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Review

Assessment of chemotherapy-associated nephrotoxicity in children with cancer

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Summary. Assessment of the toxicity caused by chemotherapy in children with cancer has become more important as the number of long-term survivors has continued to increase. It is vital to monitor both acute life-threatening adverse effects and long-term toxicity that may impair the child's development and cause permanent morbidity. Renal damage may follow treatment with cytotoxic drugs, especially cisplatin or ifosfamide, and lead to glomerular, proximal tubular or distal tubular impairment or to any combination of these. Greater understanding of nephrotoxicity and of its prevention may enable the use of more intensive schedules or of higher doses of potentially nephrotoxic chemotherapy. However, the evaluation of cytotoxic drug-induced nephrotoxicity has frequently depended mainly on measurement of the plasma creatinine concentration, which may remain normal despite substantial glomerular impairment or severe tubular dysfunction. Detailed assessment of nephrotoxicity depends on an understanding of normal renal physiology and requires evaluation of all aspects of function. A comprehensive but simple investigatory protocol that enables assessment of the nature and severity of nephrotoxicity in children is described, which can be performed without admission to hospital. *Glomerular function* is assessed by measurement of the glomerular filtration rate from the plasma clearance of [^{51}Cr]-ethylenediaminetetraacetic acid ([^{51}Cr]-EDTA). *Proximal nephron function* is evaluated in three ways: by measurement of the concentration of calcium, magnesium, phosphate, glucose and urate in blood and urine along with calculations of their fractional excretion and of the renal threshold for phosphate; by determination of the excretion in urine of low-molecular-weight proteins (e.g. retinol-binding protein); and by investigation of urinary bicarbonate excretion in patients who are acidotic. *Distal nephron function* is initially investigated by examination of the concentration (osmolality) and acidification (pH) of an early morning sample of urine. Finally, a group of general investigations is performed, including quantitation of urinary excretion of renal tubular enzymes (e.g. *N*-acetylglucosaminidase) and measurement of blood pressure.

Introduction

Over the last 30 years the introduction of effective chemotherapy, allied to major advances in surgery, radiotherapy and supportive care, has led to a great improvement in the prognosis of children with malignancy. However, in recent years there has been increasing awareness of the narrow therapeutic index of many cytotoxic drugs and of the need to balance their efficacy and toxicity, especially in children who show potential for long-term survival. Nevertheless, some children are cured of their tumour only at the cost of permanent severe and disabling side effects. Both cisplatin and ifosfamide, widely used in paediatric oncology, may cause severe renal damage, which may be permanent and ultimately require dialysis or renal transplantation.

To manage drug-induced nephrotoxicity in individual children or to reduce the frequency and severity of such damage associated with certain cytotoxic drugs or treatment regimens, it has been necessary to develop a comprehensive and sensitive investigatory protocol that provides functionally meaningful information whilst remaining simple enough to perform in children on a repeated basis. Since chemotherapy may damage any part of the nephron, it is especially important that each of the three principal aspects of renal excretory function – glomerular, proximal tubular, and distal tubular function – be evaluated. There have been few attempts to investigate all aspects of renal function in one protocol. In this paper, a 5-h protocol is described that can be performed in outpatients and enables both a distinct evaluation of glomerular, proximal and distal tubular function and an assessment of the severity of damage to each of them.

In the past, the reporting of toxicity due to chemotherapy has frequently applied the World Health Organisation

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(WHO) recommendations for grading of acute and subacute toxicity [54]. For nephrotoxicity these comprise the measurement of blood urea or creatinine and the quantitation of proteinuria. For example, grade 0 nephrotoxicity is defined as a blood creatinine value of ≤ 1.25 times the upper limit of normal for the study population, whereas a blood creatinine level of >10 times normal constitutes grade 4 renal damage. Unfortunately, these criteria give an inadequate picture of nephrotoxicity, especially in children, in whom the blood creatinine concentration is a particularly insensitive measure of early renal failure. In particular, a substantial reduction in glomerular filtration rate (GFR) or severe renal tubular impairment can occur in the presence of grade 0 nephrotoxicity. The original recommendations were not intended to be all-inclusive, and it was recognised that "investigators will undoubtedly need to add some toxic manifestations" [54].

Assessment of nephrotoxicity

The kidney's two main functions are to regulate the body's fluid and electrolyte balance and to excrete waste products of metabolism. The need for separate evaluation of glomerular, proximal and distal tubular function arises from the different roles served by each of these functional units of the nephron. *Glomerular filtration* leads to the formation of an "ultrafiltrate", which then enters the *proximal nephron*, where it is progressively modified by the processes of tubular secretion and reabsorption. Finally, concentration and acidification of the ultrafiltrate occur in the *distal nephron*, resulting in the formation of urine.

In terms of tubular reabsorption and secretion, the proximal nephron can be considered to include both the *proximal convoluted tubule* and the *loop of Henle*. For some substances, including calcium and phosphate, the majority of reabsorption occurs in the proximal convoluted tubule [79], whereas for others, notably magnesium, most reabsorption occurs in the loop [62]. Likewise, in functional terms, the distal nephron consists of the *distal convoluted tubule*, the *collecting tubule* and the *collecting duct*.

The kidney has other regulatory functions, being an endocrine organ as well. It plays a central role in the maintenance of bone homeostasis by regulating the urinary excretion of calcium and phosphorus and is the site of conversion of 25-hydroxy vitamin D to the more active 1,25-dihydroxy form. Hormones produced by the kidney include erythropoietin and renin.

There have been numerous studies of the renal damage caused by cytotoxic treatment, with several reviews appearing recently [27, 29, 48, 63, 64]. However, the incidence, nature and severity of toxicity occurring after therapy with several drugs or treatment regimens remains unclear. In particular, there have been relatively few detailed studies in children. Perhaps the most frequent limitation of previous studies has been a failure to evaluate all aspects of renal excretory function; indeed, the statement that "no nephrotoxicity was seen" has often been based on an assessment of either glomerular or tubular function alone. Furthermore, this omission has often been compounded by the use of insensitive tests, such as measurement of plasma creatinine concentration. Renal tubular damage has frequently been evaluated by observation of the biochemical end-result, e.g. a low plasma phosphate value accompanied by a high urinary concentration. Few studies have attempted to assess the underlying abnormality in tubular handling by investigating the relationship of the blood and urinary concentrations of the substance under study. Distal tubular function has seldom been specifically investigated, despite the serious consequences that may result from damage to this part of the nephron.

