within 2 months of combination therapy. Nevertheless, 2 years later new lesions appeared, but further PDT sessions were not attempted and subsequently the patient was lost at follow-up (Snider et al.,. 2020).

PDT and pediatric inflammatory skin diseases

Acne vulgaris

Acne is a chronic inflammatory disease of the sebaceous-pilosebaceous unit. In the last years, it has been clearly demonstrated that acne development is linked to the combination of predisposing genetic factors and environmental triggers, among which a prominent role is played by the follicular colonization by *Propionibacterium acnes* (*P. acnes*) (Antiga et al., 2015). Studies focusing exclusively on acne and PDT in pediatric population are scarce. In total, three possible ways of action have been

TABLE 4 PDT protocols.

DX	Publication (name first author and year)	PS	PS incub (h)	Light source	Light dose (J/cm2)	Mean no. of treat	Associat therapy	Follow-up (w, m, y)
BCCs	Loncaster et al. (2009)	16% MAL or 20% ALA	6	630 ± 15 nm (LED)	100	1–3	—	12–24 m
Acne	Hörfelt et al. (2006)	16% MAL	3	635 nm (lamp)	37	2	—	3 m
	Wang et al. (2010)	10% ALA	3	633 nm (LED)	50–70	1–3	—	6 m
	Barolet and Boucher (2010)	20% ALA	1	630 nm (LED)	70	1	Radiant infrared	1 m
	Xu et al. (2017)	5% ALA	1.5	633 nm (LED)	120	4	Oral minocycline	8 w
	Rojanamatin et al. (2006)	20% ALA	0.5	560–590 nm (IPL)	30	3	—	12 w
	Orringer et al. (2010)	20% ALA	1–1.5	PDL	6.5–7.5	1–3	—	16 w
NL	Kaae et al. (2018)	16% MAL	0.5–3	634 nm (diode), sunlight	37	4	Curettage	14 m (range 2–81)
CVW	Schroeter et al. (2005)	20% ALA	6.8	400–450 nm,580–720 nm (halogen)	—	2.3	Blunt scraping	3 m
	Caccavale et al. (2019)	10% ALA	3	630 nm red light source	75	3	Curettage, microneedling	Mean 4.3 m
GVW	Shan et al. (2016)	20% ALA	4	635 nm (laser)	—	1–4	—	3 m

Abbreviations: 5-FU, 5% topical 5-fluorouracil cream; Age (y), age of pediatric patients in years (Table 1); Age (y)= age of patients in years (Table 3); Ang TS, angiofibromas of tuberous sclerosis; Associat therapy, associated therapy; BD, Bowen's disease; CCS, corticosteroid; CL, cutaneous leishmaniasis; CR, complete response; cryo, cryotherapy; Curett, curettage; CVWs, cutaneous viral warts; DC, dissecting cellulitis; DX, diagnosis; Fmp-RF, fractional micro-plasma radiofrequency; Follow-up (w, m, y), Follow-up (weeks, months, years); FVWs, flat viral warts; gp, group; GVWs, genital viral warts; HS, hidradenitis suppurativa; imiq, imiquimod cream; LBC, lymphadenosis benigna cutis; LEBT, localized electron beam therapy; LP, linear porokeratosis; LSRs, local skin reactions, including erythema, burning, edema, crusting, desquamation, or pustule; LyP, lymphomatoid papulosis; MA, meglunime antimoniate; Mean no. of treat., mean number of treatments; min: minutes; NL, necrobiosis lipoidica; No pt, no. patients submitted to PDT (Table 3); No. ped pt, No. of pediatric/total patients submitted to PDT (Table 1); NR, no response; p-bn, plum blossom needle; PCAS, perifolliculitis capitis abscedens et suffodiens; PDL, pulsed dye laser; pigm, pigmentation changes, including hypo/hyperpigmentation; PgR, pagetoid reticulosis; PR, partial response; PS incub (h), photosensitizer incubation (hours); PS, photosensitizer; pt, patient; PT, physical therapy; sa, salicylic acid; SI, significant improvement; SR, satisfactory response; surg, surgery; VEN, verrucous epidermal nevus; Vg, vitiligo.

proposed to explain the improvement of acne by PDT: photodynamic killing of *Propionibacterium acnes*, which sterilizes the sebaceous follicle; direct photodynamic injury of sebaceous glands inhibiting sebum production; and reduction of follicular obstruction by an effect on keratinocyte shedding and hyperkeratosis. Nevertheless, these mechanisms do not appear to occur simultaneously in all cases of acne improvement. In a study on 10 patients involving a 16-year-old female, Pollock et al. (2004) found a statistically significant reduction of inflammatory acne lesions following ALA-PDT but failed to demonstrate changes in *P. acnes* numbers or in sebum excretion in the same patients. It has been hypothesized that light destroys *P. acnes* by targeting its endogenous porphyrins, including coproporphyrin III and protoporphyrin (Yeung et al., 2007). Nevertheless, according to some studies, PDT may determine a functional damage of *P. acnes* rather than a quantitative reduction (Pollock et al., 2004; Hörfelt et al., 2007). Moreover, irreversible damage of sebaceous glands may be reached only with repeated session of PDT (Pollock et al., 2004). So, other PDT effects, such as the reduction of follicular obstruction or anti-inflammatory effects, may play a more important role for acne improvement than destruction of sebaceous glands or killing of *P. acnes* (Pollock et al., 2004; Hörfelt et al., 2007).

