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In vitro testing for diagnosis of idiosyncratic adverse drug reactions: Implications for pathophysiology

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Idiosyncratic drug reactions (IDRs) represent a major health problem, as they are unpredictable, often severe and can be life threatening. The low incidence of IDRs makes their detection during drug development stages very difficult causing many post-marketing drug withdrawals and black box warnings. The fact that IDRs are always not predictable based on the drug's known pharmacology and have no clear dose–effect relationship with the culprit drug renders diagnosis of IDRs very challenging, if not impossible, without the aid of a reliable diagnostic test. The drug provocation test (DPT) is considered the gold standard for diagnosis of IDRs but it is not always safe to perform on patients. *In vitro* tests have the advantage of bearing no potential harm to patients. However, available *in vitro* tests are not commonly used clinically because of lack of validation and their complex and expensive procedures. This review discusses the current role of *in vitro* diagnostic testing for diagnosis of IDRs and gives a brief account of their technical and mechanistic aspects. Advantages, disadvantages and major challenges that prevent these tests from becoming mainstream diagnostic tools are also discussed here.

Introduction

Idiosyncratic drug reactions (IDRs), are important health problems that can cause extra patient suffering or death and high healthcare cost. Accurate diagnosis is key to effective management and prevention. Clinical diagnosis of IDRs can be difficult and often is inaccurate, if based only on medical history and physical examination. While drug provocation testing (DPT) (drug challenge or systemic re-exposure) is considered the gold standard for diagnosis of IDRs, it can be ethically problematic to perform due to possible severe consequences and it is contraindicated in patients with suspected severe reactions such as Stevens-Johnson syndrome (SJS), toxic epidermal neclolysis (TEN) and DRESS (Drug Rash with Eosinophilia and Systemic Symptoms) syndrome [1]. Other *in vivo* tests such as the patch test or transdermal applications may cause reaction flare-ups or even systemic reactions [2]. Currently, known *in vitro* tests are not in wide clinical use largely due to their complicated and expensive procedures as well as their undetermined predictive values [3, 4]. This review evaluates the role of *in vitro* testing for the diagnosis of IDRs and discusses some technical and mechanistic aspects, and challenges that prevent these tests from becoming a mainstream clinical approach in management of IDRs.

According to the classification proposed by Rawlins & Thompson adverse drug reactions (ADRs) are either type A reactions, which are predictable, dose-dependent and related to the pharmacological action of the drug or type B reactions, which are unpredictable, have delayed onset, typically unrelated to the drug pharmacology (or at least the known pharmacology) and do not have clear dose-dependency [5]. Type A ADRs are the most common and

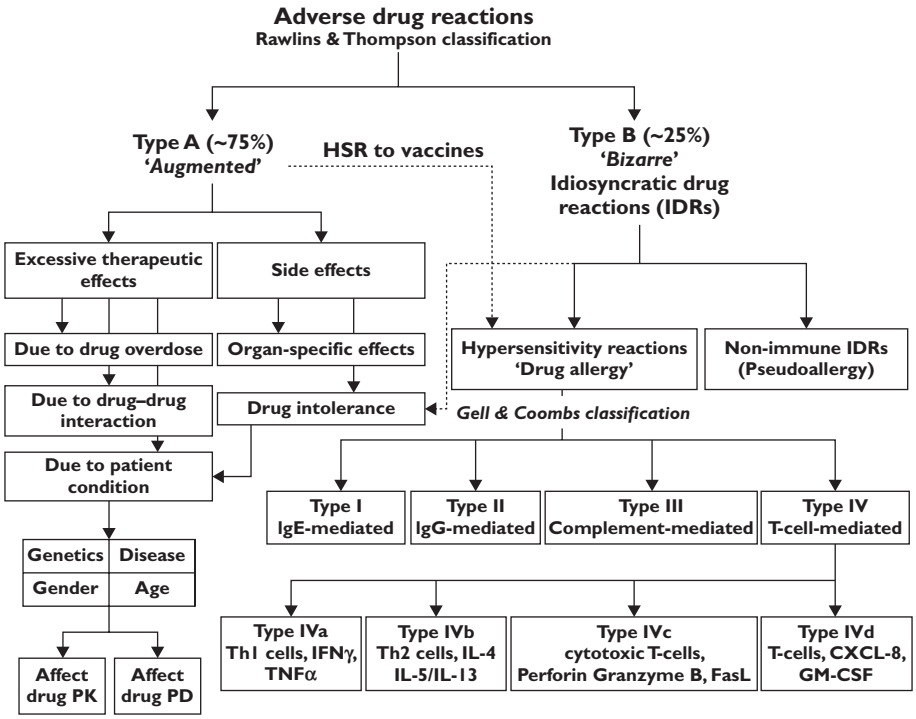

Figure 1
Classification of adverse drug reactions (see text for details)

account for 75%–80%, while type B represent 20%–25% of all ADRs (Figure 1).

