UNIVERSITÀ DEGLI STUDI DI GENOVA

SCUOLA DI SCIENZE MEDICHE E FARMACEUTICHE

Scuola di Specializzazione in Pediatria
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TESI DI SPECIALIZZAZIONE IN PEDIATRIA

Preterm newborn kidney injury: risk factors, long term consequences and multidisciplinary approach for early diagnosis

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Anno accademico 2021-2022

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ABSTRACT

Background: Acute Kidney Injury (AKI) in preterm newborns is a serious medical condition that could have significant short-term and long-term consequences. Other than prematurity, risk factors include low birth weight, sepsis and infections, hemodynamic instability and maternal factors such as preeclampsia. A multidisciplinary approach is crucial for early diagnosis, management and follow up.

Aim of the study: The aim of the study was to sensitize the awareness of AKI on the risk categories in order to reduce its incidence through prevention strategies and to investigate the eventual onset of AKI through a multidisciplinary approach.

Material and methods: 37 infants born at a gestational age < than 34 weeks and a birth weight < than 2000 grams were enrolled in the study. Data on pregnancy and events occurred at peripartum were acquired. Serial serum creatinine (SCr), ultrasound kidney dimensions, blood pressure values and urine samples were collected at different times.

Results: At every time and at every measurement the lower the birth weight the smaller the kidney longitudinal dimensions. No statistically significant correlation was found between blood pressure (BP) values measured at different times and birth weight, gestational age, kidney longitudinal dimensions, and clinical acute events. Instead, it was demonstrated that twins have lower BP than singletons, especially at 3 months. None of the neonates enrolled have met the criteria for neonatal AKI.

Conclusion: As a risk factor for developing AKI and its consequences, lower kidney dimensions relate with a lower birth weight, empathizing the awareness of IUGR, not only for preterm infants, as a category at risk. The fact that none of the neonates enrolled have met the criteria for neonatal AKI could be an ulterior confirmation of the necessity for preterm neonates to have more punctual and specific markers for AKI which would reflect kidney injury, and not its function.

ABBREVIATIONS

IUGR	Intrauterine growth restriction
NICU	Neonatal intensive care unit
AKI	Acute kidney injury
SCr	Serum creatinine
KDIGO	Kidney Disease: Improving Global Outcomes
CysC	Cystatin C
sCysC	Serum Cystatin C
NGAL	Neutrophil gelatinase-associated lipocalin
uNGAL	Urine neutrophil gelatinase-associated lipocalin
MS	Mass spectroscopy
ELBW	Extremely Low Birth Weight
H-NMR	H-nuclear magnetic resonance
PDA	Patent Ductus Arteriosus
CKD	Chronic Kidney Disease
LBW	Low Birth Weight
VLBW	Very Low Birth Weight
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MBP	Mean Blood Pressure
eGFR	estimated Glomerular Filtration Rate
BP	Blood Pressure
SGA	Small for gestational age
TEA	Term-estimated age
NEC	Necrotizing enterocolitis
GA	Gestational Age
ROP	Retinopathy of prematurity

BACKGROUNDS

1. Low birth weight at birth, a risk factor for renal damage

The formation of the metanephric kidney begins at approximately 5 weeks of gestation but nephrogenesis is normally completed by 32-36 weeks. No other nephrons appear to be formed during the lifetime of the individual.

Nephrons are the functional units of the kidney, hence, the number of nephrons correctly formed at the completion of nephrogenesis can directly influence lifetime renal function and reserve. [1]

Since the nephron number is likely attributed to differences in nephron endowment by the completion of nephrogenesis and to birth weight, given that nephrons number is directly proportional to kidney size and kidney size is proportional to body size, [2] exposure to conditions such Intrauterine Growth Restriction (IUGR) and/or preterm birth, can negatively impact on nephrogenesis and thus adversely influence the nephron endowment at the beginning of life.[1] This may be linked with an increased incidence of hypertension [3], [4] and altered renal function later in life. [5]

However, it has been widely demonstrated that in the preterm stage, the total number of nephrons tends to increase after preterm birth, indicating that nephrogenesis could continue up to 36 weeks during extrauterine life. In fact, after this period, the total nephrons number appears to be within the normal range, albeit at the lower end of the normal range at term birth. [6] However, this process, could be drastically reduced by adverse clinical conditions in the first weeks after birth. [7]

Studies in neonates with sepsis, congenital heart disease, very low birth weight infants, hypoxic ischemic injury, infants who receive extracorporeal membrane oxygenation, and neonates with other critical illnessness who required admission to Neonatal Intensive Care Unit (NICU), suggest that Acute Kidney Injury (AKI) is common and could lead to worse outcomes. [8]

2. Definition of AKI in newborns

Acute kidney injury is a clinical condition characterized by a sudden alteration of the glomerular filtration with or without kidney structural anomalies underneath. It occurs clinically by a reduction in urinary output (oliguria, ie, <0.5 mL/kg/h), and/or with an alteration in renal function measured with an increase in serum creatinine (SCr). Studies have widely demonstrated that this condition noticeably affects the morbidity and mortality of newborns with low birth weight and could increase the number of days of recovery in a neonatal care intensive unit. [9]

The diagnostic and classification criteria of the Kidney Disease: Improving Global Outcomes (KDIGO) modified for newborns, proposed in 2013, has been a powerful tool for a better approach to the diagnosis of neonatal AKI, although it is still particularly difficult to apply to newborns, especially in premature ones. [10]

In fact, for example, after birth, the SCr in the newborn reflects maternal renal function and while the glomerular filtration rate rapidly increases, the SCr declines to approximately 0.4 to 0.6 mg/dL at about 2 weeks after birth. [11] In the premature infants SCr declines at varying rates over days to weeks depending on

gestational age. Thus, the natural physiology and immature handling of SCr by the newborn kidney render its trend difficult to interpret when assessing for AKI. Finally, SCr is not a surrogate for kidney injury, but rather for function. It seems to increase late, after as 24 to 48 hours the initial injury, and not before that at least 25% to 50% of renal function is lost. In addition, SCr does not differentiate the nature or timing of the kidney insult. Moreover, AKI diagnosis based on urine output is problematic because it is often clinically difficult to monitor in neonates, especially in preterms, and non-oliguric renal failure is common in premature infants. [12]

Although the unique neonatal renal physiology we have described, this classification, shown below in Figure 1, has been described by the National Institutes of Health Neonatal Workshop, as the currently best definition of neonatal AKI available and its use has been encouraged. [10]

Box 1
Proposed neonatal AKI classification

Stage	SCr	Urine Output
0	No change in SCr or increase <0.3 mg/dL	≥0.5 mL/kg/h
1	SCr increase ≥0.3 mg/dL within 48 h or SCr increase ≥1.5–1.9 × reference SCr[a] within 7 d	<0.5 mL/kg/h for 6–12 h
2	SCr increase ≥2 to 2.9 × reference SCr[a]	<0.5 mL/kg/h for ≥12 h
3	SCr increase ≥3 × reference SCr[a] or SCr ≥2.5 mg/dL or Receipt of dialysis	<0.3 mL/kg/h for ≥24 h or anuria for ≥12 h

[a] Baseline SCr is defined as the lowest previous SCr value.
Modified from Jetton JG, Askenazi DJ. Update on acute kidney injury in the neonate. Curr Opin Pediatr 2012;24(2):191–6.

Fig. 1 criteria neonatal AKI classification

3. New markers for AKI

Because of the previously described unreliability of SCr and urinary output for the diagnosis and classification of AKI, several serum and urinary markers for early detection have recently been investigated. A recent systematic review and meta-analysis published by Kuo J. et al. has been aimed to elucidate the diagnostic accuracy of urine and serum biomarkers not currently used in routine clinical practice to predict AKI in premature infants.