Overall 31 pediatric patients with acne vulgaris were treated with various concentrations of ALA.

Ma et al. (2015) carried out a prospective study to evaluate ALA-PDT in severe acne of 21 adolescent patients. They treated patients with an average of three PDT sessions. They obtained high rates of effective response (85.7%) and observed that the efficacy of ALA-PDT tends to increase even after, reaching 95.23% of effective response after 8 weeks. PDT appears particularly useful in acne treatment when other conventional therapies have failed. Theresia et al. (2017) treated a 17-year-old male patient with severe multiple nodulocystic acne lesions on the face with ALA-PDT, who failed treatment with numerous topical and oral retinoids and antibiotics. The authors noticed a mild reduction in inflammatory acne lesions and sebum secretion already after the first session of PDT. After the fourth session of ALA-PDT, the patient had long-term remission with no new lesions during the 6 months follow-up. Borgia et al. (2018a) obtained improvement and a sustained good response in a case of acne conglobata on the buttocks in a 16-year-old boy, who did not respond to oral minocycline, topical

retinoid, and systemic isotretinoin. They used ALA-PDT for a total of six sessions. The patient experienced intense pain and inflammation during the first two sessions, then discomfort was milder. At the end of the treatment period, healing of the cutaneous nodules was observed, and at 6 months follow-up, the patient maintained good cosmetic results with no side effects. In total, two 18-year-old identical male twins with severe nodulocystic facial acne resistant to oral retinoids, antibiotics, and previous physical therapies experienced decrease in acne lesions with sustained response at 2-year follow-up after three sessions of ALA-PDT at 2 weeks interval. Both the patients suffered transient moderate pain and mild erythema during treatment with no residual pigmentation. They evaluated this method better than the previous medications (Li et al., 2018). In literature, several clinical trials or case series on PDT efficacy for acne treatment included both children and adults. Overall, 271 adult and pediatric patients with acne vulgaris were treated with various concentrations of ALA, achieving improvement of various degree, ranging from resolution of 30% of inflamed lesions and 7% of non-inflamed lesions (Orringer et al., 2010) to 90% clearance in 90% of patients (Wang et al., 2010). Itoh et al. tested the effects of 20% ALA oil-in-water emulsion and subsequent illumination by polychromatic visible light source on three men and 10 women with intractable facial acne. Among these, two 18-year-old males were also treated with one session of ALA-PDT and obtained excellent control of their acne and transient side effects. One of them experienced subsequent hyperpigmentation and was therefore treated with 4% hydroquinone cream for 10 days (Itoh et al., 2001). In a study on the efficacy of 20% ALA-PDT involving 15 patients with facial and dorsal acne vulgaris, four of them were pediatric (two of 16 years and two of 17 years). Improvement of acne lesions were recorded in all pediatric patients with facial acne after one session of ALA-PDT. There were no data about the 17-year-old male affected by dorsal acne because he was lost at 20-weeks follow-up (Hörfelt et al., 2007).

In a RCT on both pediatric and adult patients (median 18 years, range 15–28), two sessions of MAL-PDT, 2 weeks apart, determined a median reduction of 54% in the total inflammatory lesion count at week 12. Clinical response in MAL-PDT–treated patients was significantly better with respect to placebo-treated patients. Nevertheless, MAL-PDT was associated with more pain than placebo-PDT (Hörfelt et al., 2006). Wang et al. evidenced a 90% clearance rate after three sessions of ALA-PDT in a group of patients with mean age 22.9 years (range 16–37). Only 10% of patients had clearance rates between 50 and 90%. Side effects were well tolerated and transient, except for a patient who left the study because of excessive pain and discomfort (Wang et al., 2010). In a study involving patients with an age range 13–54 years, Barolet and Boucher et al. increased skin temperature of 10 patients with radiant infrared (IR) prior to ALA-PDT application to enhance the PS penetration. The authors observed a significant difference in median reduction of inflammatory lesions on the IR pre-treated vs. the control side 1 month after PDT. The authors did not report unusual treatment–related adverse effects (Barolet and Boucher, 2010). Also the combination of PDT and antibacterial therapies for acne has been investigated with encouraging early results. In a clinical trial, Xu et al. compared the effects of minocycline plus ALA-PDT and minocycline alone on moderate-to-severe facial acne of 95 patients aged 15–35 years. The authors observed a greater mean percentage reduction of lesion counts in the minocycline plus PDT group compared to the minocycline-alone group at 8 weeks follow-up for both inflammatory and non-inflammatory lesions and only mild and transient adverse events in the minocycline plus PDT group (Xu et al., 2017). Intense pulsed light (IPL) has been investigated as a light source in PDT for juvenile acne. Rojanamatin et al. treated 14 patients (range 16–27 years) with topical ALA plus IPL for three sessions. The combination determined a decrease in lesions counts of 87.7% at 12 weeks (Rojanamatin et al., 2006). Also, a pulsed dye laser (PDL) has been evaluated as a light source for ALA-PDT. Orringer et al. treated 44 patients, including pediatric patients (mean age 25 years, range 15–50) with three PDL treatments after a 60–90 min ALA application time. Nevertheless, the results with this light source were not particularly exciting with only transient decrease in mean inflammatory papule counts but no statistically significant differences in lesion counts (papules, pustules, and open and closed comedones) between treated and untreated control skin at the conclusion of the study on week 16 (Orringer et al., 2010). However, compared to topical or systemic treatments for juvenile acne, PDT did not show clear advantages in efficacy, and for this reason, it should be reserved for severe, recalcitrant cases, resistant to antibiotics and/or hormonal conventional treatments and/or not eligible to treatment with isotretinoin. In a systematic review on light therapies for acne, 25 trials on a total of 694 patients, also including pediatric patients, were analyzed. The authors observed that PDT did not show better efficacy than topical 1% adapalene gel. Nevertheless, PDT showed a benefit over light therapy alone (Hamilton et al., 2009).