When used alone, sensitive methods for the assessement of renal dysfunction may reveal subclinical renal damage but fail to clarify its clinical relevance. This may be illustrated by the finding of raised urinary excretion of *N*-acetylglucosaminidase (NAG, an extremely sensitive indicator of tubular nephrotoxicity), which does not necessitate medical intervention unless it is accompanied by evidence of more severe renal damage as shown, for example, by phosphaturia and hypophosphataemia or by a high plasma creatinine value. It is difficult to compare the results of a study in which measurement of urinary NAG is the only assessment of renal damage with those of another study using plasma creatinine levels. These two investigations display vastly different sensitivities, and abnormal results have different functional implications; they are best performed together rather than in isolation.

The philosophy underlying the investigatory protocol described in this paper is the need to study all aspects of renal excretory impairment by separate and comprehensive evaluation of glomerular, proximal and distal renal tubular damage. The aim of a standardised protocol is to enable uniform and comparable investigation of renal function in children receiving cytotoxic drugs or, indeed, other potentially nephrotoxic drugs. The protocol is also applicable to

Table 1. Investigation protocol – functional aspects

Glomerular:
GFR ([^{51}Cr]-EDTA plasma clearance)
Proximal tubular:
Blood – sodium, potassium, chloride, bicarbonate, urea, creatinine, calcium, ionised calcium, magnesium, phosphate, glucose, urate
Urine – sodium, potassium, chloride, creatinine, calcium, magnesium, phosphate, glucose, urate, with calculation of the fractional excretion of sodium, calcium, magnesium, phosphate, glucose, and urate and of the renal threshold of phosphate
Urine – low-molecular-weight proteins
Urine – bicarbonate (in acidotic patients)
Distal tubular:
Early morning urine osmolality and pH
DDAVP test (consider in patients with low urine osmolalities)
Assessment of renal control of acid-base balance (consider in patients with high urinary pH or with acidosis – see Fig. 1)
Bone chemistry:
Blood – alkaline phosphatase activity
General:
Urine – renal tubular enzymes
Blood and urine – albumin, protein
Urine analysis and microscopy
Blood pressure

Table 2. Investigatory protocol

GFR measurement:
[^{51}Cr]-EDTA plasma clearance

Early morning urine sample (10 ml):
The first morning urine passed at home on the day of investigation is collected and brought to the hospital. It should be collected in a sterile container but does not need to be kept on ice. In hospital the sample is divided as follows:
- pH
- osmolality
- low-molecular-weight proteins
- renal tubular enzymes

Blood sample (5 ml):
This can be collected when the [^{51}Cr]-EDTA is injected and then divided as follows:
- sodium, potassium, chloride, bicarbonate, urea, creatinine
- calcium, magnesium, phosphate, albumin, protein, alkaline phosphatase
- ionised calcium
- glucose
- urate

Urine sample (10 ml):
This should be collected as soon as possible after the blood sample and divided as follows:
- sodium, potassium, chloride, creatinine
- calcium, magnesium, phosphate
- glucose
- albumin, protein
- urate
- sample for urinalysis (using urine-testing reagent strips) and microscopy

General:
Blood pressure should be measured

adults. The individual components are described below and listed in Table 1. The samples required are outlined in Table 2. Non-excretory aspects of renal function are not investigated in this protocol.

Glomerular function

Measurement of the plasma clearance of radioisotopes has become widely accepted as the optimal method of measuring GFR in clinical practice. The isotope is chelated to a small molecule that is freely filtered at the glomerulus, with no subsequent tubular reabsorption or secretion. After an intravenous bolus of the chelate, the rate of disappearance of radioactivity from plasma is a reflection of the chelate's plasma clearance and, hence, of the GFR [15]. The rate of clearance of ethylenediaminetetraacetic acid labelled with ^{51}Cr ([^{51}Cr]-EDTA) from plasma is widely used. This technique involves the intravenous injection of a bolus of [^{51}Cr]-EDTA and measurement of radiolabel activity in three blood samples taken at intervals between 1 and 4 h later. The clearance of EDTA is then calculated using a single exponential pharmacokinetic model, giving the GFR.

The accuracy of the technique has been confirmed in children [14]. However, it is best avoided in the presence of oedema or substantial fluid collection, such as ascites or a pleural effusion, due to potential inaccuracies arising from delayed equilibration of the chelate within its volume of distribution. It is safe, delivering less radiation than that received daily from natural sources [14]. The technique is generally available in centres treating children with cancer, although practical details may differ slightly, and is widely used for monitoring the GFR in children receiving cisplatin.

Proximal tubular function

Fractional excretions and the renal threshold for phosphate. Calculations of the fractional excretion of glucose, calcium, magnesium, phosphate and urate and determination of the renal threshold for phosphate provide an assessment of proximal tubular function. The fractional excretion of a substance represents the percentage of the filtered load at the glomerulus that is subsequently excreted in the urine. In the absence of tubular secretion, this indicates the degree of tubular reabsorption of the substance – the higher the fractional excretion, the lower the reabsorption. For many substances, including glucose, calcium, magnesium and phosphate, most of this reabsorption occurs in the proximal nephron [10]. For other substances such as urate, which may undergo both reabsorption and secretion, the fractional excretion gives an indication of net tubular handling. The fractional excretion (FE) of a substance "a" is calculated as [9]:

$$\mathrm{FEa} = \frac{\mathrm{Ua}}{\mathrm{Pa}} \times \frac{\mathrm{Pcr}}{\mathrm{Ucr}} \times 100\%,$$

where *P* represents plasma concentration; *U*, urinary concentration; and *cr*, creatinine.

