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NEPHROTOXICITY AND FUNGAL RESISTANCE ASSOCIATED WITH AMPHOTERICIN B: A COMMENTED REVIEW

NEFROTOXICIDADE E RESISTÊNCIA FÚNGICA ASSOCIADAS À ANFOTERICINA B: UMA REVISÃO COMENTADA

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ABSTRACT

Introduction: Amphotericin B (AB) is the drug of choice for the treatment of several systemic fungal infections. Despite of its effectiveness and minimum relation to the resistance, AB is toxic to animal cells, in particular to the kidney cells. **Objective:** To address the aspects of resistance, nephrotoxicity and biomarkers available for the early detection of kidney disease caused by AB. **Method:** The bibliographic search was performed in online databases. Papers published between 2008-2019, were selected for further analysis and inclusion in the present review. **Results:** The search resulted in 42 eligible papers which the main findings were: (i) the frequency of resistance to AB appears to be substantial, however, some species should be carefully studied given their diminished susceptibility; (ii) regarding nephrotoxicity, new techniques and methods have promisingly been implemented; and (iii) a novel generation of biomarkers for kidney injury is more specific and sensitive. **Conclusion:** The adoption of conjugated drug delivery systems is a consistent alternative to minimize nephrotoxicity and fungal resistance. Considering the biomarkers, it is more likely that, instead of a punctual assessment of a single biomarker, a set of biomarkers provides a more thorough comprehension of the aspects related to AB-induced kidney injury. Nonetheless, more clinical studies are necessary for further implementation of such biomarkers in the clinical routine.

Keywords: Amphotericin B. Nephrotoxicity. Acute Kidney Injury. Biomarkers.

RESUMO

Introdução: A anfotericina B (AB) é a droga de escolha para o tratamento da maioria das infecções fúngicas sistêmicas. Apesar de sua efetividade e relação mínima com resistência microbiana, apresenta relevante toxicidade às células dos tecidos mamíferos, em particular às células renais. **Objetivo**: Abordar aspectos da resistência fúngica, nefrotoxicidade e biomarcadores disponíveis para detecção precoce da lesão renal ocasionada pela AB. **Método**: A busca bibliográfica foi realizada em bases de

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dados online. Foram incluídos artigos publicados no período entre 2008-2019, que possuíam dados completos ou parciais relacionados aos objetivos. **Resultados:** A busca resultou em 42 artigos cuja compilação dos tais sugeriu que: (i) a frequência de resistência à AB não parece ser substancial, porém, algumas espécies merecem maior atenção em virtude de sua suscetibilidade diminuída; (ii) diante da nefrotoxicidade, novas abordagens tecnológicas vêm sendo implementadas de maneira promissora; e (iii) a nova geração de biomarcadores para lesão renal aguda se apresenta de forma mais específica e sensível. **Conclusão:** A adoção de sistemas de entrega conjugados à AB parece ser uma alternativa coerente à minimização da nefrotoxicidade e da resistência fúngica. No tocante aos biomarcadores, é mais provável que, ao invés da avaliação pontual de um único, um painel de biomarcadores proporcione uma compreensão mais minuciosa dos aspectos da lesão renal mediada pela AB. No entanto, são necessários mais estudos clínicos antes que tais marcadores sejam, por fim, implementados à clínica.

Palavras-chave: Anfotericina B. Nefrotoxicidade. Lesão renal aguda. Biomarcadores.

INTRODUCTION

The discovery and use of antimicrobial agents has prolonged, at least 10 years, the life expectancy of patients with infections[1]. On the other hand, the number of infections caused mainly by fungus has increased. Among other factors, the large-scale use of antimicrobial agents and, consequently, alterations in the normal microbiota, are the causes of drug resistance[2].

Antifungal drugs currently used have enabled the progress in the management of fungal infections, although drug resistance and high toxicity have been evidenced[3]. The natural or acquired resistance of some species to antifungal agents is an important factor that, combined to side effects and toxicity, highlight the need for exploring new therapeutic approaches as alternatives to conventional drugs.

The amphotericin B (AB) is a drug rarely associated to the development of clinically important microbial resistance, but commonly associated to toxicity and side effects. Such effects are responsible for high morbidity rates, with an estimated global incidence of 80%[4, 5.] In addition, AB has been the gold standard treatment of fungal infections since its introduction into the clinical practice in the late 1950s[4].