Necrobiosis lipoidica

Necrobiosis lipoidica (NL) is a rare granulomatous disease strongly, but not exclusively, associated with diabetes mellitus characterized by yellowish-brown telangiectatic plaques with central atrophic area and erythematous edge, usually localized on the pretibial skin of females. Treatment of NL is often not satisfactory. PDT exerts positive effects in controlling the disease. In addition to its general anti-inflammatory effects, PDT seems

to influence positively the course of NL by remodeling the collagen matrix, thus stimulating the wound healing and improving sclerosis (Borgia et al., 2014). PDT is able to induce matrix metalloproteinases (MMP) production in fibroblasts (Motta and Monti, 2007) with higher production of MMP-1, MMP-9, and transforming growth factor (TGF)-β3 in wounds treated with MAL-PDT compared to untreated wounds (Mills et al., 2014). Berking et al. obtained partial results from the treatment of three adolescents among 18 patients with necrobiosis lipoidica of lower legs. The patients received two to six sessions of MAL-PDT. Pain was measured by using a 10 cm visual analog scale and pediatric patients referred a pain level ranging from 4/10 to 10/10. One of them stopped the treatment after two sessions because of treatment-related pain achieving no benefit. Of the remaining two patients, one presented partial response, whereas the other was not responder (Berking et al., 2009). Kaae et al. (2018) conducted a retrospective study on 65 NL patients treated with DL-PDT and C-PDT, including pediatric patients (median age at first treatment 35.5 years, range 12–65) and observed complete response in 66% of cases, with similar rates between C-PDT and DL-PDT. MAL-PDT was in median performed four times. The authors observed no correlation between clinical response and gender, age at first PDT treatment, duration of NL prior to PDT treatment, number of NL elements, or diabetes.

Hidradenitis suppurativa and dissecting cellulitis (perifolliculitis capitis abscedens et suffodiens or PCAS)

HS, often designed as acne inversa, is a chronic follicular inflammatory skin disease characterized by characterized by occlusion of hairs follicles and inflammation, which clinically leads to painful nodules, abscesses, and interconnecting sinus tracts involving the axillary, inguinal, anogenital, and inframammary regions. It usually manifests after puberty, but it can affect young patients especially those with familial history of HS. Bu et al. used 20% ALA-PDT as adjuvant therapy post-surgery for HS in seven patients, including two adolescents of 15 and 16 years, with Hurley grade II and III, respectively. At 5 months, they observed a marked improvement of the Dermatology Life Quality Index (DLQI) in all patients, including pediatric patients, with no recurrences at 6–12 months follow-up. Pain during illumination was well tolerated in all cases, but the 16-year-old patient, with Hurley Grade III lesions on craniofacial and neck areas, took the analgesic 30 min before PDT due to the moderate pain (Bu et al., 2017).

PCAS is considered an inflammatory bacterial process frequently caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Cui et al. speculated that PDT ameliorated PCAS by inhibiting bacterial infection. In their case series of nine patients treated with combination of surgical reverse flaps and PDT, they included a 17-year-old-boy, who reported satisfied outcome after a single session (Cui et al., 2020). Feng et al. (2019) treated a 15-year-old boy among eight male patients with dissecting cellulitis by using ALA-PDT. Before 10% ALA application and red light illumination, they cut hair and performed micropunctures in skin lesions by using a plum blossom needle, a kind of micro-needle. After 3 months, the authors observed a significant improvement in the pediatric patient, with a clearance rate > 70% and marked relief of symptoms. Treatment-related pain was tolerable and previous analgesia was not needed.