However, the fractional excretion of phosphate is dependent on the plasma phosphate concentration, due to the presence of a renal threshold for phosphate [79]. This is analogous to the renal threshold for glucose – when the plasma concentration of phosphate exceeds the renal threshold, the renal tubules can no longer reabsorb all of the filtered load and phosphate escapes into the urine. The fractional excretion of phosphate rises with increasing plasma concentration, and vice versa. This is reflected in the wide range of values for fractional excretion seen in healthy individuals (up to 20%). In the presence of hypophosphataemia with normal renal tubular conservation, the fractional excretion should approach zero. Conversely, in hyperphosphataemia, as the plasma phosphate concentration rises progressively above the tubular threshold, urinary phosphate excretion and its fractional excretion rise correspondingly.

The renal threshold for phosphate provides a measure of tubular phosphate reabsorption that is independent of the plasma phosphate concentration [7]. It is not affected by diet [77] and can be measured in the non-fasting patient. It can be quantified by the maximal rate of tubular phosphate reabsorption divided by the GFR (Tm_p/GFR). This may be calculated as [12]:

$$Tm_p/GFR = Pp - \frac{Up \times Pcr}{Ucr},$$

where *P* represents phosphate.

There is no convincing evidence for the existence of a renal threshold for calcium or magnesium, but it would appear unwise to attach undue importance to a high fractional excretion of either of these ions in the presence of a normal or high plasma concentration. However, when the plasma concentration of either of these ions is low, the normal physiological response leads to tubular conservation [79]. A high fractional excretion is inappropriate in such circumstances. Calculation of the fractional excretion of sodium may be of value in some circumstances, although the interpretation of abnormally high values is complicated by the complex physiology of tubular sodium reabsorption.

As the blood and urine samples used for calculation of a fractional excretion and of the renal threshold for phosphate should obviously correspond to each other a "spot" urine sample should be collected as soon as possible after the blood has been taken. A timed urine collection is not necessary. In calculations of the fractional excretion of calcium, plasma ionised calcium is taken to represent the plasma concentration of ultrafiltrable calcium (i.e. the concentration of non-protein-bound calcium in the blood that can be freely filtered at the glomerulus). In the case of magnesium, because plasma ionised magnesium is not routinely measured, it is assumed that 80% of plasma magnesium (P_{Mg}) is ultrafiltrable [79] and 0.8 P_{Mg} is used in the formula. More than 90% of plasma phosphate is filtered at the glomerulus [52], whereas glucose and urate are freely filtered [33]. Therefore, plasma phosphate, glucose and urate are all assumed to be 100% ultrafiltrable.

Low-molecular-weight proteins. Amino acids and certain low-molecular-weight proteins, including β_2-microglobulin (β_2-M) and retinol binding protein (RBP), are freely filtered at the glomerulus and nearly completely reabsorbed in the proximal tubule [10, 56]. A relatively small reduction in the degree of this reabsorption leads to a proportionately much greater increase in urinary excretion [56]. Therefore, measurement of the urinary excretion of these substances offers a sensitive indication of proximal tubular dysfunction. Expression of the protein concentration in ratio to the creatinine concentration of the same sample enables the use of a random "spot" sample of urine rather than a timed sample. However, it is wise to collect the sample at a constant time of day, for example in the morning, to avoid the potential effects of diurnal variation in protein excretion. Urinary β_2-M excretion is highest at this time of day [47].

Urinary bicarbonate excretion in proximal renal tubular acidosis. Hyperchloraemic metabolic acidosis may be due to either proximal renal tubular acidosis (RTA), distal RTA, or other non-renal causes. In healthy individuals urinary excretion of bicarbonate is not observed when the plasma bicarbonate concentration is below the "renal bicarbonate threshold". Although the value of this threshold varies slightly from person to person and tends to be lower in infants [25], the presence of bicarbonaturia when the plasma bicarbonate level is <20 mmol/l implies abnormal urinary loss of bicarbonate due to impairment of proximal tubular reclamation [10]. The urine container should be filled completely to the brim to avoid evaporative loss of bicarbonate as carbon dioxide. Conversely, in the presence of a plasma bicarbonate concentration of $\geqslant$20 mmol/l ($\geqslant$18 mmol/l in infants), it can be assumed that there is no significant disorder of proximal tubular handling of bicarbonate.

Distal tubular function

The first urine passed in the morning is collected at home for measurement of osmolality and pH. It has been shown that fluid restriction for 20 h in 250 children aged from 2 to 16 years resulted in urinary osmolalities ranging from 873 to 1,305 mosmol/kg [23]. Although there was a significant correlation between age and urinary osmolality, the above range was considered to describe the whole group adequately. However, newborn babies have a lower capacity to concentrate urine and during the 1st year of life there is a rapid increase in the maximal urinary osmolality that can be achieved [60]. As prolonged fluid deprivation is undesirable, indeed, unacceptable to many children, a more practical alternative of measuring the osmolality of an early morning urine specimen is used in this protocol. Pilot studies have indicated that an osmolality of $\geqslant$600 mosmol/kg reflects a normal distal tubular concentrating ability in children. Further studies of early morning urinary osmolality in normal children are in progress to validate this investigation. A urinary pH of $\leqslant$5.4 indicates adequate distal tubular acidification of urine [20]. Although such a pH may be seen in a urine specimen passed at any time, it is more likely to be obtained in an early morning urine specimen.

The failure to achieve an osmolality of $\geqslant$600 mosmol/kg or a pH of $\leqslant$5.4 does not necessarily imply distal tubular impariment, however, but merely reflects a failure to *prove* that distal tubular function is normal. The likelihood of reaching these target values is increased by the introduction of a period of fluid deprivation, such as overnight, before the urine is collected. However, prolonged fluid deprivation is potentially dangerous, especially in patients with polyuria. Collection of the second urine sample passed in the morning after overnight fasting and before breakfast or fluid intake will probably provide the best opportunity to obtain maximal concentration of urine without using a formal fluid-deprivation test. If even repeat specimens fail to exhibit an osmolality of $\geqslant$ 600 mosmol/kg or a pH of $\leqslant$5.4, further investigation may be indicated (see below).