Type B ADRs (IDRs)

Type B ADRs are also called IDRs. The term means that the reaction is specific to the individual and it is impossible to predict without an identified genetic marker. IDRs include ADRs such as 'drug hypersensitivity', which can be either immune-mediated and called allergic hypersensitivity or, if the immunologic mechanism is excluded, is called non-allergic hypersensitivity (pseudoallergy) [6, 7]. The term 'hypersensitivity' does not necessarily imply any immune system involvement and has been defined as 'objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at dose tolerated by normal subjects' [8, 9]. Drug hypersensitivity reactions have been estimated to represent approximately one-sixth of all ADRs. However, their precise incidence is not known due to under-reporting [10].

Immune-mediated IDRs are classified according to the Combs & Gells classification into four types (I–IV); Type I is mediated by immunoglobulin E (IgE), type II is mediated by IgG and IgM, type III is mediated by formation of an immune complement complex and type IV is T-cell mediated (Table 1) [11]. Despite being a useful classification, many recently understood immune-mediated reactions do not fit into the four Combs & Gells classes. A revised and more detailed system has now been introduced [12]. It is also of clinical relevance to classify IDRs according to the time required for the symptoms to appear into immediate reactions (≤1 h, e.g. anaphylaxis), intermediate (5–14 days) and delayed (2–7 weeks, Table 1) [13].

Related to their low incidence, IDRs are difficult to detect during drug development clinical trials and there are only few validated animal models to perform any mechanistic studies [14]. Also their unpredictability renders prospective studies in humans very difficult, if not impossible. Therefore, our understanding of the underlying pathophysiology of IDRs is still lacking and their classification and nomenclature remains debated. However, IDRs appear to be immune-mediated in many cases [10, 15–19]. Evidence that supports this hypothesis includes the delayed nature of these types of reactions and that the time between exposure and appearance of the symptoms shortens, if the patient is pre-exposed to the agent, although some exceptions do occur. Drug-specific T-cells have also been detected in the peripheral blood of the affected individuals [20].

Although the skin, liver and blood cells are the most commonly affected, IDRs can affect any organ and patients can present with fever, skin rash (including maculopapular, morbilliform, urticarial, fixed drug

Table 1
Classification of immune-mediated IDRs

Type	Mediator	Pathogenesis	Clinical picture	Chronology
I	IgE	Degranulation of mast cells and basophils	Urticaria, anaphylaxis, allergic rhinitis, bronchospasm, angio-oedema	Immediate (≤1 h)
II	IgG/M	FcR dependent cell lysis	Blood cell dyscrasia	Intermediate (5–14 days)
III	IgG/M	FcR-dependent immune complexes deposition	Serum sickness, vasculitis, arthus reaction	Intermediate (7–8 days)
IVa	T_H1 (IFNγ, TNFα)	Monocyte/macrophage mediated inflammatory response	Eczema	Delayed (1–3 weeks)
IVb	T_H2-IL4, IL5, IL13)	Eosinophils mediated inflammatory response	Maculopapular exanthema, bullous exanthema	Delayed (2–7 weeks)
IVc	Cytotoxic T cells (Perforine, Granzym B, FasL)	Cytotoxicity/apoptosis	Maculopapular exanthema, bullous/pustular exanthema	Delayed (1–3 weeks)
IVd	T cells (IL8, CXCL8, GM-CSF)	Neutrophils mediated inflammatory response	AGEP, Behçet's disease	Intermediate (≤2 days)

eruption or severe bullous reactions such as SJS and TEN), blood dyscrasias (eosinophilia and thrombocytopenia), hepatitis, nephritis, myocarditis, thyroiditis, interstitial pneumonitis and encephalitis. The case can present with any combination of these symptoms. Idiosyncratic drug-induced liver injury (IDILI) is a common ADR to drugs including antibiotics (amoxicillin/clavulan), non-steroidal anti-inflammatory drugs (NSAIDs), isoniazid, sulfamethoxazole, nitrofurantoin, phenytoin and anti-fungals [21]. It can be hepatocellular, cholestatic or mixed based on the biochemical pattern of liver function tests. The true incidence of IDILI is difficult to determine due to lack of data on the drug usage although some studies have estimated the annual incidence to be between 15–20 cases per 100 000 inhabitants [22, 23]. IDILI can also develop as part of DRESS. Evidence exists that implies the involvement of the immune system in the underlying pathophysiology of IDILI [24].

