ORIGINAL ARTICLE



Intravenous maintenance fluid therapy practice in the pediatric acute and critical care settings: a European and Middle Eastern survey

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Abstract

The ideal fluid for intravenous maintenance fluid therapy (IV-MFT) in acutely and critically ill children is controversial, and evidence-based clinical practice guidelines are lacking and current prescribing practices remain unknown. We aimed to describe the current practices in prescribing IV-MFT in the context of acute and critically ill children with regard to the amount, tonicity, composition, use of balanced fluids, and prescribing strategies in various clinical contexts. A cross-sectional electronic 27-item survey was emailed in April–May 2021 to pediatric critical care physicians across European and Middle East countries. The survey instrument was developed by an expert multi-professional panel within the European Society of Pediatric and Neonatal Intensive Care (ESPNIC). A total of 154 respondents from 35 European and Middle East countries participated (response rate 64%). Respondents were physicians in charge of acute or critically ill children. All respondents indicated they routinely use a predefined formula to prescribe the amount of IV-MFT and considered fluid balance monitoring very important in the management of acute and critically ill children. The use of balanced solution was preferred if there were altered serum sodium and chloride levels or metabolic acidosis. Just under half (42%, 65/153) of respondents believed balanced solutions should always be used. Respondents considered the use of isotonic IV solutions as important for acute and critically ill children. In terms of the indication and the composition of IV-MFT prescribed, responses were heterogeneous among centers. Almost 70% (107/154) respondents believed there was a gap between current practice and what they considered ideal IV-MFT due to a lack of guidelines and inadequate training of healthcare professionals.

Conclusions: Our study showed considerable variability in clinical prescribing practice of IV-MFT in acute pediatric settings across Europe and the Middle East. There is an urgent need to develop evidence-based guidelines for IV-MFT prescription in acute and critically ill children.

What is Known:

- The administration of maintenance intravenous fluid therapy is a standard of care for a lot of hospitalized children
- Maintenance intravenous fluid therapy prescriptions are often based on Holliday and Segar's historical guidelines even if this practice has been associated with several complications.

What is New:

- This study provided information on the prescribing practice regarding fluid restriction, fluid tonicity, and balance.
- This study showed considerable variability in clinical prescribing practice of intravenous maintenance fluid therapy across Europe and the Middle East.

Keywords Child · Neonate · Adolescent · Intravenous fluids · In-hospital · Balanced solutions

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Abbreviations

AKI	Acute kidney injury
ESPNIC	European Society of Pediatric and Neonatal
	Intensive Care
IV-MFT	Intravenous maintenance fluid therapy
PICU	Pediatric intensive care unit
IV	Intravenous

HCW	Healthcare worker
NICU	Neonatal intensive care unit
DKA	Diabetes ketoacidosis
ARDS	Acute respiratory distress syndrome
ERAS	Enhanced Recovery After Surgery
AAP	American Academy of Pediatrics
RL	Ringer's lactate
NS	Normal saline

Introduction

Currently, there is little consensus about the use of intravenous maintenance fluid therapy (IV-MFT) across acute pediatric practice. Maintenance fluid therapy is defined as the "volume of fluid required to meet daily metabolic needs, such as normal water and electrolyte losses, and maintain homeostasis" [1] and should be distinguished from resuscitation and replacement/redistribution fluid therapy. There has been much debate about which IV-MFT solution to use and the amount to give [2–7].

Fluids are considered to be iso, hyper, or hypo-tonic if their tonicity (also called fluid effective osmolarity, which is different from fluid osmolarity) is almost, above, or under plasma tonicity respectively. Balanced fluids are characterized by their chloride content, which is close to plasma chloride content. The use of balanced fluids has been increasingly adopted as the fluid of choice as it causes less acidosis and electrolyte disturbance than chloride-rich solutions. In these solutions, part of the chloride anion is replaced by organic anions (such as lactate, malate, acetate, or gluconate) which maintain the anion/cation balance [8, 9].