Urinary concentration. When a urinary osmolality of $\geqslant$600 mosmol/kg has not been obtained in any of several specimens collected after overnight fluid deprivation, urinary concentration can be assessed further with a DDAVP test, which avoids the need for a formal fluid-deprivation test. DDAVP (desmopressin, a synthetic analogue of vasopressin) shows a primarily antidiuretic action with very little vasopressive effect, which enables its use in diagnostic procedures [39]. It is given intranasally on the morning of the test at a dose of 10 μg in infants (<1 year of age) and 20 μg in older children, and consecutive urine samples are

collected for 5 h. The fluid intake of babies should be halved for their next two feeds after the administration of DDAVP so as to avoid the possiblity of water overload, but there is no need for fluid restriction in older children. In the presence of normal distal tubular function, this will produce urinary osmolalities comparable with those obtained after 22 h of fluid deprivation [1]. Failure to obtain a urinary osmolality of $\geqslant$800 mosmol/kg using this stimulus implies nephrogenic diabetes insipidus, due to inability of the distal nephron to respond to vasopressin [55]. As DDAVP may affect the renal tubular handling of calcium, magnesium and phosphate [62, 79], the test should be performed separately from the rest of the protocol

Renal control of acid/base balance. Similarly, when it has not been possible to achieve a urinary pH of $\leqslant$5.4 despite testing of repeated samples, further investigations of the renal control of acid-base balance may be performed as summarised in the algorithm shown in Fig. 1. The first step is measurement of the plasma bicarbonate concentration and, if necessary, clarification of a doubtful value by means of venous blood-gas measurement. A normal plasma bicarbonate value excludes proximal RTA, and isolated distal RTA can be ruled out by observation of a normal response to an acid load test. Conversely, an abnormal response to an acid load test confirms the diagnosis of distal RTA. The aim of an acid load test is to deliver enough acid to lower the plasma bicarbonate concentration to <18 mmol/l or the blood pH to <7.34 [20]; this is usually achieved by using oral ammonium chloride at a dose of 75 mmol/m^2 (4 g/m^2) [24]. A urinary pH of $\leqslant$5.4 in samples collected over the next few hours constitutes a normal response. The reduction in plasma bicarbonate concentration should be confirmed in a blood sample taken at about 3 h after ingestion of the ammonium chloride. However, the administration of ammonium chloride, which has an unpleasant taste, may provoke nausea and vomiting. It is best avoided except in cases of genuine clinical or research importance and is inappropriate in patients who exhibit a plasma bicarbonate level of <18 mmol/l.

An alternative to the acid load test involves the administration of an intravenous bolus of frusemide (1 mg/kg), which increases sodium delivery to the distal nephron, thereby enhancing hydrogen ion secretion in the cortical collecting tubule. This may enable the achievement of a urinary pH of $\leqslant$5.4 confirming the presence of adequate distal nephron acidification [65]. However, although a reduction in urinary pH to $\leqslant$5.4 following the administration of frusemide obviates the need for an acid load test, a failure to acidify urine does not necessarily imply an irreversible defect of acidification, and it may be necessary to perform an acid load test in this situation [66].

If the plasma bicarbonate concentration is <20 mmol/l, the presence of bicarbonate in the urine implicates proximal RTA as the cause of the acidosis, although a degree of distal RTA may also be present. Conversely, the absence of urinary bicarbonate when the plasma concentration is low excludes proximal RTA and narrows the differential diagnosis to either distal RTA or a non-renal cause. These can be distinguished by calculation of the urinary net charge, which is equal to the sum of urinary sodium and potassium

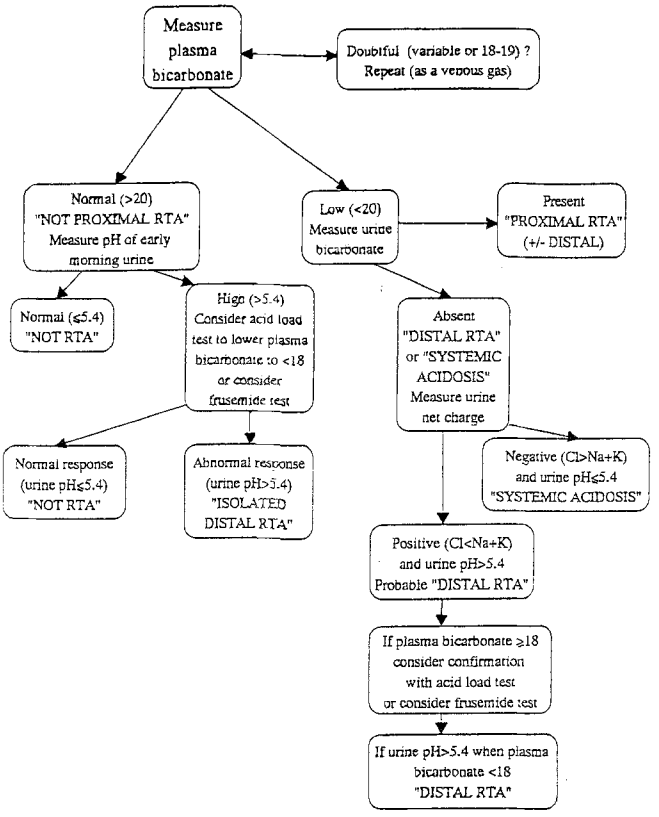

Fig. 1. Assessment of the renal control of acid/base balance

levels minus the urinary chloride value [35]. In this situation, urinary chloride excretion is taken to reflect secretion of urinary ammonium ions, which is a distal nephron function. Chronic systemic acidosis in the presence of normal distal nephron function results in chronic and usually elevated urinary ammonium secretion as a means of excreting hydrogen ions. Urinary ammonium secretion is difficult to measure directly but is inferred from the urinary net charge – with a negative value implying high urinary ammonium secretion, and vice versa. If the net charge is negative, the acidosis is of systemic (non-renal) origin and the urinary pH should be $\leqslant$5.4; alternatively, a positive net charge indicates that the acidosis is due to a lack of ammonium secretion and is therefore secondary to distal RTA. If necessary, the diagnosis can be formally confirmed by either the administration of frusemide or an acid load test.