This systematic review and meta-analysis of biomarkers of AKI in premature babies suggests that serum Cystatin C (sCysC) and urine neutrophil gelatinase-associated lipocalin (uNGAL) show the highest diagnostic accuracy, although only in a limited number of small studies involving preterm newborns. [13]

Differently from serum creatinine, Cystatin C is a cysteine protease inhibitor which is constantly formed and excreted with no reabsorption by the renal tubules. [14] Moreover, sCysC is not influenced by sex, age or muscle mass and does not cross the placenta, therefore its concentrations in the immediate period after birth, are reflective of early neonatal kidney function, rather than maternal concentrations. [15]

There are four main obstacles to incorporating routine serum cystatin C testing: the cost, which is about ten times more expensive than SCr testing, accessibility, since its measurement is through particle-enhanced immunoassays, level of clinical awareness and understanding of the results, and more importantly, for what concern preterm newborns, measurements of sCysC still relies on collecting blood samples during the neonatal period. [16]

For this reasons, larger studies on urinary biomarkers are now considered more attractive in the clinical setting of NICU, primarily because of their noninvasive nature.

A systematic review by Kuo et al. found that urinary NGAL, a marker of renal tubular inflammation, was significantly increased in AKI compared to no-AKI controls showing the best diagnostic accuracy for AKI in premature infants with a high summary sensitivity and specificity.

NGAL gene expression, in fact, wide increase in the presence of inflammation and injured epithelia, and making NGAL one of the earliest proteins induced in the kidney after ischemic or nephrotoxic insult. Consequently, NGAL significantly increases in blood and urine soon after AKI. [17]

Other urinary markers such as osteopontin, epidermal growth factor, uromodulin, CysC, [TIMP-2].[IGFBP7], annexin A5, protein S100-P and metabotype, generally provide a reliable diagnostic value but however, further studies are required. [13]

Metabolomic analysis has shown that, a chemically complex fluid such as urine, can provide information on varying physiological states, metabolism signatures and functions. In addition, its collection is non-invasive turning urine into an easily accessible biological fluid to study. However, to analyze the metabolome in this biological fluid and identify signatures associated with different health and disease states it is necessary to have access to H nuclear magnetic resonance (H-NMR) spectroscopy and mass spectroscopy (MS), which could still be a limit in daily clinical practice. [18]

Despite the encouraging results shown by the multiple studies conducted on omics methodologies, especially in the past ten years, their clinical application for

neonatal health care still requires proper addressing of the inherent inter-individual variability. However, other studies are necessary to corroborate the results on biomarkers of renal injury with the purpose of improving earlier detection of the initial insult, of improving the prediction of short- and long-term outcomes and their validation on a larger scale. [19]

4. Incidence of AKI in preterm and IUGR

In a recent review published by Wu et al. it was shown that, from fifty studies analyzed, the overall rate of AKI in preterm infants was 25% with significant heterogeneity among the studies. Moreover, the rate of AKI among the subgroup with a gestational age lower than 32 weeks was 26%, whereas the extremely-low-birthweight (ELBW) infant subgroup, whose weight at birth is less than 1000 grams, showed a rate of AKI of 37%. [20]

There are not enough studies to review the incidence of AKI in IUGR newborns. A recent single-center study published by Sinelli et al. assessed an increased risk of 34% for AKI for severe IUGR. This could be explained by a significant kidney mass reduction and a poor kidney maturation observed in IUGR newborns as observed in IUGR newborn rats and as reported in autoptic studies. [21]

Moreover, in extremely preterm born infants, AKI appears to be more frequently associated with the treatment of a hemodynamically significant patent ductus arteriosus (PDA). This may be a result as an adverse effect of indomethacin administration which was used of a first-line therapy until 2016. Other risk factors, apart from low birth weight, include the coexisting presence of congenital heart

disease such as coarctation of the aorta, aortic arch interruption, hypoplastic left heart syndrome, and transposition of the great arteries, which can cause AKI because of systemic hypoperfusion. Early-onset sepsis, however, seems to be a minor risk factor in preterm and in term newborns. [22]

5. Increased risk of cardiovascular disease and chronic kidney disease (CKD) in preterm and IUGR

Barker was the first to theorize that a fetus who experiences suboptimal nutritional uptake during intrauterine development, as usually happens to Low Birth Weight (LBW) and/or IUGR newborns, may experience a genetic reprograming that subsequently alters fetal structure, function and may lead to metabolic changes. This hypothesis, called the FOAD (Fetal origin of adult disease) or thrifty phenotype or Barker's hypothesis or “developmental origins of adult health and disease” hypothesis (DOHAD), represents a mismatch between fetal life and neonatal life, thereby increasing the risk for cardiometabolic diseases. Hence low birth weight and/or IUGR, which can be read as markers of poor fetal growth, are linked to hypertension, diabetes, obesity, and insulin resistance. [23]

Later, Barker has demonstrated that low birth weight, birth weight less than 2500 grams, could lead to high blood pressure in adult life, and moreover, that lower the birth weight, higher is the risk of adult onset of hypertension. [24]

The mechanisms proposed for the association between low birth weight and hypertension were first, that an increased pressure in fetal circulation as a compensatory mechanism in maintaining placental perfusion might persist after

birth, and then that the intrauterine growth retardation causing low birth weight, may lead to accelerated postnatal growth and thereby, an accelerated rise in blood pressure. [25]

In 1988, Brenner theorized an inverse relationship between nephron number and hypertension. Hence, a low nephron endowment at birth, as described in preterm newborns and in IUGR, in association with age- or disease-related nephron depletion could predispose to the development of hypertension.

Furthermore, in order to limit the loss of renal function related to reductions in nephron number, residual nephrons compensate by increasing their glomerular surface. However, glomerular hypertrophy, chronically contributes to the development of hypertension due to increased sodium and fluid retention. These changes could lead to a vicious cycle of increases in arterial and glomerular capillary pressure, glomerular hyperfiltration, injury and sclerosis, all of which can promote systemic hypertension. [26]

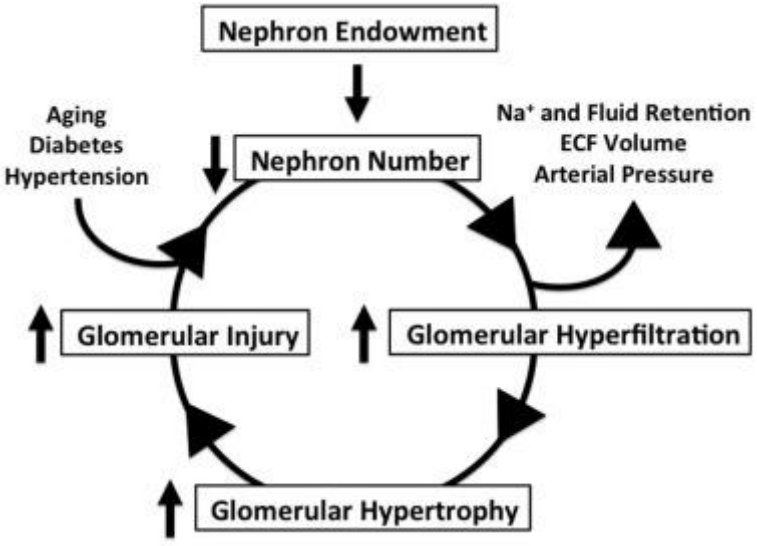

Fig. 2. Flowchart illustrating the mechanistic basis of the Brenner Hypothesis. [27]

Finally, a recent meta-analysis published by Yu et al. confirmed previous findings showing that individuals with low birth weight would have a higher risk of CKD in adulthood compared with those with normal weight.

Yu et al. also explained that there could be several explanations for the mechanisms underlying the inverse association between low birth weight and CKD. First, ulteriorly confirming the Barker hypothesis, they underlined that, due to adverse intrauterine exposure, the impaired development of kidney function and decreased number of nephrons, could lead to durable changes in the biological structures of the kidney and epigenetic modifications of gene expression, increasing the risk for cardiovascular stress and developing CKD in adult life. Another mechanism relies on pre-puberty accelerated 'catch-up' growth caused by intrauterine dysplasia, mostly expressed as low birth weight, which can cause a mismatch between kidney function and body surface area leading to increased hemodynamic pressure in the kidney. Besides, the cumulative damage to the kidney, caused by hypertension, cardiovascular and metabolic diseases that can occur in early life because of developmental programing, further increases the risk of nephrons loss and ultimately leads to CKD.