PDT and pediatric infectious skin diseases

PDT exerts antimicrobial effects on viruses, bacteria, fungi, and parasites. First, such activity is related to its ability to form high amount of reactive oxygen species (ROS), which damage biomolecules of all type of microorganisms, including viruses (Pérez-Laguna et al., 2018). In addition, as for tumoral cells, PDT stimulates the recognition of microorganisms by the immune system and mediates a local immune response against them. HPV is one of the most frequently targeted viruses in pediatric PDT. Cells infected by HPV are ideal targets of PDT because high-proliferating and can selectively accumulate PpIX compared to the surrounding non-infected cells. Moreover, selective photosensitization occurs not only in clinical HPV lesions but also in subclinical infection (Shan et al., 2016). Viruses have no capacity for PPIX production, but it has been demonstrated that the addition of exogenous ALA and its derivatives induces selective accumulation of PPIX in HPV-infected cells. PDT is able to significantly reduce HPV viral loads and to promote viral inactivation *via* cell necrosis and induction of T lymphocyte–mediated immune response against infected keratinocytes by increasing levels of IFN-α and IFN-ß (Borgia et al., 2020a). In bacterial infection, PDT-induced oxidative stress may damage multiple targets such as DNA, membrane integrity, protease activity, and lipopolysaccharide (LPS). Nevertheless, Gram-positive bacteria appear more sensitive to PDT than Gram-negative. Peptidoglycans and lipid acids in wall of Gram-positive allow penetration of cationic, anionic, and even neutral PS, while the double membrane in Gram-negative is particularly hampering and only cationic PS are active against them. Nevertheless, new technologies, including nanoparticle-based PDT, have significantly increased the PS penetration. The onset of resistance to PDT in bacteria is very unlikely because PDT-induced oxidative stress does not have a specific target but causes destruction of cell in different ways. Therefore, PDT has several advantages to antibiotics and may be considered a valid therapeutic alternative to them (Pérez-Laguna et al., 2018).

Cutaneous viral warts

PDT is indicated for treatment of cutaneous viral warts when other therapies have failed or in difficult to treat cases.

Overall 12 pediatric patients with cutaneous viral warts were treated with various concentrations of ALA, achieving complete response in almost all cases (Borgia et al., 2020b; Huang et al., 2019; Wu et al., 2019).

Borgia et al. described the case of a 6-year-old girl with multiple viral warts on the dorsal left foot. After failure of cryotherapy, the authors performed ALA-PDT for two sessions, 1 month apart. In each session, the patient experienced mild burning sensation. Complete clearance of the treated warts was seen 6 weeks after the second treatment with no recurrences at 1-year follow-up (Borgia et al., 2020b). Huang et al. described the exciting case of an 18-year-old female who completely resolved her 2 year history of resistant multiple warts in the right foot after curettage plus PDT. The patient was treated with ALA-PDT for a total of three sessions, but superficial shaving was applied only for the first session. At 3-months follow-up, the warts disappeared with no residual scar (Huang et al., 2019). Wu et al. performed on average three sessions of ALA-PDT on 10 pediatric patients ranging from 5 to 18 years among 23 total patients with multi-resistant periungual warts. The patients underwent superficial shaving before the first PDT, not performed in the following additional sessions of PDT. The authors observed an overall complete clearance in 61% of patients with a higher rate in the pediatric group (9/10 young patients had complete response, namely, 90%). All patients completed the treatment and satisfactory cosmetic outcome was obtained in almost all patients (96%). A significant decrease in DLQI at 12-month follow-up was reported. Pain was the most common adverse event, followed by secondary onychodystrophy, mild itching, and blisters (Wu et al., 2019). Also, in case of cutaneous viral warts, many studies on PDT efficacy include both children and adults.

Overall, 44 adult and pediatric patients with cutaneous viral warts were treated with 10–20% ALA, achieving complete response in 84.6–88% of cases (Schroeter et al., 2005; Caccavale et al., 2019).

Schroeter et al. (2005) treated 48 plantar warts from 31 patients (mean age 29 years, range 6–74) with 20% ALA cream and red light, observing a complete response in 88% of cases and no significant side effects. Caccavale et al. (2019) proven the efficacy of combination of curettage plus microneedling plus topical ALA-PDT for the treatment of acral resistant warts in young patients (mean age 28.8 years, range 18–52). They performed a thorough curettage on palmar and plantar warts of 13 patients, subsequent application of 10% ALA cream and microneedling. After 3 h of incubation, the warts were irradiated with a red light source. After three sessions of treatment, at 3-week intervals, the authors observed complete remission in 84.6% of cases and partial remission in further 7.7%.

Flat viral warts

PDT should be considered a useful option in treatment of flat warts, particularly in aesthetically sensitive areas such as the face of children (Borgia et al., 2020a). Overall 31 pediatric patients with flat viral warts were treated with C-PDT or DL-PDT, achieving complete response in one case (Borgia et al., 2019) and various degree of complete response ranging from 73.3% (C-PDT) to 80% (DL-PDT) in a study involving 30 patients (Borgia et al., 2020a).