In the context of developing new European evidence-based guidelines, on behalf of the European Society of Pediatric and Neonatal Intensive Care (ESPNIC), it was important to understand current practice across Europe. Previous surveys on IV-MFT have focused on the tonicity of fluids prescribed, with a preference for hypotonic fluids in the early 2010s [10, 11] shifting toward a preference for isotonic fluids approaching and during 2020 [12]. Historically, the amount of IV-MFT prescribed has been dictated by the Holliday and Segar formula produced in 1957 [13]. The aim of our study was to describe the current European and Middle Eastern practice of prescribing IV-MFT, not only on tonicity, but also of the use of balanced fluids, the amount prescribed, fluid composition (i.e., glucose, potassium, calcium, potassium, magnesium, and micronutrient content), and IV-MFT prescription strategy within different pediatric clinical contexts for children admitted to general pediatric wards (acutely ill children) and pediatric intensive care units (PICU) (critically ill children).

Methods

Study design and method

We conducted a cross-sectional electronic survey of physicians (including other healthcare professionals who were prescribers) prescribing IV MFT for acute and critically ill pediatric patients. Acutely ill patients were defined as patients admitted to the pediatric ward and/or intermediate care units requiring urgent treatment, and critically ill patients as patients admitted to the PICU. We included all physicians and advanced nurse practitioners within the ESP-NIC Network.

Survey instrument development and content

The survey was developed in English by a multi-professional ESPNIC group, leading the project to develop ESPNIC guidelines on IV-MFT in acute and critically ill children (Survey as Supplemental Digital Content 1). The scope of the survey included term neonates (> 37 weeks gestational age) and children up to 17 years of age. It was made clear that fluid boluses for resuscitation, replacement/redistribution fluid therapy, and intraoperative fluid therapy were outside the scope of the survey. Following a review of the literature and previous surveys, a new 27-item-cross-sectional survey was constructed and reviewed by an expert panel of six members of the ESP-NIC IV-MFT group (one of whom (LNT) is an expert in questionnaire design) for content validity. This was then tested for face validity on four physicians, and following this, minor changes were made to the wording to improve clarity.

The final 27-item survey included the demographic characteristics of respondents, then questions were divided into five different sections, which correspond to the five main domains of the future ESPNIC IV-MFT guidelines: (i) indications for IV MFT, (ii) amount of IV-MFT, (iii) tonicity of IV-MFT, (iv) balanced solutions, and (v) composition of IV MFT (glucose, K, Mg, Ca, P, micronutrients) (Survey instrument Electronic Supplementary File 1). The questions also sought clarification of prescribers' practice and the factors that affect their decision-making around IV-MFT. The survey used several common clinical scenarios such as gastroenteritis, status epilepticus, bronchiolitis, and post-appendectomy in different age groups (7-day-old neonate, 5-month-old infant, and 12-year-old adolescent) to elicit these responses. Questions were a mixture of Likert scale, rating scale, or multiple choice.

Data collection

The electronic survey was disseminated online in April-May 2021 within the ESPNIC network, via Survey-Monkey® software (San Mateo, CA, USA). The survey began with an invitation letter and instructions clarifying the scope of the survey and how to answer it, specifying answers should describe pre PICU transfer ward practices and local current PICU practices rather than ideal practices. Completion of the survey implied voluntary consent to participate in the research. Fourteen members of the ESPNIC IV-MFT guideline group acted as references in their region/country with their respective networks. We specified only one response per center. In view of the ESPNIC network, we anticipated answers from 100 different units. We aimed for a response rate above 60%, so a maximum of two reminder emails were sent to non-responding centers. To avoid bias with one country dominating the survey, no reminders were sent within a country if more than 15 responses were received.