Finally, it is mandatory to remind that most of infants with extremely low birth weight often receive at least one nephrotoxic treatment before discharge, for example, gentamycin, indomethacin and vancomycin, which could contribute to damage the kidney function. [28]

6. Tendency to hypertension in prematurity: when does it start?

There are many complexities to the changing patterns of blood pressures (BPs) in the newborn period and it's known that contributory factors such as gestational age at birth, postnatal and postconceptional age, and appropriateness of size for gestational age must be taken into consideration to derive reference tables useful in clinical practice. [29]

Pejovic et al. have been evaluated the variation of BPs in the preterm and VLBW in the first month of life. As resulted in this study BP systolic, diastolic, and mean arterial blood pressure seems to progressively increase from birth to the first month of life in preterm infants more significantly than in term infants. The most predictive factors for this increase were gestational age and birth weight. As shown in the figure below (Fig. 3), the entire sample was divided into four subgroups ≤28 weeks, 29–32 weeks, 33–36 weeks, and ≥ 37 weeks, resulting in a more rapid rate of rise of BPs the lower the gestational age. [30]

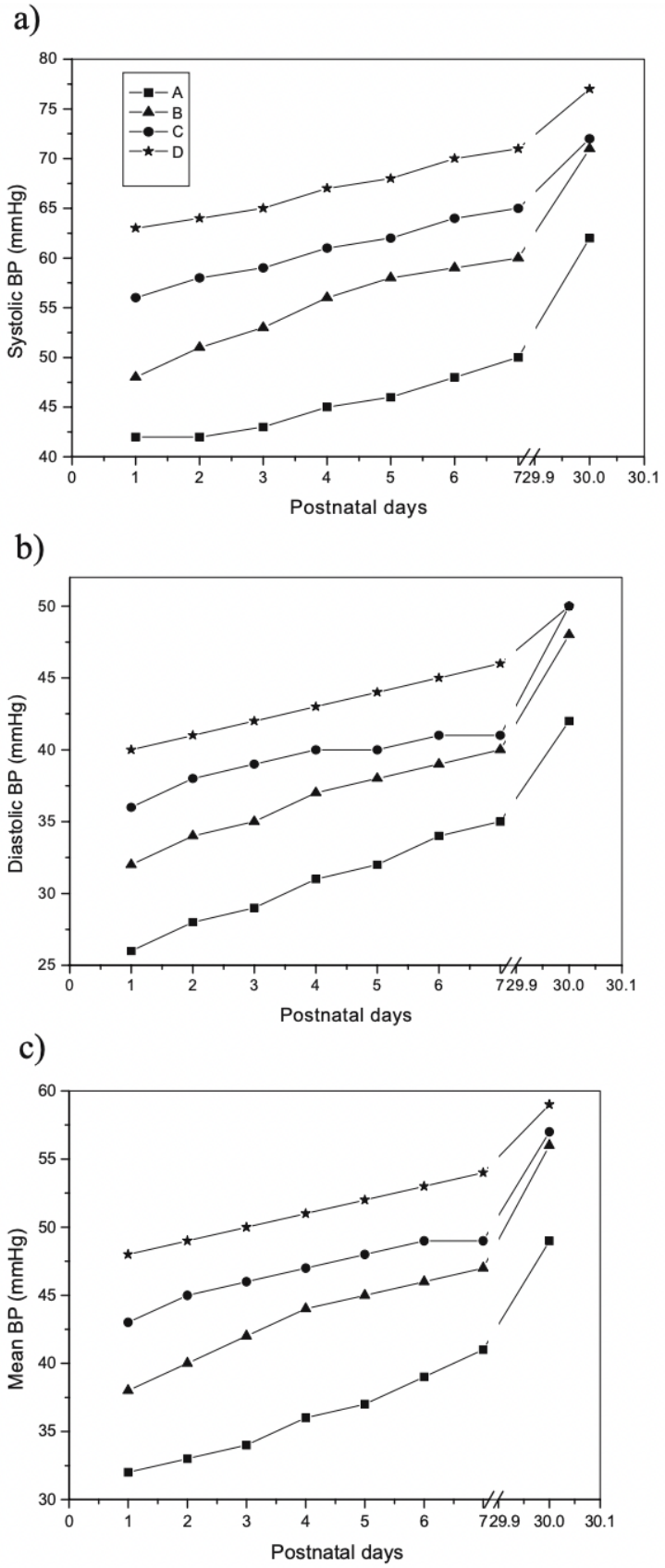

Fig. 3: Increase in systolic (a), diastolic (b), and mean (c) BP during the first month of life: ≤28 weeks (squares), 29–32 weeks (triangles), 33–36 weeks (circles), and ≥ 37 weeks (stars).[30]

To fill the lack of reference tables, Dionne et al. derived one of the estimated BP values after 2 weeks of age in infants from 26 to 44 weeks postconceptional (Table 1). Despite its value for clinical quick assessment, it should be noted that the results are based on the best synthesis of available data and are not the product of a prospective clinical study, which is still truly needed. [29]

Postconceptional age	50th percentile	95th percentile	99th percentile
44 Weeks			
SBP	88	105	110
DBP	50	68	73
MAP	**63**	**80**	**85**
42 Weeks			
SBP	85	98	102
DBP	50	65	70
MAP	**62**	**76**	**81**
40 Weeks			
SBP	80	95	100
DBP	50	65	70
MAP	**60**	**75**	**80**
38 Weeks			
SBP	77	92	97
DBP	50	65	70
MAP	**59**	**74**	**79**
36 Weeks			
SBP	72	87	92
DBP	50	65	70
MAP	**57**	**72**	**71**
34 Weeks			
SBP	70	85	90
DBP	40	55	60
MAP	**50**	**65**	**70**
32 Weeks			
SBP	68	83	88
DBP	40	55	60
MAP	**48**	**62**	**69**
30 Weeks			
SBP	65	80	85
DBP	40	55	60
MAP	**48**	**65**	**68**
28 Weeks			
SBP	60	75	80
DBP	38	50	54
MAP	**45**	**58**	**63**
26 Weeks			
SBP	55	72	77
DBP	30	50	56
MAP	**38**	**57**	**63**

Table 1 Estimated BP values after 2 weeks of age in infants from 26 to 44 weeks postconceptional age [29]

In another study by Bonamy et al., at 2.5 years of corrected age, ELBW children had significantly higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) than infants born at term, according to pediatric BP nomograms by age, gender, and height. In particular, the proportion of SBP ≥90th percentile was more significant among boys. [31]

Furthermore, has been recently shown that in preterm and VLBW infants seen at 1–3 years, obesity, elevated SBP, and low estimated glomerular filtration rate (eGFR) are observed. At 10-13 years, 45% were classified as overweight/obese, 48% had SBP ≥ 90th centile (77% considered hypertensive), and 34% had low eGFR (<90 mL min^{-1} [1.73 m^2]).[32]

Finally, it is widely known that adults born preterm at very low birth weight, have a major risk of increased blood pressure. Among those, higher values were found among women and those whose mothers had preeclampsia during pregnancy. These findings reveal the higher risk, especially in this subgroup, of coronary heart disease or stroke later in life, and confirm the necessity for clinicians, to strictly follow up to a precocious diagnosis and treatment of the hypertension. [33]

Thanks to all the studies conducted, especially in the last fifteen years, it is widely knowns that individuals born preterm have a major risk to develop higher blood pressure (BP) in childhood and adolescence. However, little is known about the onset of the initial deviation from the normal BP range, and moreover, data are still lacking for the new generation of survivors after preterm birth. [31]

Even though neonatal BPs have been measured for decades, clinicians are still in the early phase of identifying the normal patterns of infant BP, especially if born prematurely and/or with a low birth weight, and there are still many physiologic

changes that need further investigation before definitive reference data can be generated.[29]

7. Ultrasonography, a tool for diagnosis, treatment and follow-up of kidney diseases

Nephrons low endowment, defective capacity to filter waste products, inability to produce and concentrate urine, peripartum complications, drugs and long admission in NICU, make preterm newborns a risk category of patients for kidney diseases. Therefore, the evaluation of renal dimensions could be an effective tool for the diagnosis, treatment and follow-up of kidney diseases in this group. Ultrasonography is an easy, less expensive, non-invasive, could be performed bedside and it's a reliable technique to visualize and measure the renal dimensions rapidly without the risk of radiation. Moreover, since in the newborn period, renal size chenges with age and renal abnormalities are common, measurement of renal dimension could play an important role in the identification and follow-up of renal pathology, particularly in low-birth-weight infants, at major risk for kidney diseases. [34]