The molecular mechanism(s) underlying IDRs is not fully understood although thought to be immune-mediated in cases where the immune-mechanism is demonstrated [10, 25, 26]. It is noteworthy here that some IDRs exist, which are not mediated through the immune system (e.g. IDRs to NSAIDs). For immune-mediated IDRs, generation of reactive nucleophiles that are able to modify covalently endogenous macromolecules (proteins and DNA) through metabolism is thought to be an important step in the cascade of events leading to activation of the immune system and eliciting the reaction [14, 15, 21, 27]. Several hypotheses have been proposed in an attempt to explain the mechanistic pathophysiology of IDRs [10, 12, 16, 28–31]. Briefly, accumulated reactive nucleophiles metabolites (the reactive metabolite hypothesis) can modify endogenous macromolecules rendering them antigenic (the hapten hypothesis) and also provide, through causing damage and stress to neighbouring cells, 'danger signals' (the danger hypothesis) resulting in maturation of antigen presenting cells (APCs) and T-cells involved in the immune response. The parent molecule and, possibly its reactive metabolite can also interact with the immune receptors directly and non-conelantly producing direct stimulation as per the 'p-i hypothesis' (pharmacological interaction of drugs with the immune system) [32].

Discussing the molecular pathophysiology of IDRs in detail is beyond the scope of this review. For further details we suggest these recent references [12, 17, 18, 25, 30, 33].

Diagnosis of IDRs

Clinicians should bear in mind the possibility of an ADR once a patient has presented with an unexpected event. Patient medical history including a history of drug allergy may give an important clue to the case. Drugs that are known to cause IDRs should be treated as a red flag even if other differential diagnoses exist. Patients on polypharmacy represent a major challenge as identifying the culprit drug among multiple drugs has often proved to be difficult and discontinuing important drugs is not always a feasible option. Diagnosis of IDRs is two-fold: i) identifying the reaction as an IDR and ii) determining the culprit drug. Different strategies exist to achieve both goals. Currently the diagnosis of drug hypersensitivity is made on clinical grounds. The process of clinical diagnosis begins with the development of a differential diagnosis factoring in all possible aetiologies. The differential diagnosis is narrowed as the findings of the history and physical examination are factored in, which can include certain entities and exclude others. Classical laboratory tests can also be included in this consideration, although they frequently

are not very helpful in the case of the evaluation of possible drug hypersensitivity. When all of the elements of history and physical examination have been considered, the clinician must then decide on the basis of probabilities, which is the most likely diagnosis. The nature of this process highlights the importance of accurate reporting of findings. As an example, SJS is characterized by erythema multiforme associated with mucositis. The mucositis is typically inflammatory and often very painful. In this context, drugs are often the causative agent. In the case of a patient with erythema multiforme and swollen lips with no evidence of mucositis, the diagnosis is more likely erythema multiforme major and not SJS, in which case the aetiology is much more likely to be infectious. The difficulty in making an accurate clinical diagnosis points to the need for a more objective standard for diagnosis.

In vitro approaches to IDRs

A reliable and safe *in vitro* diagnostic test for IDRs would have a profound effect on the clinical management of IDRs. Although several *in vitro* tests for IDRs have been recently developed and optimized, their real predictive values are yet to be determined accurately [3, 4, 34–36]. Selection of an *in vitro* diagnostic test for IDRs depends on the type of reactions and the underlying pathophysiology predicted from the clinical picture and the natural history of the reaction. Immediate, IgE-mediated and delayed T-cell mediated reactions require different sets of *in vitro* tests for their diagnosis.

In vitro *tests for immediate IgE-mediated IDRs*

Detection of drug-specific IgE antibody Measuring drug-specific IgE is the most commonly used diagnostic test for allergic diseases [37]. The test is based on quantification of specific IgE antibodies using different laboratory techniques. The radioallergosorbent test (RAST), cellular fluorescent assay-IgE (CAP-IgE) and enzyme-linked immunosorbent assay (ELISA) are commonly used technologies. Although the radioactive technique is no longer used, 'RAST' has become a generic name for the technique.