The present study aimed to describe aspects of nephrotoxicity related to AB, mechanisms and novel biomarkers to assess the kidney injury, as well as mechanisms and frequency of the fungal resistance to the drug.

METHODS

Surveys assessing AB resistance and nephrotoxicity, as well as biomarkers related to kidney injury were extracted from three electronic databases: LILACS-Bireme (*Biblioteca Virtual em Saúde*), PubMed/NCBI (US National Library of Medicine National Institutes of Health) and SciELO (Scientific Electronic Library Online). The search was performed between September and January 2019, using the terms: “amphotericin B”, “nephrotoxicity”, “acute kidney injury” and “biomarkers”.

Studies were eligible for inclusion if they met the following criteria: i) publication between January 2008-January 2019; ii) presence of the keywords in accordance with the objectives of the present study; and iii) studies performed with animals (because the mechanisms of acute kidney injury are usually known by the histopathological analysis). References of papers were also analyzed for further inclusion. Studies related to parasitosis (Leishmaniasis) were only incorporated to the search when topics on biomarkers and mechanisms of nephrotoxicity were discussed. Review and original papers, short communications and letters to the editor written in Portuguese and English were included, whereas congress abstracts, theses and dissertations were excluded.

RESULTS

The search resulted in 492 papers. After applying the inclusion and exclusion criteria, 73 papers were analyzed. Then, after reading the full text, a total of 42 papers were selected for the present study, among them, 19 reported the relationship between AB and fungal resistance, 13 discussed about nephrotoxicity and 10 evidenced the biomarkers used to assess kidney injury.

THE AMPHOTERICIN B

For decades, it was postulated that AB induced the pore formation in the cell membrane, causing the loss of small molecules, mainly K^+, and thereby permeabilizing and killing yeast cells. As a consequence of this process, cell death was caused by oxidative damage[6]. Nonetheless, recent studies show that the channel-forming capacity of AB is not required for fungicidal activity, whereas binding to ergosterol is essential. This process is only possible because of the capacity of the drug to interact with the lipophilic membrane[7].

MECHANISMS OF RESISTANCE

Ergosterol is an essential element for several aspects of fungal physiology[8], which makes difficult for the cell to produce mutations related to resistance[9]. Most strains of the main fungal pathogens for humans have the inhibition of growth in vitro when exposed to concentrations from 0.05 to 1.0 μg/mL[10]. It means that strains presenting the minimal inhibitory concentrations (MIC) above this interval are considered potentially resistant.

Since this is a non-expected phenomenon and, therefore, reported in the scientific community only when present, data available on the occurrence of AB resistance might be underestimated. Nonetheless, when present, mechanisms involved in drug resistance can be listed.

The low fungal susceptibility to AB is directly associated to the ergosterol biosynthesis[12]. For instance, *Candida tropicalis* presents a low concentration of ergosterol in the membrane[13], and alters its mitochondrial activity, reducing the levels of reactive oxygen species (ROS) produced by the drug. Consequently, *C. tropicalis* is reported as one of the few fungal species presenting low susceptibility to AB.

The ergosterol deficiency can also result from gene mutations that codify enzymes involved in the ergosterol biosynthesis, or due to substitutions of ergosterol for other sterols with low affinity for the polyene, as fecosterol. Thus, higher concentrations of the drug are necessary to inhibit the growth of fungi with alterations in the concentrations of ergosterol and sterols[14].

High levels of catalase were found in *Candida albicans* strains resistant to AB resulting in an oxidative effect. Mutant strains of *C. neoformans* presented, similarly to *C. albicans*, alterations in the sterols compositions, being the ergosterol absent or reduced[15].

Mutations recognized to be involved in the ergosterol biosynthesis and which determine the in vitro resistance profile are: ERG2, ERG6 or ERG3/ERG11. All strains presenting these mutations were resistant to AB, although such strains are known as non virulent[9]. However, the sudden emergence of a new *Candida* species – *Candida auris*, detected for the first time in 2009 in Japan, has alarmed the scientific community. From 54 isolates, 35% were resistant to AB, and 94% were resistant to fluconazole – a drug commonly used in the treatment of candidiasis[11].

As new studies have been proposed, the susceptibility of *C. auris* to AB varied in the literature, from 0.25 to 8 mg/L in two retrospective studies[16]. Other studies evidenced a lower MIC (0.28-4 mg/L), but one third of the isolates presented a MIC ≥ 2 mg/L[60]. The strain is becoming eminent in all continents, which indicates a potential outbreak.

