

# Drug-related perinatal damage from the pathological point of view

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## Proceedings

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*The role of the clinical pathological dialogue in problem solving*

**Guest Editors: Gavino Faa, Vassilios Fanos, Peter Van Eyken**

## Abstract

Drug dosage in the perinatal period represents a continuous challenge for the neonatologist because of interindividual variability of drug metabolism. The human liver plays a central role in the uptake, transport, metabolism and excretion of the vast majority of xenobiotics and drugs. The protein products of human CYP3A account for the largest portion of CYP450 proteins in human liver. At least 50% of currently used drugs in neonatal intensive care units (NICUs) are substrates of CYP3A4 including antibiotics, antivirals, antifungals, immunomodulators, benzodiazepines, proton pump inhibitors, steroid hormones and acetaminophen. The variable CYP3A4 and CYP3A7 expression recently reported in neonatal liver suggests the existence of a marked interindividual variability in drug metabolism during the intrauterine and neonatal lives and, as a consequence, the need of an individualized tailored therapeutic approach in NICUs. The increased risk for adverse effects reported for some drugs in neonates could be related to pharmacokinetic peculiarities of the newborn liver. The fetal and neonatal liver in infants undergoing drug-induced liver injury (DILI) is always characterized by the overlapping between developmental and pathological changes, the differential diagnosis between these changes representing often a challenge for the pathologist. Data here reported clearly evidence the peculiarity of the histological examination of the newborn liver, as compared to the adult liver. In conclusion, the role of the pathologist in the interpretation of liver reactions to drugs may be relevant, only when supported by the dialogue with neonatologists. A deep knowledge of the events taking place during

liver development at different gestational ages is necessary for a dedicated neonatal pathologist, in order to avoid misinterpretation of the histological changes related to liver development, giving them a pathological significance.

### Keywords

Drug, liver, newborn, CYP, cholestasis, hepatitis.

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### Introduction

Drug dosage in the perinatal period represents a continuous challenge for the neonatologist because of interindividual variability of drug metabolism. Genetic polymorphisms as well as enzymatic induction or down-regulation are at the basis of the unpredictable drug response in neonates. Moreover maturation of organ systems, paralleled by ontogeny of drug-metabolizing enzymes probably represents other chief factor accounting for the drug clearance. [1]. There is hard data showing that newborn infants are more likely than adults to experience adverse reactions to drugs [2]. The bioavailability of drugs in newborns shows marked differences then in adults and children according with the maturity of drug metabolizing system [3]. In fact, the human neonate should be considered a particular unique patient, according with the immaturity at birth and the progressive evolution of many metabolic functions, especially drug metabolizing enzymes in liver cells [4]. Developmental aspects should be considered in drug conjugation and glucuronidation, that are decreased in neonates in the vast majority of drugs. Gestational age and size body weight at birth should be taken into account in evaluation of drug dosing and toxicity. Besides, the pharmacokinetic parameters in the premature neonates differ so significantly from those of full term newborns, that a new research field defined developmental pharmacology begins to acquire more weight

[5]. This branch of pharmacology deals with the influence of genetic variation on drug response in the perinatal age, by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. Drug dosage should be continuously adapt for a single newborn, given the daily maturation of the drug metabolizing system according with the maturational changes of the perinatal period [6]. Effective and safe drug therapy requires a full understanding of human developmental biology and of the dynamic ontogeny of drug absorption, drug disposition, drug metabolism and drug excretion. The maturation of organ system during gestation and neonatal period exerts a significant effect on the disposition of drugs particularly in preterm infants [7]. Drug absorption may be influenced by multiple factors, including gastric pH, gastric emptying time, duodenal pH, intestinal transit time and bacterial colonization. Drug bioavailability and drug distribution may be modified by membrane permeability, fat content, total body water, plasma protein concentration, regional blood flow, and by endogenous substances in plasma as hormones [8]. Drug metabolism may be affected by ontogeny, CYP activity, expression of reductive and hydrolytic enzymes, glucuronosyl transferases, N-methyl-transferases and transporters important in drug absorption, distribution and excretion [9].

### Metabolism of drugs in the neonatal liver

The human liver plays a central role in the uptake, transport, metabolism and excretion of the vast majority of xenobiotics and drugs [10]. The hepatocytes represent highly specialized cells, polarized into two distinct poles: a sinusoidal and a biliary one. Particular transporters located on the cell membrane allow the passage from the sinusoidal lumen across the hepatocytic membrane into cytoplasm of lipid-soluble drugs weighing more than 500 daltons [11]. Specific cytosolic proteins and chaperons bind and transport internalized drugs towards the enzymes of the drug metabolizing system [12]. Two main enzyme groups can be recognized. One, related with phase 1 enzymes, is involved in oxydation, and hydroxylation reactions, the other, consist of the phase 2 enzymes, in reactions mainly mediated by cytochrome P-450 (CYP). The former are enzymes mainly involved in esterification reactions, conjugation with sulfate, glucuronic acid, amino acids and glutathione molecules, generally ensuing increased water

solubility, decreased pharmacologic activity and eventually detoxification of xenobiotic compounds [13]. The excretion of drugs, metabolites and conjugates into bile or into the sinusoidal circulation characterize the final step of the hepatic drug metabolism, recently referred to as phase 3 of the hepatic drug metabolism, and take account of the multidrug resistance (MDR) protein and the multidrug resistance-related proteins [14]. The same mechanisms involved in hepatic drug metabolism, on the other hand, may cause the production of toxic metabolites, leading to drug-induced liver injury (DILI), under the following conditions: increased levels of drug, phase 1 and phase 2 enzymes that does not work suitably, or genetic alterations responsible of hepatobiliary defective drug-transporters or drug-metabolizing enzymes [14].