The mean range of renal dimensions at a given age was provided by Erdemir et al., who measured both longitudinal and anteroposterior dimensions of both kidneys in four hundred ninety-eight preterm newborns. Mean anteroposterior dimensions of both kidneys versus gestational age were plotted and are shown below in Figs. 4, 5, 6, and 7. The importance of this study relies easily on the fact that mean dimensions of the kidney must be known before an abnormality can be diagnosed. [35]

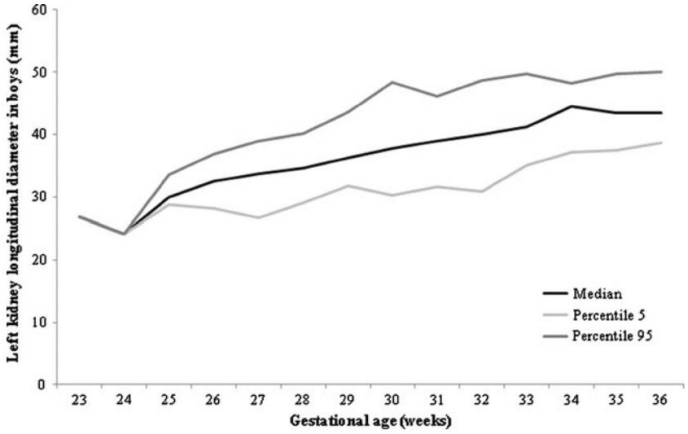

Fig. 4 Left kidney, longitudinal diameter in boys. Sonographic longitudinal dimension of the left kidney (mm) by gestational age based on the last menstrual period (weeks) in boys.

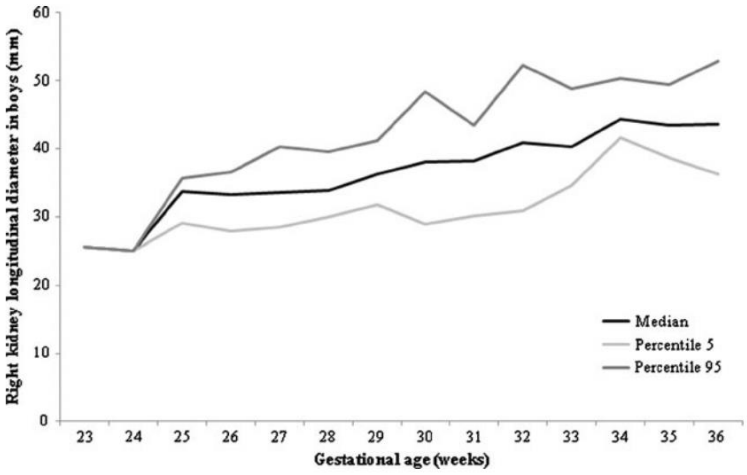

Fig. 5 Right kidney, longitudinal diameter in boys. Sonographic longitudinal dimension of the right kidney (mm) by gestational age based on the last menstrual period (weeks) in boys.

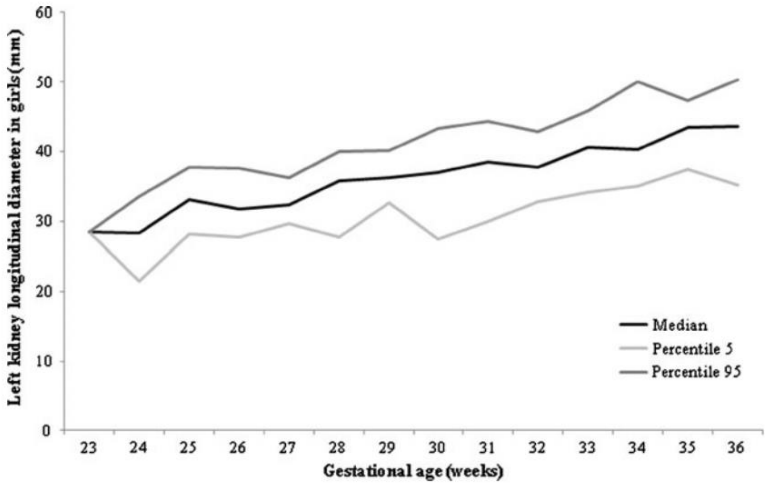

Fig. 6 Left kidney, longitudinal diameter in girls. Sonographic longitudinal dimension of the left kidney (mm) by gestational age based on the last menstrual period (weeks).

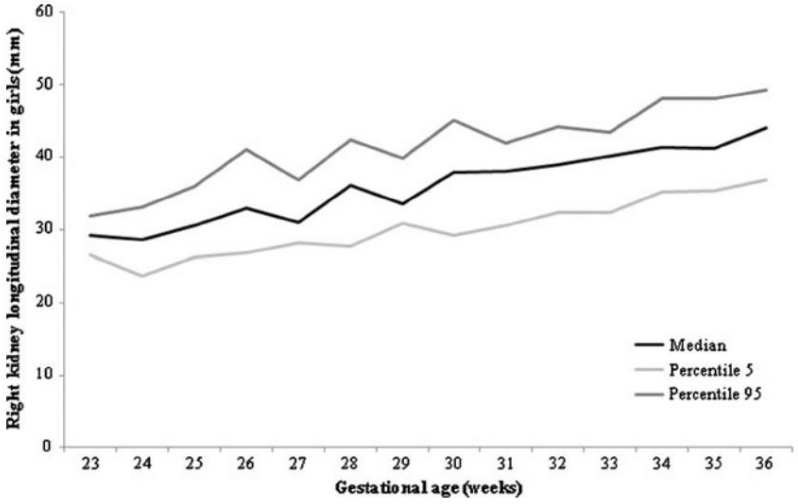

Fig. 7 Right kidney, longitudinal diameter in girls. Sonographic longitudinal dimension of the right kidney (mm) by gestational age based on the last menstrual period (weeks).

Recently, Ziauddeen et al., investigated the relationship between fetal kidney volume, function and blood pressure in children between 7 and 11 years of age. By first assessing kidney volume, in the third trimester of pregnancy and then, after 6 years, they affirmed that lower fetal growth was associated with lower childhood kidney volume and function independently of childhood growth. These findings confirm that reduced fetal growth influences birth weight and fetal kidney size and that this could have persistent effects on kidney development and function in childhood and may affect adult CKD risk.[36]

8. Importance of follow up and lack of a consensus management

Despite the growing of literature regarding neonatal AKI, it remains unclear how clinicians should incorporate these findings into practice. The Neonatal Kidney Collaborative (NKC) sought to explore differences in perceptions and practices among both neonatologists and pediatric nephrologists regarding the field of neonatal AKI, including diagnostic criteria, incidence, management, and follow-up.[37]

Puljiu et al. identified a high prevalence of renal insufficiency at a median age of 8 years in children born before 30 weeks' gestation. It has been shown that one-third of children with neonatal stage 1 AKI history had signs of renal insufficiency at follow-up, but this was not significantly different from children with no AKI history. This confirms that larger prospective studies are needed to investigate the impact of mild neonatal AKI on long-term renal outcomes in the ex-preterm population, determine the optimal timing and frequency of screening for CKD, and define

thresholds for nephrology referral and/or intervention. Moreover, it underlines that follow up of high-risk populations should incorporate multiple markers of renal function including blood pressure, urine protein excretion, and eGFR. [38]

The recent important findings such as the increasing use of staged neonatal AKI definitions, poor recognition of early AKI stages, the lack of SCr surveillance guidelines for nephrotoxic medications, and knowledge and practicalities around dialysis options in neonates, highlight the lack of renal follow-up of neonatal AKI. Collaboration in clinical care and research is required to improve the management and outcomes of neonatal AKI and to completely evaluate its long-term consequences. [37]

AIMS OF THE STUDY

As already widely explained, prematurity, low birth weight and IUGR are related to nephrogenesis abnormalities and could be related to CKD later in life.

For this reason, the primary aim of the study was to sensitize the awareness of AKI on the risk categories and to reduce its incidence through prevention strategies. With strict monitoring of the renal function routinary, and especially during critical events, it is possible to increase the awareness not only of risk populations but also of risk factors that could lead to AKI.