The resistance mechanism involves several different mutations related to ergosterol biosynthesis. The ability of forming biofilms in surfaces, as well as the potential of causing outbreaks[17] can explain the resistance of *C. auris* to AB. Moreover,

the aspects related to the immunosuppression associated to AIDS, and the low susceptibility of strains from the hospital environment are among other possible causes of resistance to the polyene[18].

It is well known that the ergosterol biosynthesis is coordinated by 25 different enzymes, and that variation in this process can lead to cell damage. A study aimed to assess and report functional alterations of overexpression of gens involved in the ergosterol biosynthesis in *Saccharomyces cerevisiae.* The main findings include increased period of duplication, respiratory deficiencies on glycerol media, cell wall insufficiencies on Congo red media, and disrupted ion homeostasis under iron or calcium starvation conditions. Alterations related to the AB susceptibility profile were also observed on the gene overexpression or deletion[18]. Thus, it is important to investigate aspects related to the development of resistance by the cell to antifungal agents targeting the ergosterol molecule through genotypic mutations.

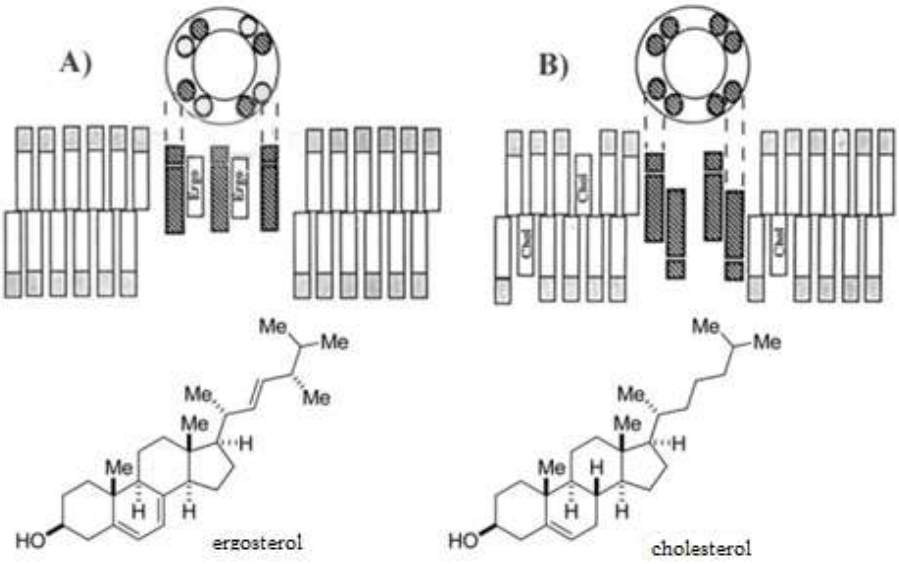
Every living organism is constantly exposed to stress as a result of direct or indirect DNA damage. However, it is known that eukaryotic cells have repair systems. In this context, mechanisms associated to repair systems using deletion of genes linked to the process (RAD53 and CHK1) were studied in *C. neoformans*. In this experiment, the cell became highly susceptible to stress induced by drugs such as AB, attenuating the virulence of the tested organism[19]. Thus, similar studies are essential to provide insights into the regulatory mechanism of fungal DNA damage repair systems and their functional relationship with fungal virulence and antifungal drug susceptibility.

Finally, considering the several cases of resistant vulvovaginal candidiasis, an alternative therapeutic scheme has been suggested: local application of antifungal agents controlled by drug delivery systems using nanofibers. In vitro and in vivo fungicidal activity of AB released from nanofibers were assessed using agar diffusion method and an experimental murine model of vulvovaginal candidiasis, respectively. No resistance was evidenced in the in vitro model and, in vivo, the drug eliminated the fungal burden after three days of treatment. These results suggest that the use of AB-loaded poly (lactic-co-glycolic acid) nanofibers may represent a strategy for local treatment without the induction of fungal resistance, assuring the adhesion and safety to the patient[20].

NEPHROTOXICITY MECHANISM

All fungi and other microorganisms susceptible to polyene present ergosterol in cell membrane – similarly to cholesterol in mammalian cells (Figure 1)[4].