Pharmacokinetic and pharmacodynamic differences have been reported between children, mainly between newborns and preterm infants, and adults [3]. Several studies show that the metabolic profile of the neonate significantly differ in the absorption, distribution, metabolism and excretion of many drugs, according with marked biochemical differences in sulphation, glucuronidation, conjugation and elimination [3, 15]. A quantity of biotransformation pathways, as hydroxylation and glucuronidation, demonstrate restricted activity at birth [16], whereas other pathways, such as sulphate or glycine conjugation, appear more efficient in the newborn than in adult subjects [17]. In clinical practice, the drug dosing for diverse drugs is based on this physiological diversity of metabolic biotransformation pathway in different ages. Newborns present a greater capacity, when compared to adults, to synthesize glutathione, thereby more effectively inactivating toxic metabolites produced by the activity of phase 1 enzymes on many drugs [16]. Some enzyme activity, including thymidine kinase and ornithine decarboxylase, is high during foetal life and at birth, and falls during the postnatal period. On the contrary, other liver enzymes, including aspartate aminotransferase, show a low expression in the human foetus, but increase their expression after birth [18]. Another group of liver enzymes is expressed only in the perinatal period, and increases after birth [14]. Drugs that are metabolically eliminated solely by specific CYP isoenzymes decreased in children, need weight-corrected doses in children, higher than adult doses. The preterm infant and the developing foetus have different risk

from drug-toxicity as compared to adults, and are responsible for the different effects of the same drug on liver at various stages of development. For example, the lower expression in the newborn of the liver enzymes involved in glucuronide conjugation, has been proposed as a possible responsible for the gray infant syndrome from chloramphenicol [16]. The balance between the activating phase 1 enzymes and the detoxifying phase 2 enzymes is crucial in order to eliminate the drug metabolites [19]. Many factors may influence this key balance, counting enzymatic inducers, affecting unduly phase 1 or phase 1 enzymes [14]. Taken at once, children cannot apparently be regarded as “small adults” with respect to drug therapy: they often need a different fraction of drug per kg of body weight as compared to adults.

### **Drug excretion and metabolism in the newborn kidney**

In clinical practice, data on the functional maturation of the drug transporters' system in preterms, in low birth weight newborns and in neonates mainly depend on the aptitude of the neonatologist to look into each neonate and be aware of the degree of functional maturation. In the neonate drug elimination relies heavily on renal excretion as a consequence of the slower rate of biotransformation activity typical of the newborn liver, which consist of slower rate of biotransformation and slower overall elimination of drugs [20]. A detailed examination of the expression pattern of the main drug transporters in the newborn kidney, as well as in all stages of human development, has hitherto to be carried out. Drugs eliminated by renal excretion or metabolism involving N-acetyltransferase 2, or uridine diphosphate glucuronosyltransferase (UDGT) are similar in newborns and adults, so necessitate the sole weight-corrected doses [21]. For example as for digoxin, a drug excreted by glomerular filtration but also secreted by the tubular renal cell, newborns need threefold higher doses per kg of body weight than adults [22]. The common observation in clinical practice of clear interindividual differences in drug responses among neonates, particularly among preterms, is related in neonatology to the concept of “tailor made” drug therapy, specifically a customised drug therapy based on the peculiar metabolic system of each neonate, irrespectively of the “general”, often adult-related therapeutic protocols [4].

## The CYP450 superfamily

The cytochrome P450 superfamily in humans is formed by 57 genes and 58 pseudogenes, encoding for 57 functional enzymes (monooxygenases) critical for the metabolism of numerous endogenous and exogenous compounds. The superfamily is organized into 18 P450 families and 44 subfamilies. CYP nomenclature is based on the identity of amino acids: members of each family share more than 40% identical sequences; members of each subfamily, are characterized by more than 55% identical sequences. A clan is formed by different families derived from a common ancestor [23]. In particular CYP3 clan members derive from a common ancestor which developed 800-1,100 million years ago: in invertebrates, CYP3 clan includes two families, named CYP3 and CYP5. The latter is also known as thromboxane synthase, and it is involved in metabolism of prostaglandin H<sub>2</sub> to thromboxane A<sub>2</sub>, a key molecule in hemostasis and thrombosis. The most important functions of monooxygenases of the CYP450 superfamily are related to:

1. metabolism of endogenous compounds, including growth factors, hormones, fatty acids, prostaglandins, cholesterol, bile acids, vitamin D;
2. detoxification of exogenous xenobiotics, including environmental pollutants, foreign chemicals and carcinogens;
3. decomposition of the vast majority of currently used drugs.

The expression of CYP450 enzymes in the human body is characterized by a marked substrate and tissue specificity. In humans, the most important isoenzymes for drug metabolism are localized in the liver, seven isoenzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4) being responsible for the metabolism of more than 95% of drugs [24]. CYP450 isoforms involved in drug metabolism are also present in kidney, lung, brain, breast, prostate and in the small intestine [25]. The clinical importance of CYP450 is represented by the myriad of enzymes encoded by the 57 CYP genes, enzymes that are implicated in important life processes, including drug metabolism, foreign chemicals metabolism, arachidonic acid metabolism, cholesterol metabolism, bile acid biosynthesis, steroid synthesis, vitamin D<sub>3</sub> synthesis and retinoic acid hydroxylation. The function of this CYP enzyme is mainly associated to hydroxylation of retinoic acid and to 16 alpha-hydroxylation of steroids, playing a key role in normal development, as well as in carcinogenesis [26].

Mutations in CYP450 genes may result in the following human diseases:

1. glaucoma (mutated CYP1B1);
2. familial hypercholesterolemia (CYP7A1 mutations);
3. congenital adrenal dysplasia (mutations in CYP11B1);
4. congenital hypoaldosteronism (CYP11B2 mutations).