The secondary aim is to document eventual onset of AKI during the admission and to individuate early term complications such as arterial hypertension at discharge to develop a targeted clinical follow up for this category of patients by neonatologists and then, family doctors.

Third, is to assess the volumetric dimensions and the echogenicity of the preterm kidneys during the hospitalization with the aim to an early identification of eventual ultrasound abnormalities predictive of CKD. Furthermore, to assess renal dimensions in a large cohort of preterm newborns to generate reference tables of normal dimensions to be used in routine clinical practice.

The last aim of the study, retrospectively conducted, to find new urinary markers of AKI. Through the collection of urine samples at different times, related related to risk factors and adverse outcomes, and by metabolomic analysis, the aim of the study is to confirm the presence of already known urinary markers of AKI or to find new ones.

MATERIALS AND METHODS

This is a prospective observational monocentric study which will likely have the complex duration of 24 months.

From February 2023, we consecutively enrolled all the neonates with a gestational age lower than 34 weeks and with a birth weight lower than 2000 grams. All newborns who met these two inclusion criteria were born at IRCCS Giannina Gaslini, inborn, or born in regional peripheral centers, who were transferred to the Neonatal Intensive Care Unit within the first day of life, outborn.

At birth, we collected data about maternal and pregnancy anamnesis and events that occurred at peripartum such as: gestational age, birth weight, single or twin pregnancy, neonates small for gestation age (SGA), IUGR and history of maternal preeclampsia.

Blood tests to measure SCr and uremia were collected during the first day of life, after 48- 72 hours, at 7 days of life and at 30 days of life. These times of collection were chosen because of the clinical necessity in running other routine blood tests, aiming to avoid extra venous-or-capillary punctures. In case of acute events or clinical conditions suggestive for AKI, such as hypotension, necrotizing enterocolitis (NEC), shock and sepsis, another blood sample was collected.

Urinary output was measured daily, due to clinical necessity and to early detect the onset of AKI through the definition and classification of neonatal KDIGO.

An ultrasound to measure the longitudinal diameter of the kidneys, and to early discover eventual anatomical abnormalities, was performed within the first week of life, at 30 days of life, at term-equivalent age (TEA), at 3 months and at 6 months.

(Fig. 8) Other measurements at 12 months, 18 months and 24 months will be collected.

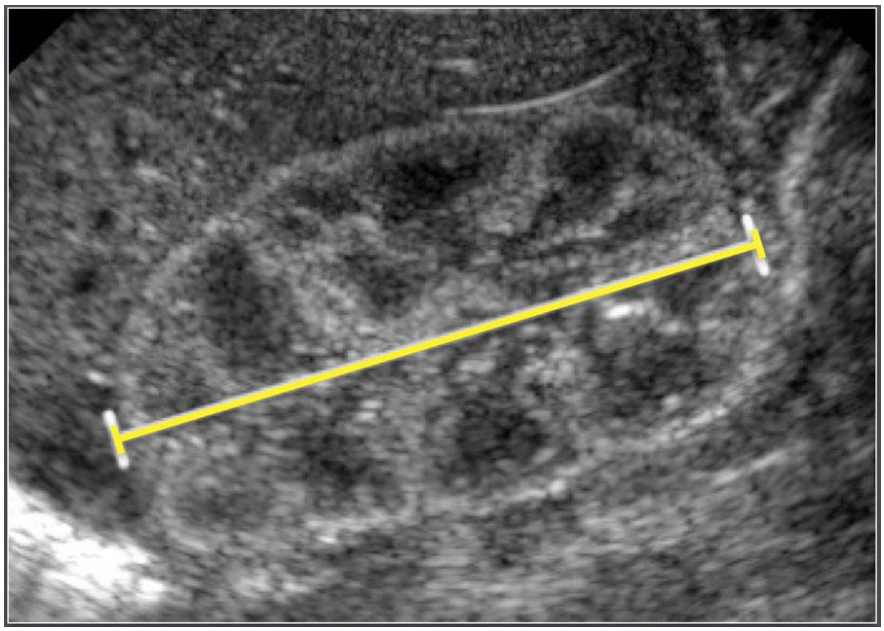

Fig. 8: kidney ultrasound longitudinal measurement

Arterial blood pressure (BP) was measured with a neonatal cuff placed at the right arm at term-equivalent age and correlated to the percentile blood pressure tables specified for age and sex, as a parameter of early renal outcome. BP and specifically, Mean Blood Pressure (MBP), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP), was also collected at 3, and 6 months of life during the follow up checkups. It will be measured at 12, 18 and 24 months.

Finally, urine sample was collected with a pediatric sterile collection bag at the first day of life, at 7 days of life, at 30 days of life and at TEA for each neonate. Right after collection, the urine samples have been refrigerated at -80°C and stored and will undergo metabolomic analysis through mass spectroscopy. This analysis has the

aim to confirm the presence of well-known urinary marker for AKI and to retrospectively look for others in defined categories of patients known to be at risk.

During the hospitalization we strictly monitored the eventual onset and reported clinical conditions at risk for acute kidney injury such as NEC, Patent Ductus Arteriosus, hypotension, sepsis and the consensual necessary administration of drugs with a toxic renal effect like vancomycin, gentamycin and furosemide.

To a clear comprehension, the design of the study is summarized in Tables 2.

	Serum creatinine	Urine sample	Kidney ultrasound	Blood Pressure
Day 1 (T0)	x	x		
Day 3 (T48)	x		x	
Day 7 (T7)	x	x		
Day 30 (T30)	x	x	x	
TEA (T37)	x	x	x	x
3 months (T3m)			x	x
6 months (T6m)	x		x	x
12 months (T12m)	x		x	x
18 months (T18m)			x	x
24 months (T24m)	x		x	x

Tab.2 time of collection of each sample or values for each neonate enrolled in the study.

1. Statistical analysis

Descriptive statistics were generated for the whole cohort; data were expressed as mean, standard deviation (SD), or median and inter-quartile range (IQR) for continuous variables, and absolute and relative frequencies for categorical variables.

Normality of distribution for all variables was assessed graphically or using the Kolmogorov-Smirnov R test, where appropriated. Comparisons of categorical variables among subgroups were carried out performing Fisher's exact tests, while for continuous variables a Mann-Whitney U test or a Student's t-test were selected.

A linear regression model was built to assess relationship between kidney dimensions and birth weight, results were expressed as Pearson's rho coefficients.

A p-value of < 0.05 was considered statistically significant, and all p-values were based on two-tailed tests.

Statistical analysis was performed using the Jamovi® project interface software, based on R language for statistical computing.

RESULTS

From February 2023, we enrolled 37 newborns who fulfilled the inclusion criteria of birth weight < 2000 grams and gestational age (GA) of < 34 weeks who managed to survive their severe condition of low birth weight and prematurity.

Of the 37 babies, 19 were girls and 18 were boys, 51% and 49% respectively, and 16 were couples of twins (43%). The median estimated gestational age at birth was 31 weeks, IQR 3, with a mean birth weight of 1388 grams $\pm$ 326. 7 babies had history of intrauterine growth retardation (19%) whereas only 4 were small for gestational age at birth (11%).

The cohort was divided in three groups based on birth weight: group 1 was represented by extremely low birth weight (ELBW), 7 premature neonates (19%) with a birth weight lower than 1000 grams, group 2 was represented by very low birth weight (VLBW), 17 newborns (46%) with a birth weight lower than 1500 grams, and group 3, represented by the remaining part, was represented by 13 neonates (35%) with birth weight within 1500 and 2000 grams.

Of 29 pregnancies, 8 were twin pregnancies (28%), only 1 was monochorionic diamniotic. 9 of the totals were complicated by maternal preeclampsia (31%), of these, and only 3 were twin pregnancies (33%). In all cases complicated by preeclampsia, maternal hypertension was one of the primary causes of premature birth.

During hospitalization critical events were recorded. Of the 37 patients, 12 (32%) had patent ductus arteriosus susceptible to treatment with paracetamol, 19 (51%) had signs of clinical sepsis and had been administered with vancomycin, 9 (24%)

had hypotension and needed hemodynamic support with inotropes, 1 patient (3%) had NEC and underwent surgery, 4, (11%) were diagnosed with retinopathy of prematurity (ROP) and 4 (11%) were diagnosed with bronchopulmonary dysplasia. All neonates were administered with gentamycin as prophylaxis for early-onset sepsis and necessary umbilical venous catheter placement.