Flat viral warts appear to be responsive not only to conventional PDT but also to DL-PDT. In the case of an 8-year-old female child with multiple facial flat warts resistant to previous topical tretinoin and cryosurgery, Borgia et al. performed two sessions of DL-PDT with 10% ALA ointment obtaining complete response with no recurrence at 1 year follow-up (Borgia et al., 2019). Borgia et al. also compared efficacy and safety of C-PDT and DL-PDT for treatment of facial flat warts in pediatric patients. They studied 30 young patients, who were divided in two group, with mean age of 9.67 ± 4.48 years (range 4–17) in group A and 9.13 ± 2.77 years (range 5–15) in group B. The two groups were randomly assigned to receive treatment with C-PDT or DL-PDT. The authors noted that in the early 12 weeks the treatment with DL-PDT seemed to fail. In fact, none of patients treated with DL-PDT reached an excellent response (75–100% reduction of total wart count), compared to 53.3% of patients treated with C-PDT. Nevertheless, this gap was filled in the following 12 weeks. At 24 weeks follow-up 80% of patients of DL-PDT group showed excellent response compared to 73.3% of patients of C-PDT group. So, in the long-term follow-up DL-PDT and C-PDT showed similar clinical efficacy for the treatment of pediatric facial flat warts. Adverse effects were also similar in the two group, with transient pain, irritation and hyperpigmentation reported (Borgia et al., 2020a). At 1-year follow-up, 60% of patients of both group (DL-PDT and C-PDT) maintained excellent response (75–100% reduction of total wart count compared with baseline). After 24 weeks, among the responders, 13.3% of C-PDT–treated patients and 20% of those treated with DL-PDT experienced mild relapses in terms of lesions' number and size. None of non-responders at 24 weeks achieved improvement at 1-year follow-up. No long-term side effects were reported in both groups (Borgia et al., 2021).

Genital viral warts

PDT may be particularly useful for genital warts difficult to treat due to their localization.

Shan et al. (2016) described the efficacy of PDT in the treatment of genital viral warts in male urethra. They treated 76 men including pediatric patients (mean age 32.6 years, range 16–65) applying 20% ALA solution with a thin cotton swab

gently inserted into the urethra. After a 3 h incubation period, they irradiated the lesions with a urethral cylindrical semiconductor laser fiber emitting light of 635 nm wavelength. The treatment was repeated once every week for 4 weeks. At the 3 months follow-up, almost all patients had a complete response and only five (6.6%) patients relapsed. Of these, three received four more sessions of PDT resulting in clearance of lesions without further recurrence.

Overall nine pediatric patients with genital viral warts were treated with ALA-PDT, achieving complete response in all cases (Xu et al., 2018; Macca et al., 2022).

Xu et al. (2018) reported eight treatment-resistant cases of pediatric genital warts successfully treated with 20% ALA-PDT. They irradiated perianal and intra-anal areas with red light from a semiconductor laser. Pretreatment by using microwave ablation was applied for lesions larger 5 cm. The patients were sedated with oral chloral hydrate (0.5–0.8 mg/kg) half an hour before light exposure. The majority of patients achieved complete response after three to six PDT sessions, but one patient required up to 12 sessions. The patients experienced mild to moderate pain during light exposure, according to a pain score. At a 6-month follow-up, neither other side effects nor recurrences were detected.

Macca et al. (2022) proven the effectiveness of ALA-PDT on non-sexually transmitted genital warts of a 5-year-old female. They applied 10% ALA ointment and irradiated with red light after an incubation period of 3 h. At 3 months follow-up, only a few flat elements were still visible but after further 3 months complete clearance was detected. The patient experienced mild to moderate burning sensation during light exposure and a transient hyperpigmentation, with no long-term side effects at 6 months follow-up. In conclusion, PDT may be considered as first-line therapy in patients with high number of genital warts, for which other topical therapies are excessively expensive or painful, or in patients with warts in urethral or anal and perianal areas. Xu et al. (2018) proven that the rapid healing of PDT makes it an optimal therapeutic option for pediatric genital warts in perianal and intra-anal areas, where other invasive treatments may cause anal stenosis and difficult defecation. Similarly, Shan et al. (2016) successfully used PDT for urethral genital warts in patients of all ages, including children.

Cutaneous leishmaniasis

In addition to treatment of viral and bacterial disease, PDT in pediatric age has been studied for management of parasitic infection from Leishmania. Mechanisms underlying effects of PDT on cutaneous leishmaniasis are largely unknown. Amastigotes are proven to accumulate very low amounts of protoporphyrin IX and some species of Leishmania lack enzymes of heme synthesis. Despite this, PDT may exert its effects on this type of protozoa by increasing the local temperature of the skin: hyperthermia treatment has been described as an effective therapeutic option for cutaneous leishmaniasis (Fink et al., 2016). Johansen et al. described an ulcerative resistant case of cutaneous leishmaniasis by Leishmania major in a 15-year-old boy successfully treated by 10% ALA-PDT. They performed conventional PDT twice weekly for 12 weeks. The patient had previously received unsuccessful treatments, including meglumime antimoniate, topical ketoconazole, and oral fluconazole. The authors noted full healing of the ulcer 1 month after PDT. Pain experienced during treatment was controlled by topical application of lidocaine (Johansen et al., 2019). Leishmania species that can cause mucocutaneous (*L. braziliensis* complex) or visceral leishmaniasis (*L. donovani* complex) should not be treated with PDT.