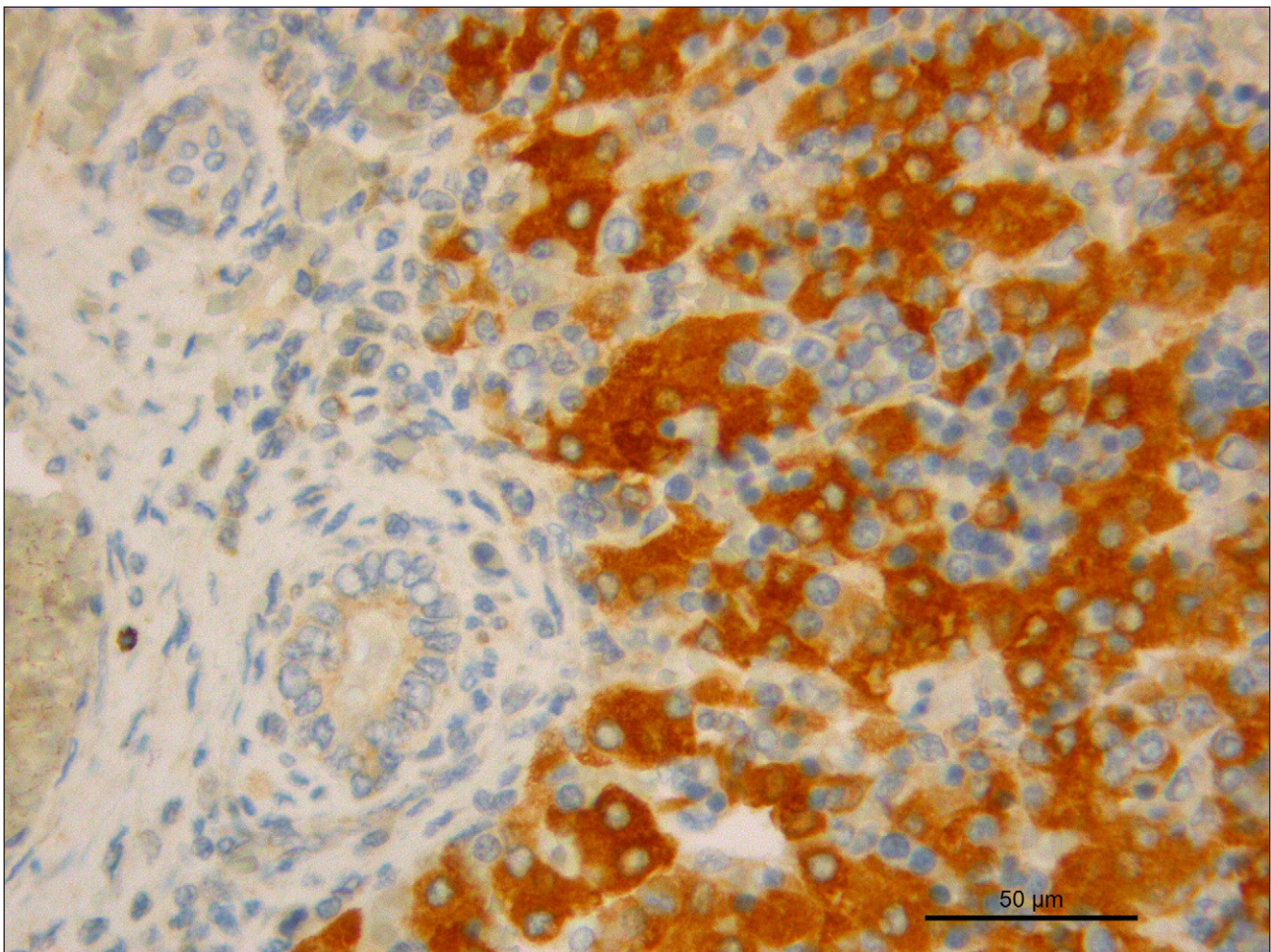
## CYP3A4 and CYP3A7 expression in the neonatal liver

The protein products of human CYP3A account for the largest portion of CYP450 proteins in human liver. The human CYP3A subfamily consists of four genes that encode for CYP3A4, CYP3A7, CYP3A5 and CYP3A43. CYP3A4 and CYP3A7 are the most abundant hepatic members of the P450 family and account for the majority of the oxidative metabolism of clinically relevant drugs in humans [27-31]. CYP3A4 has been reported to be undetectable during gestation and at birth, dramatically increasing after the first postnatal week and reaching adult levels around the first month after birth. At least 50% of currently used drugs in neonatal intensive care units (NICUs) are substrates of CYP3A4 including antibiotics, antivirals, antifungals, immunomodulators, benzodiazepines, proton pump inhibitors, steroid hormones and acetaminophen [1, 12, 32, 33]. It has been claimed that the low content of CYP3A4 in the neonate at birth might be the main responsible for the impaired metabolism of cisapride in the perinatal period, resulting in accumulation of the drug and cardiac toxicity [34]. CYP3A7 has been reported to be predominantly expressed in the fetal liver, the CYP3A7 gene being silenced in the perinatal period, normally within the first postnatal week. Catalytic activity is detectable as early as 50-60 days of gestation in embryonic liver, where it catalyzes 16-alpha-hydroxylation of dehydroepiandrosterone 3-sulphate (DHEA-S), an intermediate metabolite in the estriol biosynthesis [35]. CYP3A7 expression is characterized by interindividual variability: in some adult livers, the enzymatic activity does not end at birth, but it continues in adulthood, being detectable even extra-hepatically. CYP3A4 represents 30-40% of the total CYP content in human adult liver and in small intestine mucosa cells. CYP3A7 is the major isoform detected in embryonic, foetal and newborn liver, being also detectable at low levels in adult liver cells [36]. A marked interindividual