FEMALE	19
MALE	18
IUGR	7
SGA	4
TWINS	16
PREECLAMPSIA	9
PDA	12
GENTAMYCIN	37
VANCOMYCIN	19
ROP	11
NEC	1
INOTROPES	9
BPD	4
ELBW (group 1)	7
VLBW (group 2)	17
LBW (group 3)	13

SCr values, expressed in milligrams over deciliter (mg/dl), blood arterial mean, systolic and diastolic pressure (MBP, SBP and DBP) expressed in millimeters of mercury (mmHg), and ultrasound longitudinal kidneys dimensions expressed in millimeters (mm) were collected for each patient at different times as explained in the section above. The mean value for each measure and the time of collection are summarized in the tables below. (Tab. 4 and 5)

	SCR (mg/dl)		MBP (mmHg)		SBP (mmHg)		DBP (mmHg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T0	0,81	0,14	\	\	\	\	\	\
T48-72	0,71	0,16	\	\	\	\	\	\
T7	0,57	0,11	\	\	\	\	\	\
T30	0,38	0,06	\	\	\	\	\	\
T37	\	\	56	8	76	6	43	9
T3m	\	\	66	8	82	10	50	6
T6m	\	\	71	5	86	5	55	8

Tab. 4 mean values and SD for SCr and blood arterial pressure collected at the defined time.

	Right Kidney longitudinal measure (mm)		Left Kidney longitudinal measure (mm)	
	Mean	SD	Mean	SD
T0	33,4	4,42	33,8	4,69
T48-72	\	\	\	\
T7	\	\	\	\
T30	35,7	4,08	35,6	4,66
T37	36,7	3,61	36,9	4,35
T3m	40,5	4,37	41,5	4,61
T6m	49	5,04	49,4	4,89

Tab. 5 mean values and SD for right and left kidney longitudinal measure collected at the defined time.

Serial collections of SCr values at birth, after 48 - 72 hours, at 7 days and at 30 days of life demonstrated what already discussed about the unreliability of the SCr to assess the neonatal renal function. With a SCr mean values of 0,81 $\pm$ 0,14, and 0,71 $\pm$ 0,16, respectively at birth and after 48 to hours, and SCr mean values of 0,57 $\pm$ 0,11 and 0,38 $\pm$ 0,06, respectively at 7 and 30 days of life, the reflection of maternal renal function for the first days of life and the SCr downward trend at least after 7 days of life could be demonstrated.

Another outcome was the confirmation of the statistically significant correlation between birth weight and kidney longitudinal dimensions, as shown in table 6. At every time and at every measurement, as expected, the lower the birth weight, the smaller the kidney longitudinal dimension.

	Longitudinal measure	R	p-value
T0	right kidney	0,7	< 0,001
	left kidney	0,6	< 0,001
T30	right kidney	0,7	< 0,001
	left kidney	0,5	< 0,001
T37	right kidney	0,5	0,005
	left kidney	0,4	0,004
T3m	right kidney	0,7	< 0,001
	left kidney	0,5	0,005

Tab 6. Correlation between birth weight and longitudinal dimensions of the kidney at every time of assessment

In Figure 9 is shown a graphic example of the correlation between birth weight and the longitudinal dimensions of the right kidney at 30 days of life.

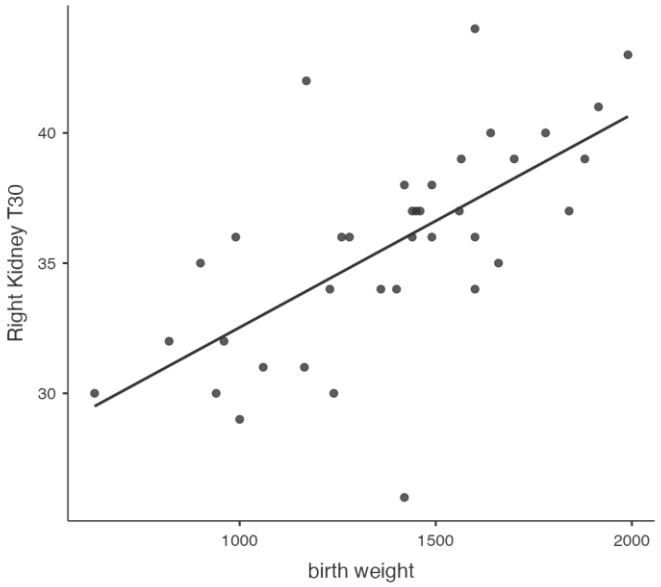

Fig. 9 linear regression showing the correlation between birth weight and longitudinal dimension of the right kidney at 30 days of life

Moreover, different from what was expected, there was no statistically significant correlation between the values of BP measured at term-estimated age, at 3 months and 6 months with birth weight, gestational age at birth, kidney longitudinal dimensions, and clinical acute events.

What has been demonstrated and shown in the Boxplot graphic below, is that being a twin seems to be a protective factor for an increase of BP, especially at 3 months. (Fig 10)

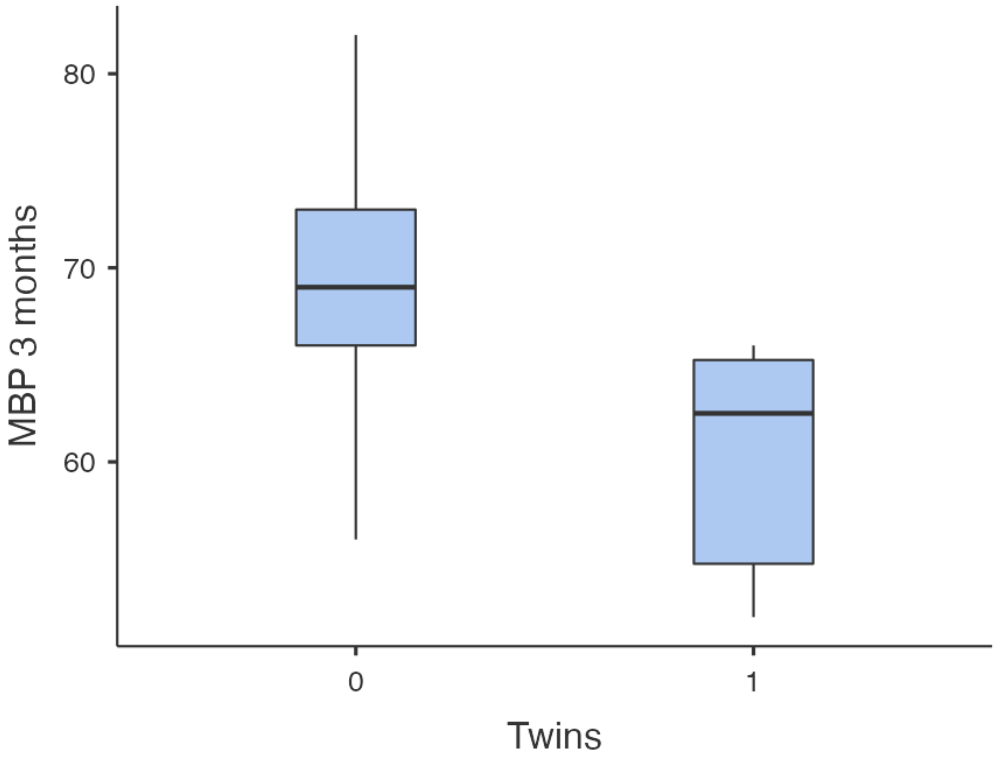

Fig 10. Boxplot showing median MBP at 3 months in neonates born from single pregnancies and twin pregnancies.

Furthermore, dividing the cohort of patients into two groups based on the value of SCr higher or lower than 0,38 mg/dl, mean value of the total cohort and value of estimated SCr at 30 days described by Allagaert et al. [39], it has been demonstrated an increase of the SCr values in the group of patients who have been administered with vancomycin due to clinical symptoms related to late-onset sepsis. This trend is shown in the graphic below (Figure 11).