However, currently PDT is indicated only for cutaneous leishmaniasis resistant to other treatments and in aesthetically sensitive parts of the body (Morton et al., 2020).

Miscellanea

Vitiligo

Vitiligo is a common progressive depigmentation of the skin due to selective destruction of melanocytes (Vaccaro et al., 2015; Vaccaro et al., 2017a). Although the pathogenesis remains scarcely known, it seems to be related to genetic predisposing factors, oxidative stress and autoimmune dysregulation (Vaccaro et al., 2016; Vaccaro et al., 2017b; Custurone et al., 2021). PDT is proven to inhibit melanogenesis *in vitro*, reducing melanocytes melanin content and tyrosinase activity. *In vivo*, PDT reduces mottled hyperpigmentation of photoaged patient skin (Kim et al., 2018). These observations may partially explain why PDT did not achieve brilliant results in treatment of vitiligo in pediatric patients. Zhang et al. (2018) conducted a study to determine the effective PS concentration, PS application duration, irradiation duration, and irradiation dosage for the treatment of vitiligo. They selected ALA concentration of 1.5%, PS application duration of 3 h, irradiation duration of 20 min, and irradiation dosage of 80 mw/cm^2 as the better parameters for treating their patients. They treated vitiligo in two pediatric patients (4 and 17 years). Nevertheless, the results were contrasting and characterized by alternating periods of worsening and improvement. In the 17-year-old patient, pigment islands around skin follicles of vitiliginous areas of abdomen increased significantly with the number of early treatments. Nevertheless, during subsequent treatments at long intervals, pigment islands decreases progressively and, at the end of follow-up, no significant changes in pigmentation were detected compared to baseline. In the 4-year-old patient, vitiligo was found on forehead. During PDT treatment, pigment islands increased significantly first near the left eyebrow, then on the

forehead and finally near the hairline, with an apparent general improvement of vitiligo area compared to baseline.

When compared to topical corticosteroids, a standard treatment for vitiligo, PDT does not demonstrate any additional therapeutic effects (Rahimi et al., 2021). For that reason, there are no reasons to prefer it to other available therapies.

Angiofibromas of tuberous sclerosis

Weinberger et al. (2009) associated ALA-PDT with pulsed dye laser (PDL) to treat angiofibromas of tuberous sclerosis of six young patients. Two of these were in pediatric age (10 and 18 years). They combined 417-nm blue light with 595-nm PDL after application of 20% ALA solution and obtained decrease of lesions number and size. Transient side effects included erythema, swelling and superficial desquamation.

Verrucous epidermal nevus

Zheng et al. (2018) combined fractional micro-plasma radiofrequency (RF) technology and PDT to treat a facial verrucous epidermal nevus (VEN) in a 17-year-old girl. They carried out local anesthesia by using lidocaine cream under occlusion an hour before the therapy. Then, they performed the fractional micro-plasma RF treatment, which caused a temporary volume decrease of verrucous papules, and the first treatment of ALA-PDT 4 h later. Other three ALA-PDT session without RF pretreatment were performed. After treatment, the patient achieved complete disappearance of warty lesions on her face and no recurrences of VEN were detected at 24 weeks follow-up.

Linear porokeratosis

MAL-PDT has proven to be very effective in the treatment of Linear porokeratosis (LP), is a disorder of keratinization typically occurring in pediatric age. García-Navarro et al. (2009) obtained a cosmetic and clinical improvement of a LP on lower leg of a 13-year-old boy by using 16% MAL-PDT and red light. They performed two PDT sessions 1 month apart. No recurrences were observed after 11 months.

Curkova et al. (2014) performed three MAL-PDT sessions on extensive LP of a 16-year-old girl. They noted a progressive improvement of her multiple reddish-brown macules and depressions on right arm and at 1-year follow-up the cosmetic and clinical response was considered satisfactory. They did not performed pretreatment except for removal of superficial scale before second PDT session. The patient experienced only a transient burning sensation during illumination.

Garrido-Colmenero et al. (2015) reported the case of an 11-year-old girl with LP on her left breast. They did not obtain clinical improvement by using topical therapies, including adapalene and tacrolimus 0.1% ointment so the patient underwent two sessions of MAL-PDT with good result. Four months after PDT, only few lesions remained.

Gracia-Cazaña et al. (2015) used MAL-PDT to treat porokeratosis in children with bone marrow transplant. A 12-year-old boy presented three round lesions in the right popliteal fossa and two in the cervical region, histologically diagnosed as porokeratosis. After three sessions of MAL-PDT, he achieved complete clearance and remained free of disease after 5 years follow-up.