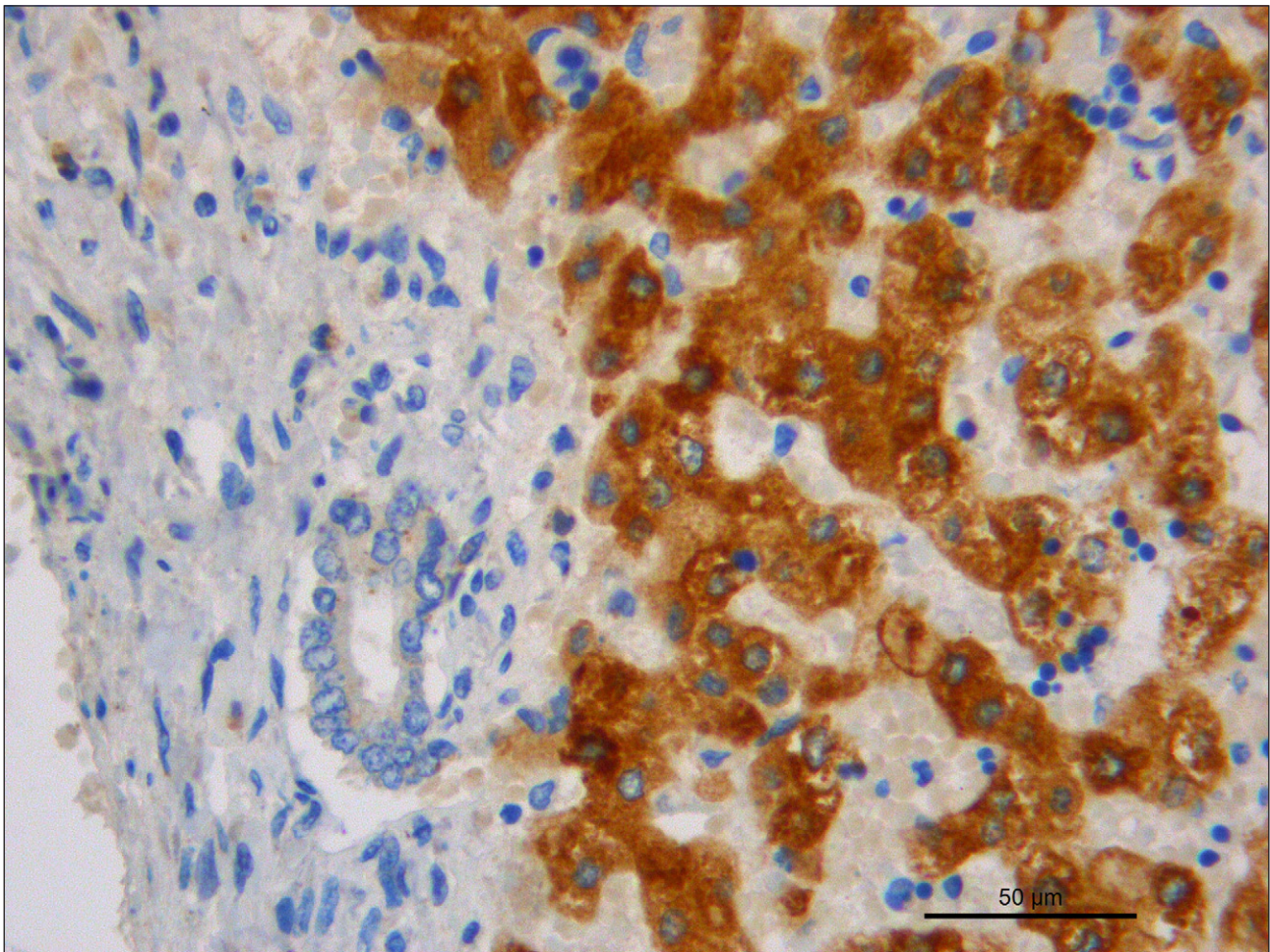
variability has been reported regarding CYP3A isoforms' expression and activity: in particular, the variable expression of CYP3A7 in fetal liver, starting from the ninth week of gestation, suggests that significant interindividual differences in drug metabolism may already exist at the prenatal stage, these differences contributing to individual pharmacological responses in the intrauterine life [37]. CYP3A7 is reported to be the dominant enzyme in fetal liver, with a subsequent age-related shift between CYP3A7 and CYP3A4 [38]. Our data on the immunohistochemical detection of CYP3A4 and CYP3A7 during development show, for the first time, that the relationship between these two CYP450 isoforms should be considered much more complex than previous thought [39]. First of all, accordingly with our data, CYP3A4 expression (**Fig. 1**) is not restricted to the postnatal age. In fact, it was detected in the vast majority of fetal livers, at all gestational ages tested and the expression was strong, diffuse and detected in all acinar zone.

Immunoreactivity for CYP3A7 (**Fig. 2**) was similar with very few differences. Just in one case both CYP3A4 and CYP3A7 were not detected in liver cells. Taken together, these data clearly show that the previously reported schemes base on restriction of CYP3A7 to the prenatal life, with a shift between CYP3A7 and CYP3A4 occurring immediately after birth [38] cannot be accepted in the human liver. Another relevant finding emerging from this study is the high interindividual variability observed among subjects at all gestational age. Liver from fetuses with similar gestational age frequently showed striking differences in CYP3A4 and CYP3A7 expression. The spectrum of the complex relationship between the two CYP450 isoforms was completed by cases in which CYP3A4 expression was higher than CYP3A7 [39]. These data clearly evidence that gestational age is not the most important variable during fetal life regarding the hepatic expression of CYP3A4 and CYP3A7. High and low expression of both enzymes being detected



**Figure 1.** Immunostaining for CYP3A4 shows a strong reactivity in the cytoplasm of periportal zone hepatocytes in newborn at 23 weeks; 40 HPF.