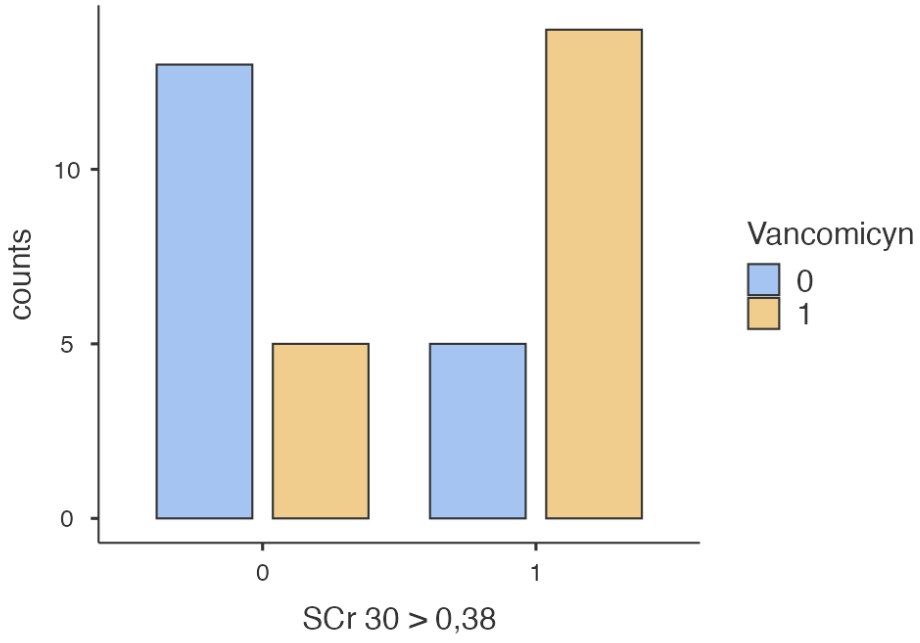

Fig. 11 number of patients showing an increase of SCr at 30 days related to the administration of vancomycin.

More, collecting for each patient serial kidneys dimensions, we have been able to create kidney length nomograms for groups 1, 2 and 3, respectively ELBW, VLBW and LBW. A graphic representation is shown below. (Fig.12, 13)

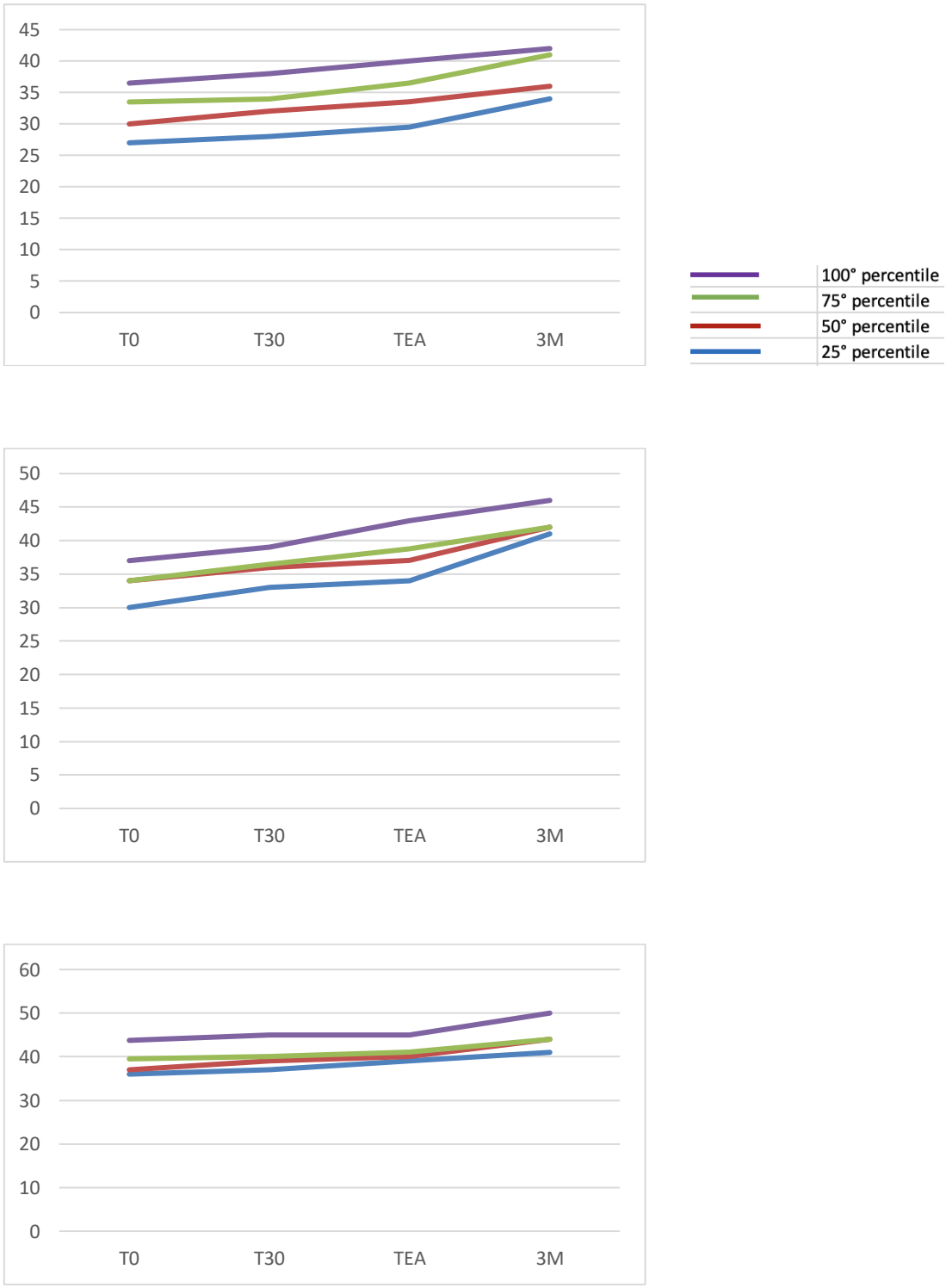

Fig. 12 right kidney length nomograms, express in millimeters, for group 1 (ELBW), group 2 (VLBW) and group 3 (LBW)