Figure 1 – Schematic representation of AB mechanism of action



A) Fungal membrane model. B) Mammalian membrane model. Chemical structures of ergosterol and cholesterol are below. AB is represented by black retangules.

Adapted by Bolard et al. (1991)[21].

Toxicity is a result from the interaction between the drug and the human cells membrane which, similarly to fungal cells, contain cholesterol[4]. Experimental studies on AB nephrotoxicity (mainly the ones assessing histopathological endpoints) enable the comprehension of two basic mechanisms in the process. Moreover, these mechanisms might be related.

The first is the vascular mechanism, comprising dysfunctions in the renal blood flow. The second is described as a direct damage to the structure of the tubular cell. The combination of these processes leads to the reduction of the glomerular filtration rate (GFR) and to hydroelectrolytic and acid base disorders.

Vascular mechanism

Since 1978, several studies have documented the reduction of renal plasma flow and the GFR after AB administration[22]. Thus, it was possible to conclude that the injury induced by the drug is more related to functional aspects rather than to morphological.

A study demonstrated that the AB administration (2.5 mg/kg) in dogs increased renal vascular resistance and decreased GFR and urine flow in approximately 94%. The same study pointed that the administration of dopamine and saralasin could reduce the AB-induced kidney injury[23].

A number of studies have been conducted in order to elucidate the mechanisms involved in the AB-induced renal vasoconstriction. Apparently, the combined action of direct (endothelial metabolism) and indirect (by vasoactive substances) alterations could result in renal vasoconstriction. Natural renal vasoconstriction substances, such as angiotensin II and vasoactive amines, do not seem to be involved in this process[22].

Thereby, in 2000, molecular analyses were performed aiming to assess the mRNA synthesis of the enzyme nitric oxide synthase in kidney endothelial cells. The concentrations of AB between 2.5 and 5.0 µg/ml caused the inhibition of the gene transcription. Given the knowledge on the oxide nitric effect on the vasoconstriction function, it is possible to assert that its inhibition leads to complications to the maintenance of the contractile state, causing an acute vasoconstrictor and dose-dependent effect, which can be reversible in some cases[24].

Likewise, when fluids rich in sodium reach the kidney filtering units, they promote arteriolar vasoconstriction, reducing the blood flow and the GFR[25]. Thus, it is clear that the vasoconstriction response is also dependent of the patient's saline status. The reduction of the renal perfusion itself can damage the tubules – posterior region to the glomeruli – by ischemia.

The drug seems also to affect the mesangial cells (located among the capillaries of the glomerulus), inducing the contraction of these cells, causing a reduction of the glomerular flow and the filtration rate. This effect may be secondary to the calcium entrance in the extracellular space through voltage-gated ion channels[26].

Direct structural damage to tubular cells

The mechanism related to the direct toxicity to renal tubules are associated to the AB antifungal actions and also to its physicochemical characteristics, as follows: (i) amphipathic molecule (soluble in acid and base media, but not in water); and (ii) present a lipophilic portion[27, 59].

Since 1964, studies have reported the histological injury of tubular and interstitial cells. Therefore, important issues addressed to the interaction of AB with phospholipids present in human cell membranes were elucidated[28].

The integration promotes the membrane rupture, so that, by the gradient of concentration, Na^+ ions are pumped into the cell and K^+ and Mg^{++} are pumped out of the cell. As a result of injury to the cell membrane, the process of ions exchange occurs in large quantities, which induces the activation of the $3Na^+/2K^+$ATPase (adenosine triphosphatase) pump as an attempt to maintain the cell stability. Thus, the mitochondrial activity (dose-dependent) and the oxygen consumption are elevated[29]. When the mechanism of injury exceeds the capacity of adenosine triphosphate (ATP) synthesis, depletion of energy, formation of reactive oxygen species (ROS) and accumulation of calcium in the intracellular space occur[30]. Thus, sub lethal and lethal injuries occur in the cells by necrosis or apoptosis.

The reason why direct injuries to mammalian cells are restricted to the kidney are not well elucidated. However, since the AB administration is intravenous, without liver metabolization, high concentrations of the drug in the kidney filtering units (an epithelium of cells rich in cholesterol) are found, causing a restrict tissue injury[31].