Data analysis

Data was exported from a CSV file into Microsoft Excel for further checking and descriptive analysis. Questionnaires

Table 1 Characteristics of survey respondents

Characteristics	n=154	%
Role of prescribers:		
Consulting pediatric intensivist (attending) physician	131	85%
PICU fellow/resident/junior	13	8%
Anesthetist	10	7%
Types of PICU:		
General PICU only (without cardiac ICU)	58	38%
Mixed general PICU & cardiac ICU	40	26%
Mixed general PICU & cardiac ICU & NICU	12	8%
Mixed general PICU & NICU (without cardiac ICU)	32	21%
Cardiac ICU only	4	2%
Specialised PICU, e.g., burns/neuroscience	6	4%
Adult ICU (that admits children)	2	1%
Type of Hospital:		
Specialist children hospital	87	57%
Local hospital	45	29%
University hospital	22	14%

Results are expressed in number and percentage (%)

PICU pediatric intensive care unit, *NICU* neonatal intensive care unit, *ICU* intensive care unit

with more than 10% incompleteness were excluded from the study. Percentages were used to summarize categorical data. We used a summative score to summarize the results from Likert scale questions for each participant. Continuous variables were presented as median and inter-quartiles (IQR) or mean and standard deviation (SD) depending on the variables' distribution and frequency and proportions for categorical variables. Comparisons between both groups were made by paired t test or a paired samples Wilcoxon test according to the distribution for continuous variables, as appropriate. Results were considered statistically significant at p value less than 0.05. and two tailed tests were used. Tests were performed using BiostaTGV. Ethical approval was obtained from Caen-France institutional review board (reference number 2474) for the study.

Results

Participants' characteristics

One hundred and fifty-four respondents from 154 units responded from 35 European and Middle Eastern countries of the 240 PICUs contacted in 43 countries (response rate 64%). The characteristics of the respondents are detailed in Table 1 and Fig. 1. Complete response data is available in the supplementary electronic material (Supplemental Digital Content 2).

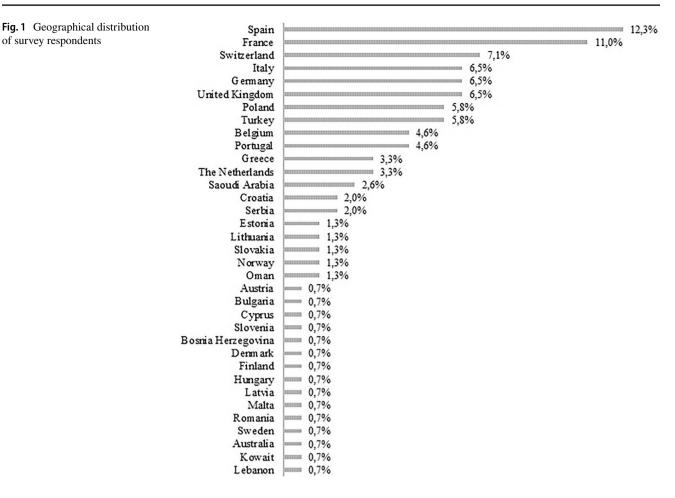
Indications for IV maintenance fluids

Responses regarding the indications for IV-MFT were heterogeneous among centers and respondents. The child's condition was the main criteria to prescribe IV-MFT (Table 2). Respondents all indicated that they would always prescribe IV-MFT rather than enteral hydration/nutrition in severe DKA, 84% (128/154) and post-abdominal surgery 74% (113/154). Regarding other clinical scenarios, practices varied between respondents (Table 2), except bronchiolitis, in which more than half of respondents (54% (82/154) rarely to never prescribed IV-MFT.

Amount of IV fluids

Fluid balance monitoring was considered important by all respondents in the management of critically ill children (mean score $10/10 \pm 0.8$) and in acutely ill children (mean score $8/10 \pm 1.9$). In relation to the calculation of fluid

of survey respondents



balance, which fluids were included in the total fluid intake varied by center and these are shown in Fig. 3.

All respondents indicated they routinely used the following formulas to prescribe the amount of an IV-MFT, with Holliday-Segar 76% (117/154) being the most common, followed by Oh 23% (35/154) then Adelman and Solhaugh 15% (23/154) [13-16]. However, a fluidrestrictive strategy was used in different categories of patients such as children with cardiac conditions (87% of respondents, 130/154), children following cardiac surgery (86% of respondents, 113/154), children with renal failure (78%, 118/154), and in children on invasive mechanical ventilation (56% of respondents, 84/154) (Fig. 2).