Bone chemistry

Abnormal renal handling of calcium and phosphate (which may be evaluated as previously described) may lead to changes in their plasma concentrations, which may in turn cause abnormal bone metabolism. The effect on bone homeostasis is assessed by measurement of the plasma alkaline phosphatase activity, with identification of the bone isoenzyme in cases of doubt.

General aspects of renal function

Renal tubular enzymes. Measurement of the excretion of renal tubular enzymes, which are released into the urine in

increased quantities as a result of tubular damage, provides sensitive evidence of renal tubular toxicity [61]. Enzymes that may be measured include the brush-border enzymes, alanine aminopeptidase (AAP) and alkaline phosphatase (ALP); a cytoplasmic enzyme, lactate dehydrogenase (LDH); and a lysosomal enzyme, *N*-acetylglucosaminidase. These enzymes are present throughout the length of the tubule, but in differing quantities in different sites. For example, NAG is found in cells all along the nephron but occurs in greatest quantities in the proximal tubule [34]. Therefore, elevated urinary excretion of these enzymes reflects renal tubular damage but may not distinguish proximal from distal damage.

Adenosine deaminase-binding protein (ADBP) is a proximal tubular membrane protein that is liberated into the urine in increased quantities as a result of tubular cell damage [31]. By virtue of the more specific localisation of ADBP within the nephron, its measurement can potentially identify toxicity specific to the proximal tubule.

All of these substances may be measured in a random "spot" urine sample and their concentration related to that of creatinine. However, as with low-molecular-weight proteins, diurnal variation in enzyme excretion may occur in healthy individuals [51]; thus, it is best to collect the sample at a constant time in all patients.

Albuminuria and proteinuria. Proteinuria can be classed as either glomerular or tubular. Glomerular proteinuria occurs due to an increase in permeability of the glomerulus and is characterised by the presence of proteins of high molecular weight. Tubular proteinuria is the result of either a failure of the proximal tubule to reabsorb low-molecular-weight proteins from the ultrafiltrate or an increase in the excretion of protein due to shedding from damaged tubular cells. The investigations performed in this protocol may help discriminate these two types of proteinuria. Measurement of the ratio of urinary albumin: β_2-M excretion has been used to distinguish glomerular proteinuria (in which the ratio is high due to a predominance of albumin excretion) from tubular proteinuria (in which the ratio is low since β_2-M excretion is relatively greater) [59].

Urinalysis and microscopy. Analysis of urine with reagent strips is a simple bedside test that can provide a rapid, semi-quantitative estimate of urinary pH, proteinuria, albuminuria, glycosuria, haematuria and haemoglobinuria. The use of microscopy may enable the detection of blood cells (red or white) or casts (hyaline or cellular). Although abnormalities identified by urinalysis and microscopy are non-specific, being found in a variety of renal lesions, they may provide an easy method of detecting certain features of nephrotoxicity, e.g. glycosuria due to impairment of glucose reabsorption in the proximal nephron.

Blood pressure. Acccurate measurement of blood pressure is important in any assessment of renal function. However, attention should be paid to detail: the correct choice of cuff is important, the child should be seated and have rested for 3 min prior to blood pressure measurement, and determination of systolic blood pressure is preferable to that of diastolic pressure [80].

Table 3. Age- and sex-related variation in reference ranges

Plasma creatinine	≤75 μmol/l	(3–6 years)
	≤90 μmol/l	(7–14 years)
	≤105 μmol/l	(14–18 years, male)
	≤85 μmol/l	(14–18 years, female)
Plasma phosphate	1.1–1.85 mmol/l	(2–5 years)
	1 –1.80 mmol/l	(6–12 years)
	0.9–1.75 mmol/l	(13–16 years)
Plasma urate	0.05–0.40 mmol/l	(1–9 years)
	0.11–0.50 mmol/l	(10–19 years, male)
	0.11–0.42 mmol/l	(10–19 years, female)
Plasma alkaline phosphatase	≤350 IU/l	(2–9 years)
	≤450 IU/l	(10–15 years)
	≤300 IU/l	(16–18 years, male)
	≤130 IU/l	(16–18 years, female)
Renal threshold for phosphate	1.21–1.71 mmol/l	(1–2 years)
	0.92–1.70 mmol/l	(3–4 years)
	1.05–1.54 mmol/l	(5–6 years)
	1.07–1.72 mmol/l	(7–8 years)
	1 –1.59 mmol/l	(9–10 years)
	0.92–1.66 mmol/l	(11–12 years)
	0.75–1.64 mmol/l	(13–15 years)

Normal ranges used by the Department of Clinical Biochemistry, Royal Victoria Infirmary and Universtiy of Newcastle upon Tyne, Newcastle upon Tyne, with the exception of the renal threshold for phosphate (see Brodehl et al. [11]). Plasma alkaline phosphatase is measured by a Technicon SMAC Auto-Analyser, using enzymatic hydrolysis of *p*-nitrophenylphosphate with 2-amino-2-methyl-1-propanol buffer to produce *p*-nitrophenol, which is measured colorimetrically

Reference ranges

Normal ranges for some of the investigations included in this protocol vary with age or sex, as shown in Table 3. Accurate assessment of renal function depends on an awareness of such variations. Detailed tables of age-related reference ranges for many biochemical investigations are available [16, 53]. Unfortunately, however, specific age-related reference ranges for children have not yet been obtained for some of the investigations in this protocol. In these cases, it has been necessary to use alternative data, obtained in some cases from adult studies; the results are nonetheless likely to be valid in most cases, since many aspects of normal renal physiology change relatively little after early infancy. The reference ranges that we use are detailed in Table 4 along with their sources, and those based on adult data are marked with a superscript *a*. Normal ranges for commonly performed investigations, such as those for plasma electrolyte determinations, are not included since the values applied by the laboratory performing the assays should be used.