**Figure 2.** Immunostaining for CYP3A7 shows a strong reactivity in the cytoplasm of periportal zone hepatocytes in newborn at 29 weeks; 40 HPF.

at all gestational ages. On the contrary, this data suggest that other covariable contribute to a high phenotypic interindividual variability in CYP3A isoforms expression during intrauterine life. From a practical point of view, this variable CYP3A4 and CYP3A7 expression suggests the existence of a marked interindividual variability in drug metabolism during the intrauterine life and neonates and as a consequence the need of an individualized tailored therapeutic approach in NICUs.

### Liver injury

The vast majority of drugs are metabolised inside the hepatocytes, generating toxic metabolites and predisposing the liver to drug toxicity [40]. Hundreds of drugs have been implicated in the aetiology of toxicity in the liver and have been associated with adverse events related to primary liver involvement [41]. The clinical picture of hepatitis belonging to drug toxicity is as well better defined as DILI [42] or

drug-induced liver disease (DILD) [43] and ranges from a mild reversible increase of transaminases in serum levels to acute fulminant hepatic failure [44]. Because of the significant low ability of neonates to metabolize the vast majority of drugs, even with drug conjugation and glucuronidation, the developmental aspects should be considered [15]. The pharmacokinetic peculiarities between neonates, children and adults may play a relevant role in the different populations age-related drugs adverse effects. In neonates an increased risk for adverse effects has been reported for some drugs, while others drugs seems to give a protection against hepatotoxicity [45]. A recent issue underlines the scarcity of drug studies in intensive care newborns and point out the widely different dosing guidelines in the four most commonly used drug formularies in the intensive care units, regarding doses per kilogram, dose description and dosing regimen [20]. Reliable guidelines for intensive care newborns are required. Many factors may play a role in

pharmacokinetic of drugs. Among them, biological rhythms, for example the sleep-wake, the circadian hormones secretion and the daily temperature trend have been proposed to deeply influence the action and toxicity of drugs [46]. Asphyxia enhances gentamicin ototoxicity [47]. Therapeutic whole body hypothermia is useful for treatment of hypoxic-ischemic encephalopathy in neonates, but has been shown to influence the metabolism of many drugs. In fact the administration under hypothermia of phenobarbital, because of longer half-life, results in higher plasma concentrations than in normothermic newborns. This evidence suggests a lower rate of liver metabolism by the hypothermic liver [48]. Moreover, topiramate in neonates under whole body hypothermia effect delayed time of maximal serum concentration, higher serum values and lower total body clearance. Again, lower absorption and elimination when compared to normothermic newborns were reported [49]. Size and gestational age at birth should also be taken into account when drug dosing and toxicity are considered: the pharmacokinetic parameters in the premature neonates have been shown to differ significantly from those of full term neonates [50]. The developmental pharmacology aims quite to study during the perinatal period the impact of birth and of gestational age on drug response [51], in order to induce the NICUs neonatologists to continuously adapt drug dosage for each newborn, according with the daily drug metabolizing system maturation and the relevant consequences in the pharmacokinetics of many drugs [14].

### Pathological features

The diagnosis of drug and toxic hepatic injury begins with careful morphological evaluation of the liver for the types of cellular injury as well as the overall pattern [52]. While it is true that hepatotoxicity can replicate essentially every other type of liver disease, individual hepatotoxins display a more limited range of injury. Hepatotoxins may injure any or all of the cell types found in the liver, but particular attention should be paid to hepatocytes, bile duct cells and endothelial cells. Thus, identification of the pattern of injury can be very helpful in both including and excluding hepatotoxicity as the cause of liver dysfunction. Cell injury may result in necrosis, apoptosis, cholestasis, steatosis, cytoplasmic inclusions, or pigment accumulation depending on the degree of injury and the cellular elements involved. Cellular injury may

vary across the hepatic lobule and may affect the different cell types in different ways [53]. At last, the peculiar type of cellular injury will depend on the particular agent involved (**Tab. 1**). Severe injury may lead to fulminant hepatic failure by several pathways, while subacute and chronic injury can lead to fibrosis or cirrhosis. The overall outcome is a pattern of injury that is characteristic of the agent involved, and this pattern may be modified by the genetic and biochemical background of the host as well as the response of the host's immune system to the injury [54]. It is important to remember that when reporting instances of DILI, that pathological descriptive words like hepatitis, necrosis, steatosis and cholestasis should only be used if tissue is available for examination. Toxic injury frequently takes the form of either necroinflammatory injury or cholestatic injury [53]. Necroinflammatory injury is characterized by relative degree of cell death and inflammation. The necrosis observed in necroinflammatory injury may be non-zonal, zonal or massive. As a general rule, the necrosis might be zonal or not. Both zonal and non-zonal necrosis can result in massive necrosis. Individual cells involved in necroinflammatory injury can undergo a variety of changes. Cell death can occur by two processes: necrosis and apoptosis [54, 55]. Necrosis refers to destructive disintegration of the cell, apparently initiated by plasma membrane injury and yielding only debris. Apoptosis (programmed cell death) is an energy-dependent event leading to cell shrinkage, nuclear fragmentation, condensation of chromatin and production of cytoplasmic blebs yielding distinct fragments of the cells or entire condensed shrunken cells. The condensed cells are referred to as apoptotic or free acidophilic bodies. The mechanisms for the two processes are different [56, 57]. Necrosis results when there is massive injury to the cells. Frequently this is due to massive injury to the cell membrane and organelles. Apoptosis may occur when the cell suffers similar direct injury, but in lesser degrees. Thus, apoptotic hepatocytes are sometimes seen adjacent to zones of confluent necrosis. Ballooning hepatocellular changes, steatosis and other degenerative features may also be seen as components of necroinflammatory injury. Pure zonal necrosis may involve the perivenular (zone 3), periportal (zone 1) or rarely the mid-zone (zone 2). Histologically, confluent coagulative necrosis may be observed. As the injury becomes more severe it will extend to involve other zones with bridging necrosis. Massive necrosis of the zonal type tends to retain its zonal



**Table 1.** Type of cellular injury depending on the particular agent involved. Hepatocellular injury: mainly degeneration and necrosis with or without inflammation. Cholestatic injury: bilirubin casts in canaliculi with or without portal inflammation and with or without mild parenchymal injury.