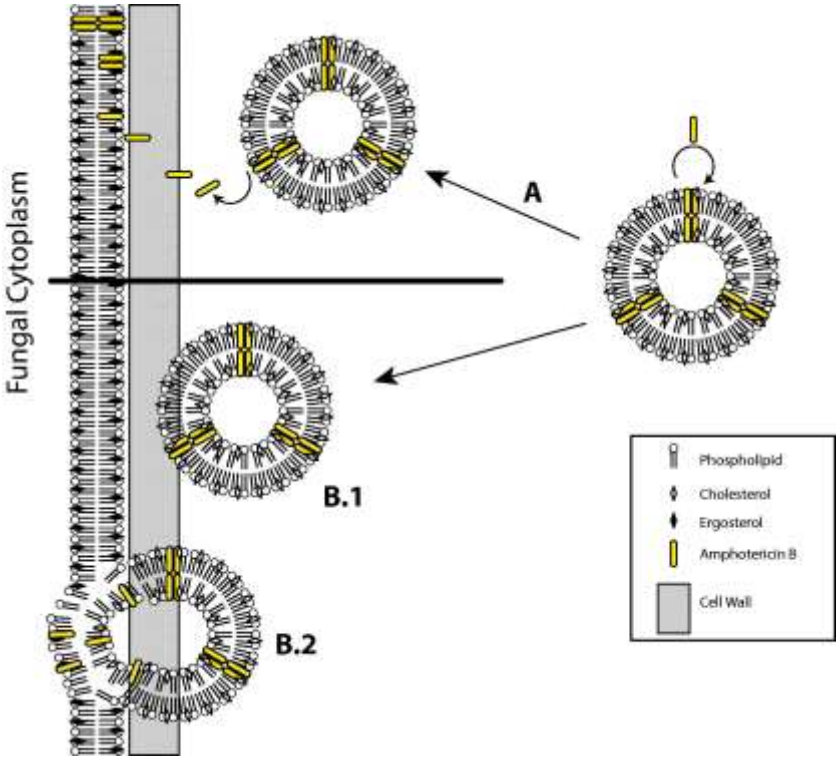
In 1996, it was suggested that the local pH could influence in the process of cell toxicity to AB[32]. Such study expused tubular cells in different pH to concentrations of AB ranging from 2.5 to 10 mcg/mL. Although the potassium depletion occurred independently of pH, higher pH (above 6.0) was associated to cell recovery in 6 h after the exposure to AB. Nonetheless, at low pH, cells became progressively depleted of ATP; they leaked lactate dehydrogenase and became irreversibly damaged after approximately 6 h. This means that low urinary pH, especially in distal tubules, could also explain why kidney cells are the main target of the AB-induced toxicity.

Alternative treatments to nephrotoxicity

Several studies have been proposed to investigate the safety and efficacy of these formulations. More recently, considering that lipid-based formulations reduce the nephrotoxicity and maximize the therapeutic use, data from a retrospective study assessing the safety of liposomal AB (LAB) in patients with kidney dysfunction, suggested that LAB was a safer agent than AB for the treatment of fungal infections in patients with a GFR lower than 60mL/min, being the reference value >90mL/min for adults[33, 34].

Furthermore, new technological approaches related to AB administration are being evaluated. For instance, oral and topic AB, where there is the incorporation of drug release systems (nanoparticles) and the higher solubility in aqueous medium, which results in oral absorption; and, to enable the topic use, the increased cutaneous permeation[31]. However, the mechanism of action of these formulations are not well elucidated (Figure 2). In the presence of cholesterol, free AB (i.e. outside the carrier) is almost inexistent (<1%), which explains the reduced incidence of effects related to infusion and to nephrotoxicity[35]. Likewise, an AB of oral administration incorporated to nanoparticles was developed and a reduction of nephrotoxicity in vitro was observed. Additionally, the low permeability over the membranes and poor stability in the gastric environment were attenuated, suggesting that chitosan functionalized (ε-caprolactone) nanoparticles may be an alternative treatment to nephrotoxicity in the future[36].

Figure 2 – Possible mechanisms of action related to AB incorporation to release systems.



(A) amphotericin B leaving the liposome that is perturbed by fungal wall and binds to ergosterol in the fungal membrane, or (B.1) the liposome crosses through the fungal cell wall (B.2) to fuse with the fungal cell membrane and facilitates the transference of AB[35].

Studies related to dose of lipid formulations and to parenteral routes are promising and invaluable to reduce the AB-induced toxicity and injury of the renal tissue.