Discussion

Despite its worldwide use in adult patients that has provided strong evidence about efficacy and safety not only for oncologic conditions but also for inflammatory and infectious diseases, PDT applied to the pediatric population appears to be a substantially unexplored continent. Our review has in fact evidenced that this peculiar kind of photochemotherapy has been investigated in few diseases, with a very limited number of RCT and small case series, while most of our knowledge in children originates from sporadic case reports on single patients. This find an obvious justification regards to skin tumors, which occurrence in pediatric age is very rare, mainly represented by keratinocyte cancer in syndromic patients. The consequent almost complete absence of data about its effectiveness in the face of the not estimable risk of worsening or relapse strongly suggest, for ethical reason, its use only in exceptional cases. PDT could be considered therapeutic alternative in case of benign lymphocytic infiltration of the skin, while its use in cutaneous lymphomas may be hypothesized in a near future, as proposed for adult patients, only in localized form when other therapies are contraindicated or have failed (Morton et al., 2020). Moreover, as demonstrated in adults patients (Hooper et al., 2021) and suggested for children by Heng et al. and Mendese et al., repeated sessions of PDT are needed to obtain a clinical response in cutaneous lymphomas such as MF, thus potentially limiting the feasibility of this type of treatment. The anti-inflammatory effects of PDT have been studied in diseases mainly affecting the pilosebaceous unit, such as acne and HS. Both acne and HS have a dramatic burden, negatively conditioning the everyday life of the patients, especially in a delicate period such as adolescence, with disastrous effect on affective, social and sexual aspects resulting in low self-esteem feelings and depression. The point of strength of PDT in such cases seems to be its ability to hit with one shot three different pathogenic mechanisms, inhibiting the proliferation of *P. acnes*, targeting activated T lymphocytes thus reducing the release of cytokines which attract leucocytes to dermis and improving

follicular hyperkeratosis acting on keratinocytes differentiation and proliferation. Examining the available data on this topic, no significant advantages emerge respect to both topical and/or systemic conventional therapies. To date, PDT may be considered as a valid second-line treatment in patients resistant to antibiotics and/or retinoids or when such therapies are contraindicated. Some types of acne, especially the nodulocystic form of the face and trunk, may largely benefit from PDT not only to achieve sustained clinical improvement but also to reduce the risk of permanent scarring. PDT is known to promote the remodeling of the dermal matrix architecture *via* keratinocyte photoactivation with subsequent paracrine induction of matrix metalloproteinases production in fibroblasts. Its use at an early stage of the disease may accelerate resolution of the cystic lesions, reducing the risk and the severity of disfiguring scars. On the basis of these consideration, PDT could find growing application also in pediatric HS patients, a highly disabling disease with an always increasing incidence in pre-puberal and puberal patients, especially in those affected by concomitant predisposing factors such as obesity. More than in acne, PDT could help to control inflammation in a non-invasive way lowering the necessity to recur to prolonged systemic therapies not free from long-term side effects (antibiotic resistance), partially contraindicated (tetracyclines) and often not well accepted by both little patients and their parents. Moreover, its use since the onset of the disease may reduce the frequency and the severity of inflammatory episodes, preventing the development of undesirable scars with both cosmetic and functional impairment. An interesting field of application of PDT in pediatric patients is undoubtedly the antimicrobial one, with particular regards to HPV infection. With respect to the aforementioned indications, there are enough experimental and clinical experiences to affirm that PDT can be considered an effective, safe and well tolerated solution for both cutaneous and mucosal warts. Conventional therapies, including topical keratolytic agents, electrosurgery, cryotherapy and carbon dioxide laser may cause scars, inflammatory reactions and hyper- or hypopigmentation, with high risk of treatment failure and recurrence. Furthermore, such treatments are often contraindicated or not tolerated, especially in children. In general, lesion-directed therapies are not fully effective to eradicate HPV infection, in particular in subclinical and latent conditions. Conventional PDT has been used in children with good cosmetic results, better compliance and lower recurrence rates. However, it shows some limits: it is accompanied by pain during illumination, is time-consuming and requires dedicated equipment. DL-PDT offers advantages over C-PDT in terms of tolerability, time and cost, making this procedure more suited to the pediatric setting. DL-PDT is a novel procedure in which the activation of the topical photosensitizer is induced by exposure to natural daylight, without requiring preliminary occlusion. The absence of occlusion, with consequent less time spent at the clinic and the possibility to perform the treatment in an outdoor setting, may increase the compliance of young patients. In addition, pain intensity during DL-PDT is significantly lower than with C-PDT, probably because of gradual and continuous production and photoactivation of smaller amounts of protoporphyrin IX, minimizing the little patient's discomfort during irradiation.