Agent	Injury pattern
<b>Antibiotics</b>	
Chloramphenicol	Hepatocellular injury Hepatocellular injury and cholestatic injury
Cephalosporins	Cholestatic injury
Amoxicillin	Abnormal transaminase levels
Amoxicillin-clavulanic acid	Cholestatic injury
Ampicillin	Hepatocellular injury
Macrolides	Cholestatic injury
Erythromycin	Cholestatic injury
<b>Anti-retroviral</b>	
	Hepatocellular injury
<b>Non-steroidal anti-inflammatory drugs (NSAIDs)</b>	
Ibuprofen	Hepatocellular injury Hepatocellular and cholestatic injury (rare) Steatosis
Indomethacin	Hepatocellular injury Microvesicular steatosis Cholestasis
Acetaminophen	Hepatocellular injury
<b>Glucocorticoids</b>	
	Steatosis and cholestatic injury

character. The zonality of necrosis may be related to the mechanism of injury. Lobular, lymphocytic infiltrate with scattered apoptotic hepatocytes can be detected. Increased numbers of eosinophils can be seen and suggest the diagnosis of DILI. There may be other evidence of hepatocellular injury, such as ballooning degeneration and steatosis and regenerative changes such as mitoses, hepatocyte rosette formation and the proliferation of ductular hepatocytes. Portal inflammation and interface hepatitis are often present, as is bile duct injury, but they do not dominate the overall inflammatory pattern [53]. Reticulin stains will show the zones of collapse and periportal fibrosis may be present. More extensive fibrosis may occur as a result of extensive necrosis if the patient survives. In case reports of DILI, necrosis has been one histological feature clearly associated with a fatal outcome [58], although the degree of necrosis is undoubtedly important. The presence of cholestasis (either canalicular or hepatocellular) should lead the pathologist to consider mixed injury. In some cases the injury mainly spares the parenchyma and instead causes arrested bile flow [53]. This mild form of cholestatic injury consists mainly of bile accumulation in the cytoplasm of liver

cells (hepatocellular cholestasis) and in canaliculi (canalicular cholestasis). Hepatocellular cholestasis is frequently marked by cell swelling, and the bile itself may be difficult to identify without the use of special stains. Iron and copper stains may both be more useful in this regard than a hematoxylin and eosin (H&E) stained section because of their light counterstains that allow the bile pigment to be more easily seen. An iron stain is doubly useful in that it can be used to distinguish iron (blue) from lipofuscin (granular and dirty brown) from bile (pale green to greenish brown). The cholestasis is usually most prominent in zone 3, and one should be careful to exclude other processes that may result in zone 3 cholestasis. There is sometimes a mild degree of parenchymal injury and inflammation (particularly portal inflammation) associated with acute cholestasis. Intrahepatic cholestasis accompanied by significant portal or lobular inflammation should be classified as a mixed hepatocellular and cholestatic injury. When acute intrahepatic cholestasis is associated with a necroinflammatory pattern of acute or chronic hepatic injury, the result is a mixed form of hepatic injury. This is a common manifestation of DILI due to many agents and should strongly suggest a drug aetiology when



present [52]. Histologically, there is hepatocellular or canalicular cholestasis present, as in the acute cholestatic injury described above, along with portal and lobular inflammation. The inflammatory component may resemble either the acute or chronic hepatic patterns described above. Variable degrees of portal and lobular inflammation may be present, as well as hepatic apoptosis, necrosis, steatosis or other evidence of hepatic injury [53].

## Discussion

Data here reported clearly evidence the peculiarity of the histological examination of the newborn liver, as compared to the adult liver. The fetal and neonatal liver is always characterized by the overlapping between developmental and pathological changes, the differential diagnosis between these changes representing often a challenge for the pathologist. Major differences regard the following aspects: cholestasis, the immaturity of the intrahepatic biliary tree, the presence of hematopoietic cells in the fetal liver, steatosis, liver cell death.

### *Cholestasis*

The ability to synthesize and excrete bile components, including bilirubin and bile acids, is immature in the neonate, and, when associated with low glucuronidation activity, may favour the insurgence of physiologic jaundice. The developing fetus and the preterm infant are at differential risk for cholestasis from toxic injury caused by different drugs (**Fig. 3**). Moreover cholestasis is one of the distinctive consequences to drug hepatotoxicity and the reduced capacity to synthesize and excrete bile, usual of each newborn, facilitates the neonate particularly susceptible to cholestasis from toxic injury due to drugs. Bilirubinostasis and cholestasis are the principal morphological features indicating the interference of a drug on bile formation and excretion [14].

### *The immaturity of the intrahepatic biliary tree*

The histological examination of the neonatal liver, particularly in preterm infants, is characterized by the progressive regression of the ductal plate, the redundant biliary structure developed at the periphery of each portal tract that undergoes involution during gestation, ending with the maturation of a single septal duct in every portal tract at birth. During regression, multiple biliary ductules

may be observed at the periphery of portal tracts, in close proximity to the periportal limiting plate [59]. These remnants of the ductal plate should not be erroneously interpreted as newly formed ductules, suggesting a wrong diagnosis of cholestatic disease.