BIOMARKERS OF ACUTE KIDNEY INJURY (AKI)

One of the most important issues addressed to clinical therapy of kidney injury is to diagnose and evaluate precisely the pathogenic condition of the patient. In order to achieve the therapeutic goal, it is necessary to validate specific and sensitive biomarkers[37]. In this context, the laboratorial analysis has an important role, since the early diagnosis, risk stratification and prognosis have been one of the main targets regarding the urinary system.

Endogenous metabolites, products of the reaction of substances, have a role in homeostasis. When alterations in the environment occur, their levels may also vary. Thus, more sensitive metabolites can be selected as potential biomarkers of AKI. The most commonly used biomarkers include creatinine (CR), urea (URE)[38] and other markers exclusively urinary, obtained by a physical, chemical and sediment analysis

of the urine (Table 1). Nevertheless, in case of oliguria/anuria, obtaining urine samples is complicated.

Table 1 – Markers of importance for nephrotoxicity in urine analysis

Physical analysis	Chemical analysis	Sediment analysis
Aspect	Hematuria	**Cell elements**
Collor	Proteinuria*	
Density	pH	Tubular cells/*Decoy***

*The traditional method is more efficient to detect albumin and may not detect tubular proteins.
**Detection of the presence of intact or remnant tubular cells.

Clinically, the serum CR is the marker used to detect the AKI[39], although there are several disadvantages of its use. First, it is affected by physiological interferences (dietary, age, sex, race, muscle mass and physical exercise) and other factors (infections and inflammatory condition). So, CR is not sufficiently specific to diagnose/monitor such situations. Second, serum CR and the GFR are hyperbolic (easily altered) and are not linearly related. Third, the CR can overestimate the kidney function, especially in the beginning (24 to 48 hours) of AKI[40].

The main nitrogenous metabolite derived from the protein catabolism is URE. Despite being filtered by the glomerulus and not being actively reabsorbed and secreted, URE is a poor predictor of glomerular filtration, since 40 to 70% of URE returns to the plasma by a process of tubular passive diffusion, which is dependent on the urinary flux[41]. Similarly to CR, physiological and pathological factors can alter the serum levels of URE (low, specificity). Despite this limitation, URE is more sensitive to kidney injuries in comparison to CR, presenting higher levels earlier[42].

Cystatin C (Cys C)

All nucleated cells constantly synthetize Cys C – an inhibitor protein of proteinases. Thus, this protein can be found in several fluids and, in contrast with serum CR, it is not affected by physiological factors. Because of its size and isoelectric point, Cys C is easily filtrated by the glomerulus and it is not reabsorbed by the proximal tubule (where it is catabolized), then it is not totally excreted in urine[50]. Thus this protein is clearly used as an endogenous marker to estimate the GFR, overcoming the barriers regarding the biomarkers of GFR and their detection restricted to urine.

Although there is no consensus considering the advantages of Cys C compared to CR, several clinical studies have reported the ability of both plasmatic and urinary Cys C to predict AKI. In a study conducted with 85 patients to diagnose AKI, higher levels of Cys C were detected between 1 to 2 days prior to CR detection[51]. In contrast, another study found no relevant alteration in the levels of Cys C, even when

histopathological analysis confirmed the occurrence of AKI, after the intravenous administration of AB for 10 days[46].

Considering the challenge of monitoring the kidney function in immunocompromised patients with protein malnutrition, which compromises the results when evaluating only serum CR, a study proposed to describe the use of Cys C. Indeed, the molecule was efficient to demonstrate the kidney injury and could be a reliable marker to adjust doses of drugs[49].

Furthermore, several equations have been proposed to estimate the GFR based on plasmatic levels of Cys C. Overall, the accuracy of the results is higher when compared to CR, however there are limitations of laboratory methods to determine the levels of Cys C, especially related to standardization of methods, thus reflecting in the conclusions of studies[52]. So far, it is known that higher levels of neutrophil gelatinase-associated lipocalin (NGAL) (discussed posteriorly) can be detected prior to Cys C in patients with AKI.

Kidney injury molecule-1 (KIM-1)

When the AKI occurs, there is an induction of the transmembrane glycoprotein KIM-1, which makes it a specific and sensitive biomarker in the monitoring of drug-induced kidney diseases. The function of this molecule is still not clear, but a role in the repair and regeneration of the renal tissue is postulated. Based on animal studies, KIM-1 is a promising biomarker for kidney tubular injury[53].