Type of solution: isotonic

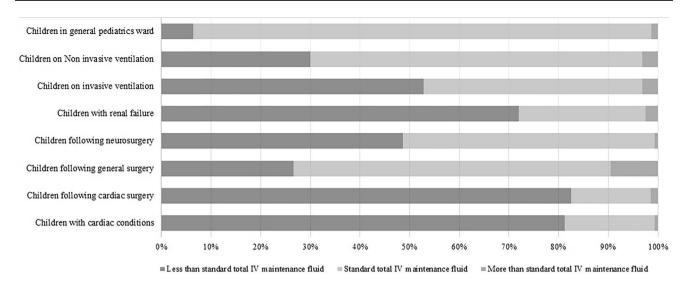
The use of isotonic IV-MFT solutions was reported as very important by respondents in the management of critically ill children (mean score $9/10 \pm 1.9$) and in acutely ill children (mean score $8/10 \pm 2.0$). The fluids selected by respondents for each clinical scenario showed considerable heterogeneity and are shown in Table 3.

Table 2 Frequency of prescribing IV-MFT in Different clinical conditions

	Always	Often/sometimes	Rarely/never	Number of respondents/ questions
Non-severe bronchiolitis	3 (2%)	48 (31%)	82 (54%)	153
Severe diabetic keto acidosis	128 (84%)	20 (13%)	5 (3%)	153
Post abdominal surgery	113 (74%)	36 (24%)	3 (2%)	152
Fasting < 24 h (nill by mouth)	73 (49%)	67 (45%)	10 (7%)	150
ARDS	52 (34%)	76 (50%)	25 (16%)	153

Results are expressed in number and percentage (%)

ARDS acute respiratory distress syndrome





Type of solution: balanced solutions

Prescribing a balanced fluid as IV-MFT for critically and acutely ill pediatric patients was considered very important by the respondents (mean score $8/10 \pm 2.4$ and $7/10 \pm 2.6$ respectively). Just under half (42% 65/135) of respondents

believed that balanced solutions should always be used. The criteria for selecting a balanced IV solution as IV-MFT were consistent among the respondents. Respondents stated they would prescribe a balanced solution in situations of altered serum chloride levels (78%, 120/154), metabolic acidosis (75%, 116/154), altered serum Na

 Table 3
 Type of fluids depending clinical situation

	Crystalloid: hypotonic solution Unbalanced	Crystalloid: isotonic solution Unbalanced	Crystalloid: Isotonic solution balanced	Number of respondents/ questions
Viral gastroenteritis not tolerating oral rehydrat	ion			
-Serum Na normal: 137 mmol/L				
5-month-old patient	10 (7%)	72 (47%)	71 (46%)	n=153
12-year-old patient	8 (5%)	70 (46%)	75 (49%)	n=153
-Serum Na above normal: 149 mmol/L				
5-month-old patient	41 (27%)	36 (24%)	76 (59%)	n=153
12-year-old patient	38 (25%)	35 (23%)	80 (52%)	n=153
Status epilepticus and still somnolent				
5-month-old patient	6 (4%)	85 (56%)	62 (41%)	n=153
12-year-old patient	4 (3%)	81 (53%)	68 (44%)	n=153
Persistent respiratory distress on NIV				
7-day-old patient	38 (25%)	59 (38%)	56 (37%)	n=153
5-month-old patient	20 (13%)	72 (47%)	61 (40%)	n=153
12-year-old patient	13 (9%)	71 (46%)	69 (45%)	n=153
ARDS ventilated on PICU (1st 48 h)				
5-month-old patient	14 (9%)	70 (46%)	69 (45%)	n=153
12-year-old patient	7 (5%)	71 (46%)	75 (49%)	n=153
Traumatic brain injury ventilated on PICU				
14-year-old patient	2 (1%)	82 (55%)	66 (44%)	n = 150

Results are expressed in number and percentage (%)

NIV non-invasive ventilation, ARDS acute respiratory distress syndrome PICU, pediatric intensive care unit