### *The presence of hematopoietic cells in the fetal liver*

Hematopoietic cells characterize the fetal liver, with marked differences in the amount of progenitors of the different cell types normally differentiating in the hematopoietic tissues (**Fig. 4**). The precursors of blood cells may be located inside the liver acini, being aggregated in clusters inside the sinusoidal lumen, and in the developing portal tracts [53, 60]. The latter should be differentiated from inflammatory infiltrates, avoiding a wrong diagnosis of hepatitis.

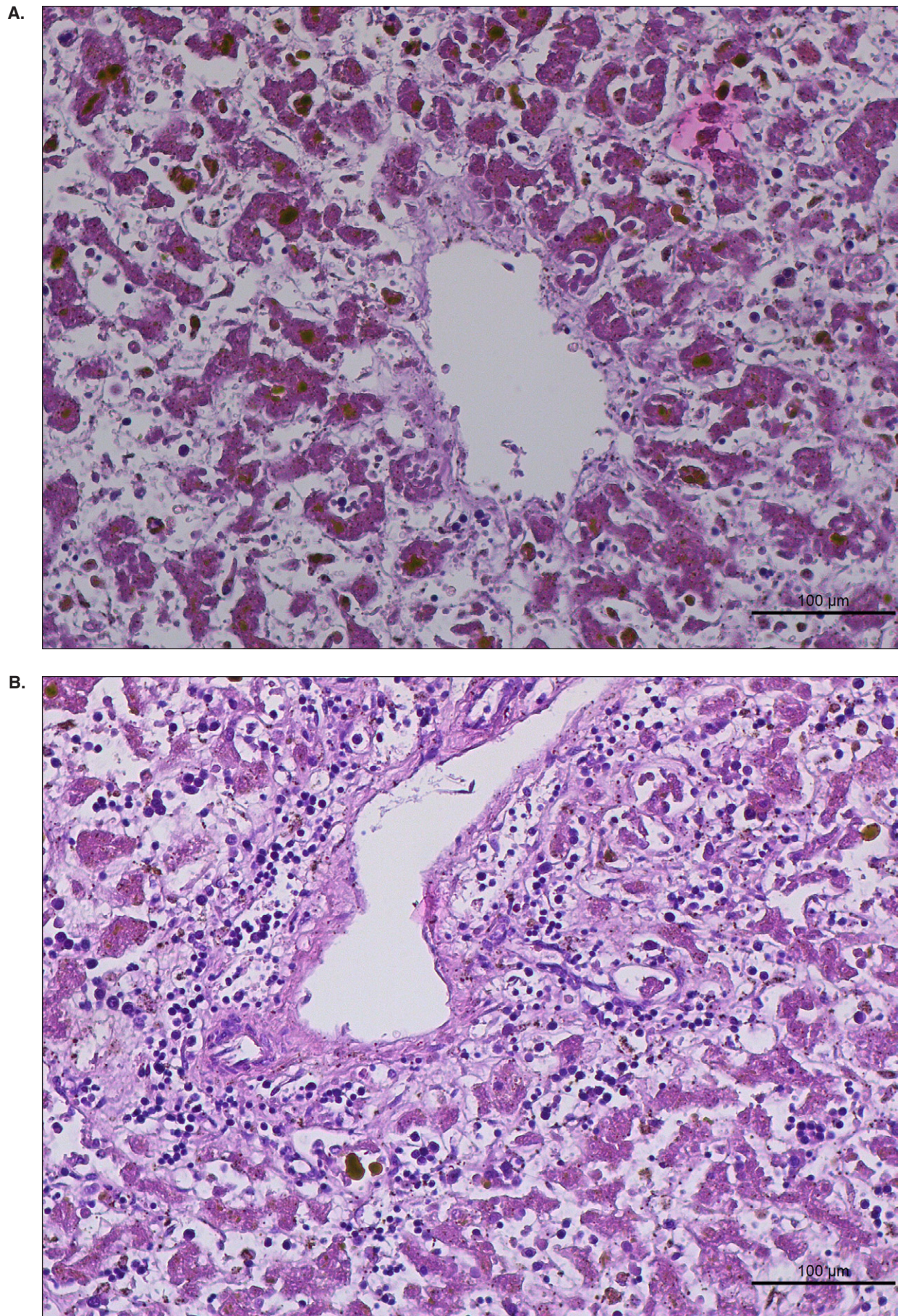
### *Steatosis*

Another very frequent lesion in drug-related liver disease is steatosis, i.e. fat accumulation inside hepatocytes, appearing as clear droplets different in size, ranging from very small (microvesicular steatosis) to large vacuoles occupying the entire cytoplasm and pushing the nucleus at the cell border (macrovesicular steatosis) [8]. Whereas macrovesicular steatosis should be considered a pathological feature in the adult as well as in the neonatal liver, the interpretation of microvesicular steatosis in the fetal and in the neonatal liver may be complex, and often impossible. In the neonatal liver, microvesicular degeneration is frequent and, according with many authors, its clinical significance is low. The differential diagnosis between the microvesicular steatosis, indicating a toxic reaction, and microvacuolization may be difficult on paraffin-included and H&E-stained sections. To this end, a particular procedure may be utilized in the analysis of the neonatal liver. A small bioptic or autoptic sample should be frozen, and utilized for histochemical stains for fat, that will allow a diagnosis of microvesicular steatosis and of toxic (possibly drug-related) liver damage.