Discussion

The treatment of childhood malignancy has improved greatly in the last three decades [78]: >60% of children and adolescents with cancer who are treated with currently available multimodality treatment can now expect to sur-

Table 4. Reference ranges

Parameter	Range	Source of data
Glomerular function:		
GFR	87–174 ml min^{-1} 1.73 m^{-2}	Barratt [2]
Proximal nephron function:		
Plasma ionised calcium[a]	1.19–1.37 mmol/l	Dept. of Clinical Biochemistry[b]
Fractional excretion of calcium[a]	<2%	Sutton and Dirks [79]
Fractional excretion of magnesium[a]	~3%	Sutton and Dirks [79]
Fractional excretion of phosphate[a]	<20%	Sutton and Dirks [79]
Fractional excretion of glucose	<1%	See Skinner et al. [73]
Fractional excretion of urate[a]	7–12%	Grantham and Chonko [33]
Urinary β_2-microglobulin	<0.01 mg/mmol creatinine	Fielding [26]
Urinary retinol-binding protein[a]	<23.4 µg/mmol creatinine	Rowe et al. [67]
Distal nephron function:		
See section on Distal tubular function		
Bone chemistry:		
See plasma alkaline phosphatase in Table 3		
General:		
Renal tubular enzymes[a]		Dept. of Clinical Biochemistry[b]
Alanine aminopeptidase	<1 IU/mmol creatinine	
Alkaline phosphatase	<0.4 IU/mmol creatinine	
Lactate dehydrogenase	<2.6 IU/mmol creatinine	
N-acetylglucosaminidase	<0.6 IU/mmol creatinine	
Urinary albumin	<10 mg/mmol creatinine	Barratt et al. [3]

[a] Data derived from adult studies
[b] Department of Clinical Biochemistry, Royal Victoria Infirmary and University of Newcastle upon Tyne, Newcastle upon Tyne

vive for at least 5 years after diagnosis [18]. Although these long-term survivors retain a small risk for late relapse or a second malignancy, the majority are probably cured. It is therefore vital that the toxicity of treatment in these children be minimised, especially that with potentially severe long-term effects [57]. Consequently, the assessment of toxicity is an important component of the management of individual patients receiving chemotherapy. Furthermore, investigation of new cytotoxic drugs and treatment protocols should include detailed assessment of their adverse effects.

Impairment of renal function is a potentially serious complication of cancer and its treatment, both in children and in adults. There are several reasons for its occurrence in childhood malignancy [64]. These include factors related both to the disease itself and to its management. Radiotherapy-induced nephropathy and drug-induced renal damage, both from supportive therapy (e.g. aminoglycoside antibiotics) and from cytotoxic drugs, may occur.

Cisplatin and ifosfamide are the commonly used cytotoxic drugs most likely to cause renal damage in children. Any aspect of renal excretory function may be affected, and damage may be widespread. Severe clinical problems may ensue, limiting therapy or even threatening life. In view of the use of these drugs in many paediatric solid malignancies, their nephrotoxicity consitutes a major problem.

Treatment with either cisplatin or ifosfamide may be associated with glomerular impairment, leading to a fall in GFR [73, 82]. Chronic renal failure may ensue in severe cases [30, 68]. Proximal tubular damage is a common effect of both of these drugs, although with different manifestations. Cisplatin tends to cause magnesuria with resultant hypomagnesaemia [71], which may cause tetany [70] or convulsions [5]. Ifosfamide-induced proximal tubular toxicity may be widespread, with aminoaciduria, glycosuria and phosphaturia leading to hypophosphataemia, with rickets occurring in some children [72]. The damage may amount to a Fanconi syndrome in severe cases [21, 73, 76]. Distal tubular damage may lead to poor urinary concentration and, in severe cases, to nephrogenic diabetes insipidus [73] or impaired acidification of urine, causing renal tubular acidosis [40]. Subclinical tubular damage may be seen with elevated urinary excretion of renal tubular enzymes [32]. Albuminuria and proteinuria are commonly documented following treatment with either drug, and casts may be seen on microscopy of urine [36]. Hypertension may follow treatment with cisplatin, especially when it is given intra-arterially [38, 46].

Other cytotoxic drugs used in children that may cause nephrotoxicity include high-dose methotrexate [44, 50], the nitrosoureas [37, 81], carboplatin [75] and cytosine [74]. It is important to emphasise that much of the nephrotoxicity caused by these drugs, as well as by cisplatin and ifosfamide, is primarily tubular in nature and cannot be detected by measurement of the plasma creatinine concentration alone.

Detailed assessment of renal function in children receiving chemotherapy is justifiable both clinically and for research purposes. A sound knowledge of normal renal physiology and an awareness of the toxicity that may be seen after treatment with a particular drug help physicians in the management of individual patients. Of even greater importance is the recognition of potentially serious renal damage due to a new drug or regimen early in the course of

its use (ideally *before* its use) rather than several years later.

Measurement of plasma creatinine is a poor indicator of glomerular function, since it is an insensitive measure of early glomerular impairment and is dependent on non-renal factors, especially the rate of creatinine production, which itself is dependent on muscle mass [45]. Its use is particularly inappropriate in children with cancer, who are often cachectic and, hence, exhibit a low serum creatinine value [19]. Although creatinine clearance has been used to measure GFR for many years, methodological difficulties inherent in the measurement of plasma creatinine (chiefly the measurement of non-creatinine chromogens in the standard colorimetric assay), the presence of a degree of tubular secretion of creatinine, and potential errors in urine collection, especially in young children, reduce the method's acccuracy. In particular, creatinine clearance may substantially overestimate a genuinely low GFR [4]. Inulin clearance remains the gold standard for measurement of GFR, but its determination requires an inulin infusion and the assay is time-consuming. Measurement of GFR from the plasma clearance of [^{51}Cr]-EDTA is accurate yet simple and avoids the need for timed urine collections. This is a considerable advantage in paediatric practice.