 Table 4
 Frequency of prescribing different nutrients and electrolytes within the fluid

	Always	Often/sometimes	Rarely/never
Glucose	70 (46%)	80 (52%)	3 (2%)
Potassium	38 (25%)	111 (73%)	4 (3%)
Phosphate	2 (1%)	89 (59%)	60 (40%)
Magnesium	3 (2%)	81 (54%)	67 (44%)
Calcium	14 (9%)	96 (63%)	43 (28%)
Trace elements	3 (2%)	58 (38%)	90 (60%)
Vitamins	6 (4%)	57 (38%)	88 (58%)

Results are expressed in number and percentage (%)

levels (62%, 96/154), or according to the child's underlying clinical condition (60%, 92/154).

Fluid composition

The median age that glucose was no longer perceived to be required to be added to the IV-MFT was 12 years (IQR 4,75–16). Forty-six percent (70/154) of respondents indicated they always prescribed glucose in the IV-MFT, and 52% (80/154) often prescribed it. For potassium supplementation in IV-MFT, 25% (38/154) indicated they always prescribed it, while 73% (112/154) often prescribed it. Calcium was always prescribed by 9% (14/154) while 63% (96/154) often prescribed it and 28% (43/154) rarely prescribed it. For the other elements such as phosphate, magnesium, trace elements, and vitamins, these were rarely or never prescribed by 51% (77/154) of participants (Table 4).

In terms of IV-MFT practices, most (70% 107/153) respondents believed there was a gap between current practice and what they considered ideal IV-MFT practice,

especially outside the PICU setting. The main reasons for this were believed to be the lack of guidelines (32%, 49/153), the inadequate training of healthcare professionals (26%, 39/153), and a lack of access to "ready to use" solutions. Although there was a wide range of ready to use IV-MFT solutions used in each center (in PICU and general pediatric wards), 4% of respondents (6/153) have no access to ready to use IV fluid solutions.

Discussion

Our European and Middle Eastern survey has described multiple aspects of practice around IV-MFT in acute pediatric and intensive care. Our results show a wide variation in practice in IV-MFT in children across Europe and the Middle East in both PICU and ward settings. The severity of illness seems to influence the indication for IV-MFT, but not the use of isotonic fluid or balanced fluids, which are probably more related to local habits or availability of ready to use products. A newly published survey of European fluid practice in invasively ventilated critically ill children has recently been published [17] but this looked only at "general" invasively ventilated children, excluded cardiac children, included fluid replacement therapy, and was only conducted within European centers. Our survey is considerably broader than this and, importantly, incorporates acutely ill children within the hospital, not just in the PICU.

IV-MFT should be considered like any other drug, with side effects and consequences [1]. The indications for IV-MFT are varied, but the Enhanced Recovery After Surgery (ERAS) protocol recommends avoiding prolonged IV-MFT

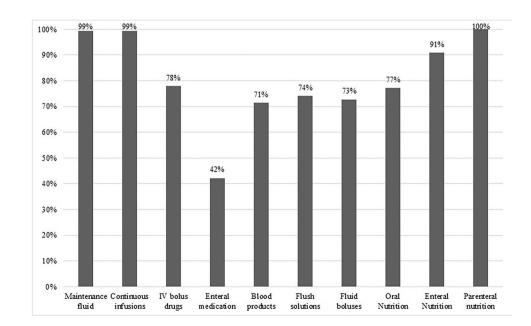


Fig. 3 The fluids considered in the total fluid intake by the respondents

by starting enteral nutrition/fluids early [10]. Whenever it is possible, the oral and/or enteral route should be favored over the IV-MFT as the IV route is associated with greater potential for loss of nutritional status and iatrogenic electrolyte disturbances [11].