### *Liver cell death*

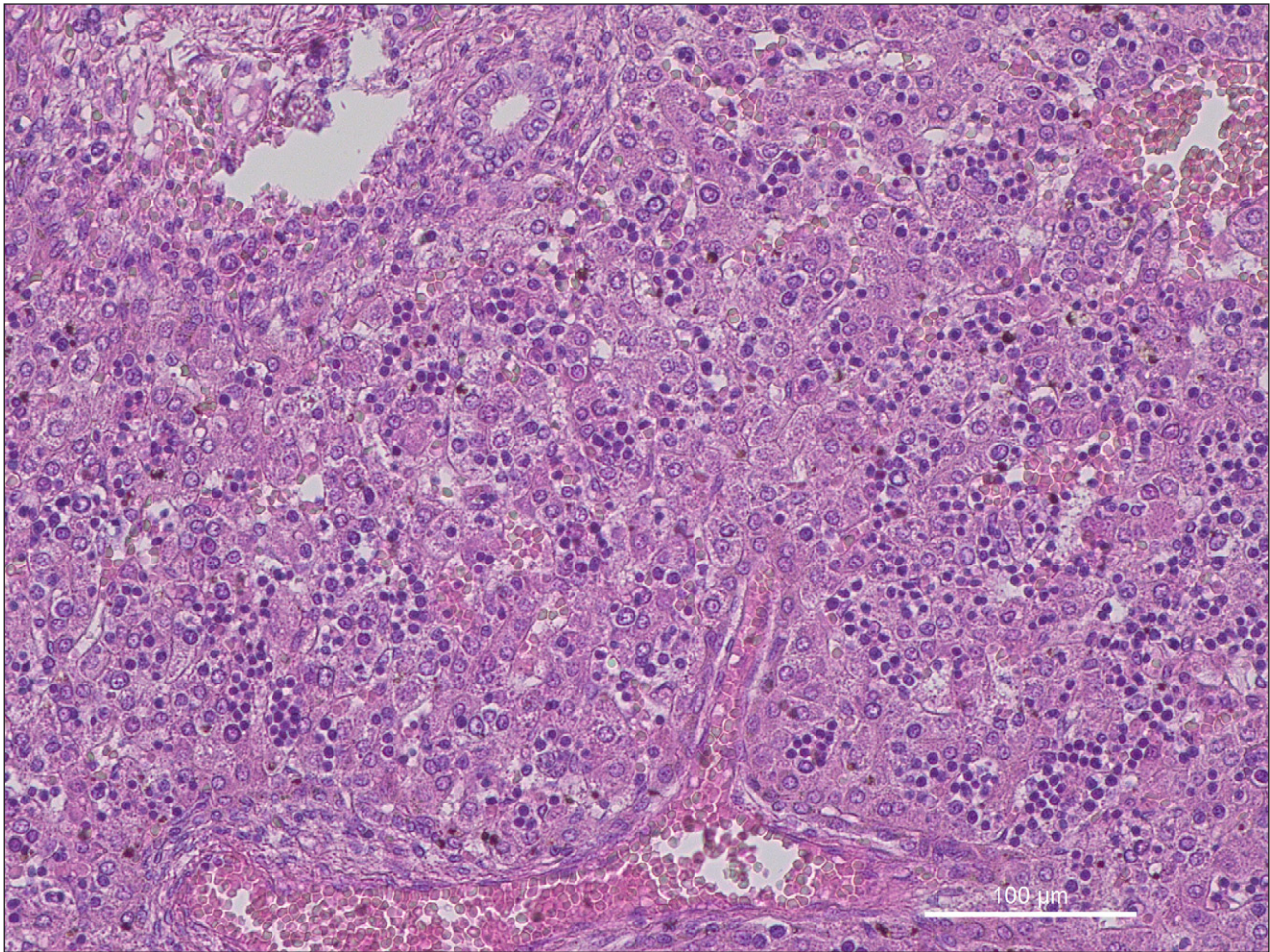
The interpretation of apoptotic liver cells may be complex in the neonatal liver. Whereas in the adult liver the histological evidence of apoptotic bodies is always considered a typical mark of liver damage [14, 56, 57], this is not the case in





**Figure 3.** Peritermal (A) and periportal (B) cholestasis in a newborn at 29 weeks; hematoxylin and eosin (H&E), 40 HPF.





**Figure 4.** Hematopoietic cells in the fetal liver in newborn at 38 weeks; hematoxylin and eosin (H&E), 40 HPF.

the neonatal liver. In fact, during development, apoptosis represents the physiological mechanism balancing cell proliferation by mitosis. As a consequence, the finding of apoptotic bodies, both in hepatocyte precursors, as well as in biliary cells and in hematopoietic cells should be evaluated with caution as a sure sign of toxic damage. On the contrary, the finding of massive necrosis, with a zonal distribution or massive, should be considered a certain marker of severe liver disease [53-55], inducing to consider, in the differential diagnosis, even a possible drug-related toxic reaction.

When a toxic drug reaction is diagnosed or suspected on morphological ground in a neonatal liver, the clinical-pathological dialogue appears mandatory. The neonatologist will give data regarding the (often numerous) drugs the newborn was under treatment and the pathologist will compare the possible toxic effect of each drug with the elementary lesions detected. Whereas, for example, non-steroidal anti-inflammatory drugs (NSAIDs) are predominantly associated with a

necroinflammatory reaction, steroids are associated with steatosis and cholestasis [8, 14].

In conclusion, the role of the pathologist in the interpretation of liver reactions to drugs may be relevant, only when supported by the dialogue with neonatologists. A deep knowledge of the events taking place during liver development at different gestational ages is necessary for a dedicated neonatal pathologist, in order to avoid misinterpretation of the histological changes related to liver development, giving them a pathological significance.

#### Declaration of interest

The Authors declare that there is no conflict of interest.

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