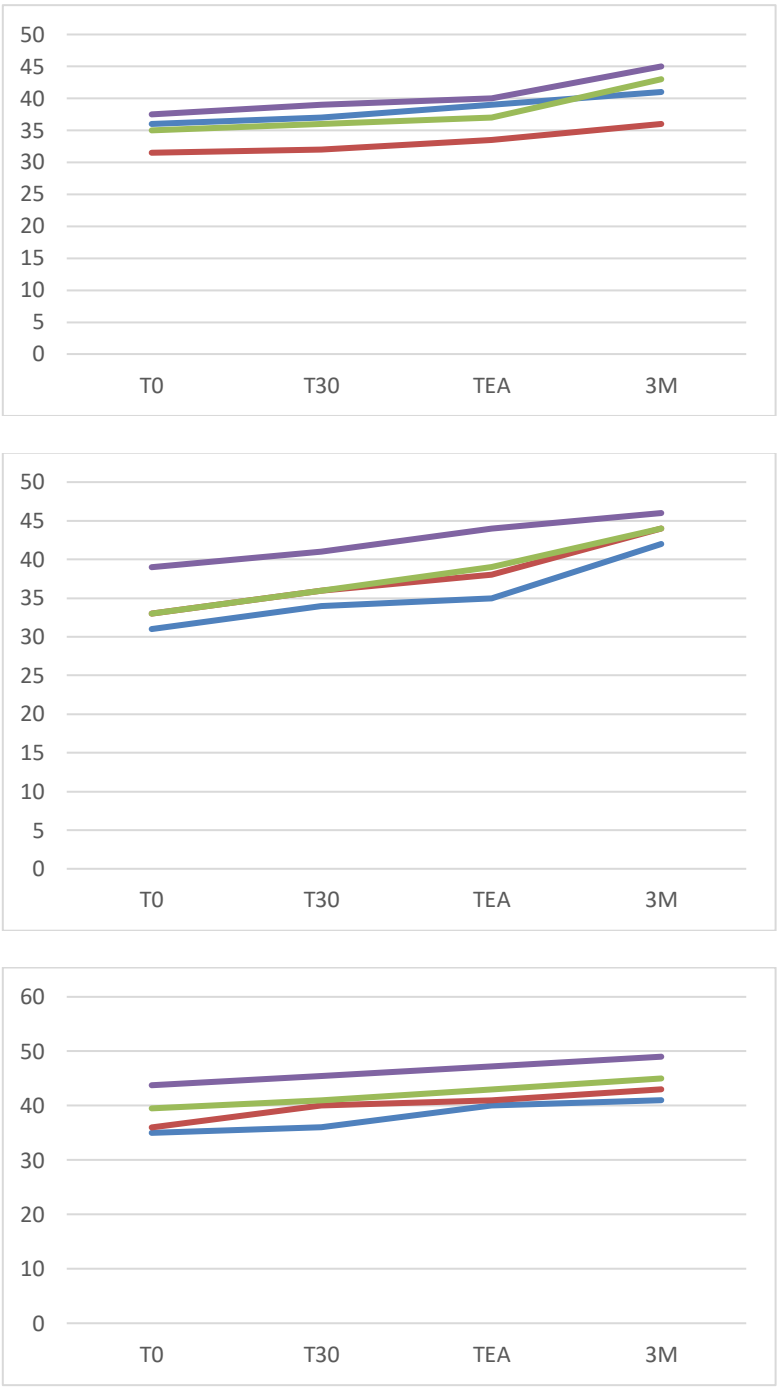

Fig.13 left kidney length nomograms, express in millimeters, for group 1 (ELBW), group 2 (VLBW) and group 3 (LBW)

Finally, out of 37 patients enrolled in the study, none has met the criteria of neonatal AKI defined by KDIGO, even though data about the values of SCr and the urinary output were collected during the onset of critical events or risk clinical conditions.

DISCUSSION

Preterm and IUGR newborns are at an increased risk of kidney injury because of their physiological immaturity and exposure to various stressors in the neonatal intensive care unit (NICU). Understanding the risk factors and long-term consequences and developing a multidisciplinary approach for early diagnosis are crucial for the well-being of these vulnerable infants.

In terms of kidney dimensions, preterm and IUGR neonates often have smaller and less mature kidneys, which can contribute to their vulnerability to AKI. Smaller kidneys may have reduced functional capacity, and therefore premature birth, nephron development is not yet complete, and their number may be lower than that in full-term infants. This can affect the kidney's ability to filter waste products and maintain fluid and electrolyte balance, making them more susceptible to injury. [1]

As shown in this study, at every time and at every measurement, as expected, the lower the birth weight, the smaller the kidney dimensions, increasing the necessity of awareness for the risk of AKI not only in preterm but also in IUGR neonates. Given the lack of correlation between the dimensions of the kidneys and the gestational age it is important to reiterate the concept that birth weight is the primary factor that influences the kidney development and hence, the associated risk of AKI.

Serum creatinine (SCr) is a commonly used biomarker for assessing kidney function in adults and older children, but its reliability can be limited in preterm infants, particularly as a sole indicator of kidney function. Its limitations are due to their

physiological immaturity, low muscle mass, administration of nephrotoxic medications and postnatal fluid imbalance due to clinical reasons. [11], [12]

For this reason, is necessary to employ a combination of clinical judgment, urine output, other laboratory markers such as serum Cystatin C, and more specific neonatal formulas to accurately assess kidney function and detect kidney injury in preterm infants. [16]

Though, given that the cost of the dosage of sCysC is ten times higher than the routinary SCr and its analysis is not available in all laboratories, the values of SCr is still the primary tool to assess renal function. Its increase from the mean estimated normal value at 30 days, described by Allagaert et al. and confirmed in our cohort, has been used to define if the administration of vancomycin could be a risk factor for increase SCr. Given that the increasing of SCr itself could not define AKI, as reported in the neonatal classification of AKI by KDIGO, this study confirms, as recently stated by Authors, the increase of SCr in the neonates that have been administered with vancomycin. [40]

Moreover, hypertension and AKI can be interconnected, and they may have long-term implications for an individual's health**.** Hypertension can be both a cause and a consequence of AKI, since pre-existing hypertension can increase the risk of AKI, and AKI can lead to hypertension as a long-term consequence. Moreover, when AKI occurs, it can result in kidney damage and impaired kidney function affecting blood pressure regulation and potentially leading to hypertension.

The outcomes can vary based on the severity of AKI, the underlying causes, and individual factors. Early detection, appropriate management, and regular follow-up

with healthcare providers are essential for improving long-term outcomes and reducing the risk of complications.

As emphasized by Authors, systolic, diastolic, and mean arterial blood pressure seems to progressively increase since birth to the first months of life in preterm more significantly than in term infants. Unexpected and not described earlier, we noticed that being born by a twin pregnancy significantly reduces the risk of higher blood pressure at 3 months of life. Although further studies will be necessary to corroborate these disclosures, we believe that it could be related with the incidence of preeclampsia in our cohort of patients. Of 29 pregnancies, 9 were complicated by maternal preeclampsia (31%), and of these, only 3 were twin pregnancies (33%), which is odd, since preeclampsia is known to be more common in multiple pregnancies. [41]

In fact, preeclampsia itself is a risk factor for low birth weight,[42] and low birth weight has been demonstrated to be one of the most predictive factors for increasing blood pressure in the first months of life. [30] As already said, other studies are necessary to corroborate these findings.

Even though with a very smaller cohort of patients than enrolled by Erdermir et al.[35], we hope that at the end of the 24 months, we will be able to provide a more updated range for ultrasound renal dimensions in preterm infants that could assess the kidney diameter not only until discharge, but also through their follow up as ex-preterm neonates.

The limitations of the study are the small cohort of patients and, because of their premature infants status, the related difficulties of obtaining a punctual and non-invasive method to define urinary output. For this reason, and the unavailability of a

better marker for AKI than SCr, none of the neonates enrolled in our study have fulfilled the neonatal KDIGO criteria for AKI during the days of hospitalization.

Moreover, the assessment of arterial blood pressure with a non-invasive method, such as the neonatal cuff, could be affected by the momentous discomfort that could lead to slight physiological increase in blood pressure and heart rate in such small infants.

Finally, it's important to note that the long-term outcomes for preterm infants with AKI can vary widely, and not all individuals will experience severe or lasting effects. Since preterm and IUGR are risk categories for AKI, it is also necessary to consider that even with the absence of laboratory and clinical signs of AKI, a close and multidisciplinary follow up is still needed to improve long-term outcomes and to reduce the risk of future complications. The specific approach to follow-up care must depend on the individual's health status and unique needs and must be discerned at every clinical control.

FUTURE PERSPECTIVE

A complex of 100 urine samples was collected and refrigerated at -80°C degrees. 36 samples were collected at birth (97%), 24 samples were collected at 7 days of life (65%), 23 samples were collected at 30 days of life (62%) and only 17 (46%), were collected at term-estimated age. The downward trend in collection showed is related to the necessity to transfer the neonates, once obtained adequate gestational age and proper clinical conditions, to other second level neonatology departments due to the necessity of admitting more critical patients.

	Urine sample
T0	36
T7	24
T30	23
TEA	17
Total	100

These samples will undergo metabolomic analysis with the aim of confirming the presence of already known urinary markers for AKI [13], [17] or finding new ones, based on the record of clinical acute events, risk factors, and on the follow up of arterial blood pressure and ultrasound kidney dimensions.

Even though the lack of widespread knowledge and the possibility of running these types of tests is still far from routine clinical practice, is has been demonstrated that the use of these urinary markers should be part of a comprehensive approach to assess kidney function in preterm infants, to improve an early detection of AKI, and to guide appropriate interventions and management in neonatal intensive care units. [19]

CONCLUSIONS

As a risk factor for developing AKI and its short- and long-term consequences, lower kidney dimensions relate with a lower birth weight, empathizing the awareness of IUGR, not only for preterm infants, as a category at risk of developing AKI. The fact that none of the neonates enrolled in the cohort have met the criteria for neonatal AKI could be an ulterior confirmation of the necessity for preterms of more punctual and specific markers for AKI, which would reflect kidney injury, and not its function.

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