The lack of abnormal glomerular histology in the kidneys of patients known to show glomerular impairment after treatment with cisplatin has led to suggestions that the fall in GFR may be secondary to alterations in renal haemodynamics. These may be detectable at an early stage by a reduction in renal blood flow, expressed as the effective renal plasma flow (ERPF) measured by the plasma clearance of hippuran [58]. However, recent work using the lithium clearance method has suggested that proximal tubular impairment of sodium and water reabsorption may be the first abnormality after administration of cisplatin, occurring before any changes in GFR or ERPF [19]. It is possible to perform simultaneous assessment of GFR and ERPF using radioisotopic methods, but this cannot be recommended for general use until further information has been obtained on the value of measurement of ERPF. Furthermore, many patients with cytotoxic drug-induced nephrotoxicity develop tubular dysfunction that impairs the uptake and elimination of hippuran, thus invalidating the use of hippuran clearance methods to measure ERPF.

Measurement of the plasma concentration of small ions (e.g. sodium, calcium, urate) may reveal obvious abnormalities such as hyponatraemia, hypokalaemia, hyperchloraemic metabolic acidosis, hypocalcaemia, hypomagnesaemia, hypophosphataemia and hypouricaemia [8, 41, 42, 49, 70–72, 76]. Quantitation of the urinary loss of the same substances may be difficult to perform and evaluate. It may be impossible to obtain an accurately timed urine collection from a young child. Even if such a sample is obtained, the results should be related to the child's age and size, complicating interpretation.

However, investigation of proximal tubular function in children is simplified by calculation of the fractional excretion of substances such as calcium, magnesium, phosphate and glucose, which are freely filtered at the glomerulus and are mostly reabsorbed in the proximal nephron [10]. This method of assessment is easy, involving the collection of one blood sample and a corresponding urine specimen and the use of a straightforward calculation. As the fractional excretion is relatively constant after infancy and is not related to size, interpretation is much simpler. The renal threshold for phosphate can also be calculated simply, using the same samples.

Although the fractional excretion and the renal threshold for phosphate are well-known concepts amongst nephrologists, their use in oncological practice is relatively novel. However, these calculations have two main advantages. First, the need for a timed urine collection is avoided. Second, the fractional excretion provides an assessment of overall tubular function since it depends on the net result of tubular reabsorption and secretion, and the renal threshold of phosphate gives a direct functional indication of proximal tubular handling of phosphate. Both of these calculations therefore fulfill the need to relate blood and urinary concentrations of the substance under study rather than examining each separately and, hence, they evaluate renal tubular handling directly. This approach provides a greater understanding of the pathophysiology of the tubular toxicity. However, it should be noted that the fractional excretion of electrolytes is affected by many other renal and non-renal factors (e.g. excessive secretion of growth hormone or other hormones) [79], although these are unlikely to cause such large changes as those seen in renal tubular dysfunction due to chemotherapy.

Specific patterns of aminoaciduria may be seen early in the course of renal damage due to cytotoxic drugs or other toxins [8, 13], perhaps as particular active transport mechanisms in tubular cells are disabled. Such damage may be transient if exposure to the toxic insult is limited [13], but repeated treatment with nephrotoxic chemotherapy may lead to apparently irreversible generalised aminoaciduria [73]. However, the value of assessment of patterns of aminoaciduria is limited by the time-consuming and expensive nature of the techniques for quantitative measurement.

Measurement of the urinary excretion of certain low-molecular-weight proteins, including β_2-M and RBP, provides a more practical alternative, but some limitations should be borne in mind. First, it is necessary to ensure that the plasma concentration of the protein is not elevated before assuming that increased urinary excretion is attributable to proximal tubular impairment, although measurement of corresponding plasma and urinary concentrations and calculation of the fractional excretion enables a correct interpretation. Increased plasma concentrations of low-molecular-weight proteins may result from glomerular impairment or from abnormal production of the protein. However, the tubular threshold for β_2-M and RBP is exceeded only when the GFR has fallen by >70% [6]. The β_2-M concentration in blood is often elevated in adults with certain lymphoproliferative malignancies and some solid tumours [17, 28] as well as in some auto-immune disorders [69]. Although we are not aware of any such reports in children with malignancy, it would seem prudent to exercise caution in the interpretation of an elevated urinary excretion of β_2-M in such children, unless there is strong corroborative evidence of proximal tubular damage.

Second, β_2-M is unstable in acid urine with a pH of <5.5 at room temperature [69]. Although alkalinisation of freshly voided urine halts further degradation of β_2-M, it cannot prevent that which has occurred in vivo during bladder filling. This may result in a false-negative result on occasions.

To our knowledge, the plasma RBP concentration does not appear to be increased in malignant conditions. Furthermore, RBP is less liable to degradation than β_2-M in acid urine, although the former is not completely stable [22]. Therefore, measurement of RBP has two distinct advantages over that of β_2-M. However, other low-molecular-weight proteins (e.g. kappa chains, α_1-microglobulin) may also be suitable.

RTA can be classified into two main groups: proximal RTA and distal RTA. In proximal RTA, the primary defect is a reduction in the proximal tubular reclamation of filtered bicarbonate, which results in excessive urinary bicarbonate loss and, therefore in acidosis [10]. Distal RTA is due to an impairment of acidification of urine in the distal tubule, the precise mechanisms of which are uncertain [20]. Both types may lead to a similar biochemical pattern of hyperchloraemic metabolic acidosis – the plasma bicarbonate concentration is reduced, but the anion gap remains normal due to an increase in the chloride concentration. However, such a picture could be due to proximal RTA, distal RTA, a mixture of both, or even an entirely different systemic (i.e. non-renal) cause of hyperchloraemic acidosis, e.g. gastrointestinal bicarbonate loss due to chronic diarrhoea or laxative abuse [35]. The algorithm shown in Fig. 1 enables clarification of the cause.

In the context of this investigation protocol, the results of the other tests may point to one particular type of RTA as the cause of hyperchloraemic acidosis in a child with chemotherapy-related nephrotoxicity. For example, in the presence of other features of proximal tubular dysfunction but not of distal toxicity, proximal RTA would be the likeliest cause. Such an impression would be confirmed by the presence of bicarbonate in the urine when the concentration in blood is <20 mmol/l.