With regards to the safety profile, as reported by all aforementioned studies, PDT is associated with only transient and mild to moderate adverse effects in pediatric population, such as those observed in the adult population. PDT side effects can be classified in early (immediately or within days after treatment) and late (after weeks or months) onset side effects. Early onset side effects include pain and local skin reactions (LSRs), namely, erythema, burning, edema, crusting, desquamation, or pustules (Borgia et al., 2018b). Pain is the more common adverse effects, but rarely requires analgesia. DL-PDT is considerably and statistically significantly less associated with pain than C-PDT. Borgia et al. observed that post-irradiation pain was similar in DL-PDT and C-PDT groups of pediatric patients, but further studies are needed to compare the two modalities and to evaluate any differences in terms of safety (Borgia et al., 2020a). Pain during PDT in pediatric patients may be also related to disease localization. Head and neck district is one of sites most associated with treatment-related pain. In their study on treatment of HS, Bu et al. proven that PDT did not requires previous analgesia except in the case of a 16-year-old boy who presented HS lesion in craniofacial and neck areas (Bu et al., 2017). Hyperpigmentation is a worrisome side effect, especially in children with high phototype skin, but may be avoided using appropriate photosensitizer concentration and incubation time and most often is transient and disappears spontaneously after a few weeks or months. Other side effects have been described following PDT, such as onychodystrophy, hair loss, erosive pustular dermatosis of the scalp and urticarial reaction urticarial reaction (Guarneri and Vaccaro 2009). Urticaria-like reaction was reported in two pediatric patients following a few minutes of light exposure. The first patient was an 11-year-old girl with Gorlin syndrome who was treated for BCC, while the second patient was a 4-year-old girl who was treated for porokeratosis. In the first patient, a subsequent provocation skin test confirmed that the reaction was produced by the combination of MAL and illumination while in the second patient, provocation testing was not carried out due to the her young age (Miguélez et al., 2013). No information regarding long-term safety of PDT in pediatric patients are available (Snider et al., 2020). The main concern is the development of PDT-induced skin cancers, as reported in some cases in adult patients (Borgia et al., 2018b). To date, there is no evidence that PDT can stimulate skin carcinogenesis in children but a continuous and

careful follow-up of PDT-treated patients is needed to verify this hypothesis.

Conclusion

Clinical trials focusing on PDT treatment in children are rare. There is a general reluctance about involving children in trials by parents and adults, especially because of fears of unpredictable side effects in the pediatric population. Moreover, trials on children involve more ethical concerns because children lack the capacity to understand the risks underlying trials and informed consent is difficult to obtain by parents (Joseph et al., 2015). Nevertheless, the review of the available data has showed promising results, with some points of strength but even a bigger number of uncertainties. It appears as a safe therapeutic procedure. Pain may limit the compliance of pediatric patients but previous local analgesia or more tolerable PDT settings, for example, daylight PDT, lower PS concentration, shorter incubation times or lower light fluences, may be useful in more sensitive patients. These parameters should be modulated in the same way to avoid the risk of hyperpigmentation, which represents a particularly worrisome side effect on a child's face. Nevertheless, PDT-induced hyperpigmentation is generally transient and responsive to local treatment. To date, there are no reports of PDT-induced skin cancers in pediatric age, so this possible adverse events remains only theoretical. PDT may be a soft procedure in children because it does not require daily treatments but limited in number and spaced in time. Nevertheless, it is time-consuming because the patients have to wait a number of hours at the hospital between PS application and illumination. Daylight PDT may be useful to reduce waiting times and it better fits to pediatric patients who would rather spend time outside than within the walls of an hospital. Data on efficacy of DL-PDT in pediatric patients are still limited, and further comparison between C-PDT and DL-PDT is needed in this population. PDT has the advantage that it can be easily combined with other therapies, thus increasing its effectiveness rates. The majority of local combination therapies, such as curettage, microneedling, fractional micro-plasma radiofrequency, radiant infrared or surgical debulking, were used to improve penetration of photosensitizer in the skin. Instead, other therapies with mechanism of action other than PDT, such as cryotherapy for viral warts or oral antibiotics for juvenile acne, can also be associated without increased risk of side effects. Nevertheless, the analysis of the literature has evidenced a number of questions that need to be addressed relatively to the most appropriate type, concentrations, and incubation period of photosensitizers, and optimal parameters of illumination sources in the different pathologies, adapting them in the light of the clinical characteristic of each single patient including age, disease severity, extent of the disease and its localization in different areas of the body. Unfortunately, the great heterogeneity of light sources, formulations, and photosensitizer types reported in literature makes comparison and analysis difficult. Moreover, PDT has been used with satisfying results in adults for other dermatologic conditions which may be explored also in pediatric patients, including connective tissue disorders, such as chronic lupus erythematosus or morphea/scleroderma, genital and oral lichen planus, lichen sclerosus, and several types of scars (Kvaal et al., 2013; Gordon Spratt et al., 2015; Wennberg, 2015; Borgia et al., 2016b; Gerkowicz et al., 2021). Specific protocols for pediatric patients as well as the length of follow-up intervals must be better standardized in larger RCT studies in order to draw up shared guidelines taking full advantage from such versatile treatment.

Author contributions

DL: conceptualization, data curation, formal analysis, writing—original draft, and first authorship. AD: methodology, data curation, formal analysis, validation, writing—review and editing, and first authorship. VM: conceptualization, data curation, investigation, supervision, and writing—review and editing. VF: data curation, investigation, methodology, and validation. SV: data curation, investigation, methodology, and validation. SF: data curation, investigation, methodology, supervision, and writing—review and editing. BF: conceptualization, data curation, investigation, supervision, and writing—review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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