In 1957, Holliday and Segar published a formula to guide the prescribing of pediatric IV-MFT volume [13]. We found that this formula still dominates practice, despite the limitations of this original paper, which was based on the energy requirements of healthy, well-hydrated children [13]. We still have little definitive evidence that Holliday and Segar is the optimum formula (4, 14-16). Indeed, there are many situations where fluids were restricted beyond this standard calculation. Our survey showed that IV-MFT fluid was commonly restricted in children with cardiac conditions, renal failure, invasive mechanical ventilation and in children following cardiac surgery, with no respondents reporting exceeding the standard maintenance fluid volumes. This is consistent with the recent survey of only non-cardiac ventilated children where most (75%) respondents restricted IV fluids by around 20% on PICU admission [17].

Most (but not all) respondents in our study reported including most fluids received by the child in calculating the total fluid intake and daily fluid balance (enteral and parenteral fluids). Under hydration has rarely been reported in the literature, in contrast to the impact of over hydration and fluid overload. The frequency and adverse effects of fluid overload are increasingly reported in critically ill children leading to longer duration of mechanical ventilation, the need for renal replacement therapy, and longer duration of ICU stay [18]. This is due to multiple factors, one being that critically ill children may have increased levels of anti-diuretic hormone (ADH) to compensate for the initial hypovolemia, which predisposes them to fluid retention and hyponatremia [19].

This study shows the preference for prescribing isotonic solutions for the maintenance of intravenous fluid for acute and critically ill children. This finding is aligned with the latest recommendation of the American Academy of Pediatrics (AAP) Clinical Practice Guidelines, which recommend the use of isotonic fluid therapy instead of hypotonic fluid therapy [20]. This recommendation and the evidence it is based on [3, 21] have markedly changed the prescribing of IV-MFT practices in children toward isotonic fluid therapy [22]. The aim of this recommendation was to prevent adverse events associated with iatrogenic hyponatremia and acute or permanent neurological impairment associated with the administration of hypotonic solutions in contrast to isotonic solutions [20]. Moreover, fatal hyponatremia has been reported in children receiving hypotonic fluid therapy [23, 24]. Conversely, children receiving isotonic fluid therapy have an increased risk for hypernatremia [3], which has previously been associated with an increased risk of mortality

if left untreated [25]. Furthermore, it has been suggested recently in the adult population that isotonic fluids produce an additional sodium burden [26-28]. This sodium burden appears to be associated with a positive fluid balance and possibly with respiratory complications [26-28]. The underlying mechanisms for this suggest that it may be related to the kidneys' inability to deal with an abrupt massive sodium load [26]. To our knowledge, there is currently no pediatric data to sustain this theory. Lastly, Lehtiranta et al. showed that commercially available solutions (Plasma-Lyte/dextrose 5%) were associated with significantly more electrolyte disorders and weight gain compared to a fluid with 80 mmol of sodium and 20 mmol of potassium [22]. Although their conclusion may be used by opponents of isotonic fluids [29], the differences highlighted in this study are mainly due to the difference in the frequency of hypokalaemia. These differences are related to the potassium concentration and not the tonicity of the fluid [22].

The results of our study are consistent with recent observational studies, indicating that unbalanced crystalloids are the most used maintenance fluids. However, more than one-third of centres used balanced solutions as first-line IV-MFT. Additional factors contributing to the decision-making around prescribing balanced salt solutions were mainly related to the serum chloride level, the presence of metabolic acidosis, and the child's clinical condition [2, 30, 31].

Ongoing debate has focused on whether chloride-rich solutions worsen patient outcomes, through the increased risk of hyperchloremic acidosis and whether the physiologically balanced solutions may improve or ameliorate these. Notably, potential side effects related to sodium chloride use have been identified, including hyperchloremic acidosis, nephrotoxicity, coagulopathy, gastrointestinal dysfunction, and increased mortality. Animal studies have shown evidence of afferent arteriolar vasoconstriction with elevated tubular chloride which in turn leads to decreased glomerular filtration rate and impaired renal perfusion [32].

Further studies found that hyperchloremia produces an increased risk of coagulopathy, renal vasoconstriction, heightened inflammatory response in the kidneys through the release of eicosanoids, resulting in reduced renal cortical tissue perfusion and has been associated with a higher incidence of acute kidney injury [31, 33–35]. In adults, several studies have reported a higher incidence of metabolic acidosis and hyperchloremia in patients who received saline compared with balanced solutions [36, 37].