The principle role of the distal tubule is urinary concentration and acidification. Traditionally, these have been assessed in children by the ability to concentrate urine during a formal water-deprivation test or to acidify urine after administration of oral ammonium chloride. However, these tests are unpleasant, being potentially dangerous in the former case, and are obviously unsuitable for routine use in this protocol. Measurement of the osmolality and pH of an early morning specimen of urine is more appropriate.

If sufficiently high urinary osmolalities or low pH values are not achieved despite repeated testing after overnight fluid deprivation, further investigation may be necessary. Awareness of the probability of at least a degree of distal tubular impairment may be sufficient for clinical needs in many children. However, clarification of the exact diagnosis may be important in other children, and such information may be of value in the assessment of the nephrotoxicity of new drugs or treatment regimens.

Changes in blood calcium and phosphate concentrations that occur as a result of proximal tubular toxicity may lead to disordered bone homeostasis, which may be reflected by an elevated plasma alkaline phosphatase activity, e.g. in hypophosphataemic rickets due to ifosfamide [72].

Abnormal degrees of enzymuria provide evidence of cellular damage, but they offer little information about the functional or clinical relevance of the toxicity. Indeed, enzymuria may be too sensitive a measure of tubular toxicity to be of practical value in the management of individual children. However, it may be of more help in the early detection of tubular toxicity and may provide a relatively simple means of comparing the subclinical nephrotoxicity of different treatment regimens. Furthermore, use of enzymes with different subcellular localisation may give some indication of the nature and severity of tubular damage at a cellular level, e.g. brush-border damage or tubular cell necrosis [32].

Although measurement of urinary albumin and protein excretion provide only a general indication of renal damage, differentiation of glomerular from tubular dysfunction may be possible using more sophisticated investigations such as protein electrophoresis. However, these may not be generally available, limiting their use in a protocol of this nature.

An advantage of this protocol is the incorporation of investigations with varying levels of sensitivity in the detection of nephrotoxicity. For example, measurement of renal tubular enzymes gives very sensitive evidence of tubular cell toxicity but does not assess the functional consequences or distinguish proximal from distal tubular damage. However, quantitation of the urinary excretion of low-molecular-weight proteins is not only similarly sensitive but also localises the damage to the proximal tubule. Calculation of the fractional excretions and the renal threshold for phosphate provides an assessment of proximal tubular function at the "whole kidney" level and, hence, defines the implications for the patient. This protocol combines investigations that are necessary for the assessment of clinical problems with more sensitive measures that demonstrate subtle degrees of renal dysfunction.

Acute and chronic toxicity should be clearly distinguished, but both may be clinically important and warrant investigation. The principle of comprehensive evaluation of all aspects of renal excretory function that is incorporated in this protocol is appropriate in the assessment of both transient and long-term nephrotoxicity. Indeed, the use of a standardised protocol enables a clearer documentation of the occurrence and subsequent reversibility or irreversiblity of acute toxicity.

Although these tests enable the assessment and localisation of renal damage in most cases, it is important that the results be viewed as a whole, since separate consideration of each test may lead to diagnostic difficulty at times. By simultaneous assessment of all aspects of renal excretory function, it should be possible to define the site, nature and severity of renal toxicity with precision.

The potential uses of this investigatory protocol include both clinical and research applications in the field of paediatric oncology. In individual patients, comprehensive assessment of both the nature and the severity of drug-induced renal damage is essential for optimal management of the resultant complications. Glomerular impairment may

lead to alterations in the pharmacokinetics of renally excreted drugs, which may be of clinical relevance if the therapeutic index is small, e.g. with other cytotoxic agents or with aminoglycoside antibiotics. The use of sensitive indicators of renal dysfunction may provide early evidence of toxicity. Likewise, repeat studies may lead to earlier recognition of partial recovery or deterioration in renal function. It may be acceptable for clinical purposes to monitor selected, drug-specific facets of toxicity in certain patients, e.g. the fractional excretion of magnesium in patients who have received cisplatin, rather than to perform the whole protocol.

In the context of research, use of a comprehensive investigatory protocol in early clinical trials is vital to reduce the risk of failure to detect potential adverse effects. Larger studies may identify certain risk factors for the development of nephrotoxicity. For example, it may be possible to identify a high-risk group of patients, such as children aged <5 years. Likewise, early subclinical damage as demonstrated by abnormalities in sensitive investigations may be predictive of subsequent overt toxicity if treatment is continued. Sequential studies performed during and after treatment courses reveal the usual time course of subclinical (or, indeed, of overt) toxicity. By alternative scheduling of the drug (e.g. using a given drug every second course of treatment rather than every course), toxicity may be reduced. Repeated studies, both during and after treatment, enable an assessment of the reversibility, if any, of the damage and, hence, of its long-term importance. The investigatory protocol may be of value in the establishment of strategies to reduce nephrotoxicity, such as the possible reduction of cisplatin-induced damage by probenecid [43]. Finally, a knowledge of the nature and evolution of toxicity may suggest possible sites and mechanisms of cell or tissue damage, leading to appropriate laboratory research.

The investigatory protocol described in this paper was developed during cross-sectional and follow-up studies in children treated with either cisplatin or ifosfamide. It has been used in >50 children with cancer, including many who have been studied on 2 or 3 occasions each. Age-related reference ranges from normal children are being compiled for investigations for which such information is not yet available.

In conclusion, nephrotoxicity is a potentially serious adverse effect of some cytotoxic drugs, especially cisplatin and ifosfamide, and is of particular concern in children, who have a potentially long life expectancy if cured of their malignancy. There is a need for careful assessment of renal function in children following treatment with such drugs, since nephrotoxicity is often clinically silent in its early stages. This has been emphasised by the recent recognition, after only several years of use, of the severity and frequency of nephrotoxicity associated with ifosfamide treatment in children. The investigation of renal damage should be based on an appreciation of the different aspects of renal function and should therefore include assessment of glomerular, proximal and distal tubular function. Such assessment of nephrotoxicity has many potential applications in the management of individual children and in clinical research, both for cytotoxic agents and for other drugs that may cause renal damage. An investigatory protocol has been described that is easy to perform in children and yet provides a comprehensive evaluation of nephrotoxicity.

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