In addition, high concentrations of KIM-1 were detected in urine four days after the administration of AB related to severe tubular damage (microscopic analysis)[54]. Hence, there is great interest in investigating the specificity and early detection of KIM-1 in urine related to kidney injury and the determination of AKI.

Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin, a low-molecular-weight, acute phase protein originally identified in neutrophil-specific granules, is found in kidney and other tissues including liver, lung and gastrointestinal tract, where it is produced in response to tissue injury, inflammation or sepsis[55]. When present, besides indicating kidney injury, this protein is related to a reduction in the number of apoptotic tubular cells and an increase in proliferating proximal tubule cells, therefore reducing the impact of damage on the epithelium. Consequently, this protein plays a protective role of the organ, related to iron chelator and regulation of heme-oxygenase-1 synthesis. Studies on the proteomic analysis of NGAL in animal models revealed this protein can be a predictor of AKI and is highly expressed in tubule cells that are undergoing

proliferation[56] and detectable within two hours after renal ischemia[57]. Currently, commercial kits for the detection of NGAL in urine and plasma are available[47]. Both urine and plasma were tested in relation to the treatment with the AB lipid formulation (less nephrotoxic) and another non lipid AB. The following aspects were reported: (i) peak urinary NGAL levels were higher in patients with AKI in comparison with the patients without AKI; (ii) the protein was increased in the group of patients receiving the non-lipid AB; and (iii) AKI could be detected 3.2 days earlier by the use of the urinary NGAL than by the use of serum CR[45].

In contrast, another study compared the performance of the urinary protein with serum and urinary CR. The overall changes in the mean values of urine NGAL were not significant during the AB treatment. Nonetheless, levels of NGAL in the first day of treatment with AB were significantly higher than serum CR for predicting AB nephrotoxicity[58]. Likewise, in a study conducted with 138 critically ill patients, elevated NGAL was associated with sepsis independent of level of acute kidney dysfunction. Thus, using plasma NGAL as a marker of AKI should be avoided[43]. However, some limitations of the study were pointed: (i) different forms of NGAL were not specifically measured; (ii) few patients with AKI were included; (iii) short/insufficient period of evaluation to detect all events related to nephrotoxicity; and (iv) gene expression in studies with transgenic animals may lead to upregulation of this protein.

One important aspect that should be considered is that AB leads to a reduction of GFR and approximately 6 hours after a kidney injury, the expression and secretion of the NGAL protein increases in different sites of the tubule – the most common site of injury induced by AB[44]. In this perspective, the concomitant measurement of NGAL can be beneficial when evaluating all aspects of AB-induced nephrotoxicity, including extension and magnitude.

Although NGAL has been considered a reliable marker of AKI, studies are necessary to confirm its use in clinical practice and define adequate cut-offs for different populations and clinical situations[47, 48].

Considering the data available, it is possible to identify the origin of these biomarkers of kidney injury. Such information contributes to the management and future systematic characterization of the use of these biomarkers (Table 2).

Table 2 – Biomarkers of nephrotoxicity

Biomarker	**Local of kidney injury**	**Matrix**	**Method**
Cys C	Glomerular and tubular filtration	Serum, plasma, urine	Immunonephelometry, espectrophotometry
KIM-1	All segments of the proximal tubule	Urine	Immunoassay
NGAL	Proximal tubule	Plasma, urine	Imounoturbidimetry, imunoassay, radioimmunoassay

CONCLUSION

The AB resistance is only reported in the literature when evident, since it is not expected. Therefore, the analysis of data available on the subject might not reflect the real scenario of the frequency of the drug resistance. Once eminent, the mechanisms of low susceptibility of species should be carefully analyzed in order to minimize the risk of occurrence of possible outbreaks.

The adoption of release systems conjugated to AB is a promising alternative in the reduction of nephrotoxicity and prevention of fungal resistance. In relation to the biomarkers, it is more likely that instead of a punctual evaluation of a single biomarker, a set of biomarkers provides a more thorough comprehension on the nature, severity and extension of the AB-induced kidney injury. Thus, more clinical studies are necessary to evaluate the chronological evolution and performance of biomarkers so that they can be eventually implemented in the clinical routine.

Overall, the implementation of such technological innovations may provide new parameters for risk stratification, early diagnosis, therapeutic guidance and management, as well as prevention, prediction and reduction of AB-induced nephrotoxicity and resistance.

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