In contrast, for the physiologically balanced salt solutions such as RL, the lactate in RL is converted to bicarbonate via gluconeogenesis and oxidation, not only in the liver but also in the kidneys and can improve pH and may ameliorate this harm associated with the chloride-rich solutions [38]. However, the benefit of balanced solutions to reduce the risk of acute kidney injury (AKI) remains controversial, with randomized trials in critically ill adults comparing balanced solutions and 0.9% saline (SPLIT, SALT trials, and BASICS) not showing any reduction in AKI while two other trials (SMART and SALT-ED trials) showing a reduction in major adverse kidney events [39–43].

Currently, there is a lack of robust evidence to recommend the use of one isotonic crystalloid over another one in children. Still, some societies/organizations such as the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (2018) and the WHO advocate the use of Ringers lactate for IV-MFT in acute pancreatitis and for the correction of severe diarrheal dehydration [44].

Although the understanding of the metabolic response to critical illness has evolved over the last decade, there is still huge variability in daily practice around the composition of IV-MFT [45, 46]. Glucose is the preferential energy substrate during acute and critical illness and a lack of glucose supply leads to ketogenesis and neurological effects [47]. Our survey showed most respondents still prescribed glucose in IV-MFT in young children. However, the age at which glucose was no longer routinely prescribed in IV-MFT was heterogeneous.

The addition of electrolytes to IV-MFT was also highly variable, probably due to the lack of recommendations to guide the clinicians. The AAP recommends using solutions with appropriate levels of potassium chloride, most commonly 2 mmol of potassium per 100 kcal metabolized [48]. However, despite this recommendation, most "ready to use" maintenance IV fluid solutions do not meet these recommendations, (e.g., Ringers lactate contains 0.4 mmol/kg/L). The practice of adding micronutrients to IV-MFT was also rare. A recent systematic review of micronutrient studies in critically ill children revealed that micronutrients should be provided in sufficient amount to critically ill pediatric patients, but there was insufficient data to recommend the routine supplementation of micronutrients at higher doses during critical illness [48].

Limitations of the study

This study has some limitations, inherent to its design. The self-report nature of the survey risks bias and may reflect individual views rather than actual practice. The selection bias, caused by the voluntary nature of the survey, may have resulted in clinicians with a greater interest in the topic answering. Moreover, as predominately pediatric intensivists completed the survey, the accuracy of the prescribing practice in the general pediatric ward setting may be less reliable. Furthermore, in the survey, the concept of tonicity was not precisely defined and may have led to some degree of confusion with osmolarity; however, pediatric intensivists are usually confident with these concepts. Finally, in some clinical conditions such as gastroenteritis and diabetic ketoacidosis, it remains difficult to clearly distinguish IV-MFT from that of IV rehydration therapy, which are managed concurrently. Despite these limitations, our response rate was high, thus improving the reliability of the survey and it is the largest survey to engage with clinicians both in PICU and in acute pediatric settings across Europe and the Middle East. It also examined broader practices around IV-MFT in children than other surveys.

Conclusions

Our study showed considerable variability in pediatric clinical practice around IV-MFT. There is an urgent need to conduct more robust research and develop evidencebased guidelines for IV-MFT in acute and critically ill children to guide clinical practice. This survey may also be used after the dissemination of future guidelines to assess the change in practice.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00431-022-04467-y.

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Author contribution FVV conceived the study; CM, FVV, DB, and LT developed the study protocol; CM, FVV, LT, DB, HM, and FA developed and piloted the survey tool; CM was responsible for data

collection; CM, FVV, LT, DB, HM, and FA analyzed the results and drafted the manuscript.

Availability of data and material Raw data are submitted as supplemental material.

Code availability Not applicable.

Declarations

Ethics approval Our protocol was analysed within the Research Ethics Committee (CLERS) and was approved on May 2021. Due to the nature of the study, the Institutional Review Board waived the need for informed consent.

Consent to participate Not applicable.

Consent for publication Not applicable.

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