Technical and mechanistic aspects In the test procedure, suspected antigen (drug) bound to the insoluble phase is incubated with a serum sample from the patient, washed, and then bound IgE is quantified using labelled anti IgE antibody. Direct detection of a drug-specific IgE in patient blood is a strong indicator of an immune reaction but this is not necessarily true in all cases. An individual may have circulating IgE that recognizes a drug molecule without having an immune reaction towards that drug. Nevertheless, the test is known to have high positive predictive value (PPV) when combined with good clinical and medical history. Its negative predictive value (NPV) is inherently low, which is probably due to the low sensitivity of the currently used techniques to detect low titres of circulating immunoglobulins [38, 39]. Thus, positive results strongly indicate immune mediation of the reaction but negative results do not exclude the reaction. In such cases and if the clinical history is highly suggestive of an allergic reaction, either skin tests or DPT are required to determine safety of future therapy. The low clinical sensitivity of IgE measurement renders the test of limited usefulness as a diagnostic tool. One commercially available test (CAP-FEIA, Phadia®) has a sensitivity and specificity of 0% to 25% and 83.3% to 100%, respectively, in diagnosis of immediate reactions to β-lactam antibiotics [40]. Measurement of drug specific IgE antibodies is widely used for diagnosis of immediate reactions to β-lactam antibiotics, muscle relaxants and some NSAIDs [41, 42]. Levels of specific IgE antibodies tend to decrease over time in patients with immediate allergic reactions, which leads to a decrease in the test sensitivity over time. Therefore, the test must be done as soon as possible after the reaction [43]. Another pitfall of *in vitro* measurement of drug-specific IgE is the high false positive results in patients with high total IgE levels and high false negative in patients with high IgG levels [37].

Basophil activation test (BAT) The BAT is a useful diagnostic tool for immediate IgE-mediated reactions to both foods and drugs. Its major limitation is the low count of basophil in peripheral blood, but recent flow cytometric techniques have allowed the use of whole blood samples and more accurate determination of the levels of different markers. Its other pitfall is the low sensitivity and this problem is tackled by using different cut off values when evaluating activation markers by flow cytometry [44]. However, the timing of the test with regard to the initial reaction is very critical as the test tends to lose its sensitivity with time [45]. On the other hand, time during which basophils maintain their activity after blood sampling seems to be short and it has been recommended that samples are processed within 3 h of sampling [46], which limits the availability of the test. The test is quite reproducible but only when used with a limited number of standardized drugs [47].

Basophils are effector cells in immediate-type hypersensitivity reactions and they respond to antigen stimulation *in vitro* by degranulation (e.g. release of histamine and leukotrienes) and expression of certain surface markers including CD45, CD11b, CD11c, CD62L, CD203c and CD63. Originally, the BAT was performed by measuring the release of histamine. Alternatively, flow cytometry based techniques are now used to measure specific surface markers that are up-regulated during basophil activation. The most commonly used are the antigens CD63, also known as lysosomal-associated membrane glycoprotein-3 (LAMP-3) and CD203c, a glycosylated type II transmembrane molecule [48].

The BAT has been validated for type I reactions to muscle relaxants [49], β-lactam antibiotics [50], pyrazolones and NSAIDs [51, 52]. Sensitivity of the test for reactions to β-lactam antibiotics, quinolones and rocuonium was reported as 33–67%, 71.1% and 80%, respectively [53–55].

The lymphocyte transformation test (LTT) The LTT is discussed in details below as part of the *in vitro* tests for delayed type IDRs. Nevertheless, positive LTT results have been obtained on samples from patients suspected with immediate type I (IgE-mediated) reactions to β-lactam antibiotics [56, 57]. This observation is attributed to the involvement of activated T-cells in the process of producing drug-specific IgE in immediate type allergic reactions. However, the meaning of detecting drug-specific T-cells in IgE-mediated reactions remains unkown [58].

In vitro *tests for delayed (T-cell-mediated) IDRs*

Delayed, T-cell-mediated, IDRs are believed to be a result of a complex interplay of many different pathways. Biochemical and genetic approaches have recently begun to shed some light on the pathophysiology of these types of ADRs. Understanding this pathophysiology is a prerequisite for development of evidence-based approaches for better management. Two key players in the in the underlying molecular pathophysiology of immune-mediated IDRs are the drug (or its reactive metabolites) and the immune cells, particularly circulating lymphocytes (when isolated from peripheral blood samples often referred to as peripheral blood monocytes, PBMCs). In addition recent development has led to the use of blood platelets as a surrogate cell model for *in vitro* toxicity assays. According to the 'reactive metabolite' hypothesis, metabolic activation of drugs to reactive metabolite(s) represents the first step in a series of events [28]. The 'reactive metabolite' hypothesis postulates that IDRs develop as a result of imbalance between metabolic activation and detoxification of drugs in the biological system leading to accumulation of one or more toxic reactive metabolites [59–61]. It is important to understand that reactive metabolites may not be the principle direct activator of the immune system as parent, non-reactive drugs can activate isolated T-cells *in vitro* without need for any bioactivation or processing [32]. However, chemically reactive electrophilic metabolites seem to be the major, if perhaps not the only, products capable of supporting two important pathways in the immune system activation process: generation of haptenated endogenous proteins (act as antigens, signal 1) and generation of danger signals from stressed and dying cells (signal 2, Figure 2) [16, 29]. Signal 2 can also be provided by factors such as trauma, bacterial and viral infections, or co-administered drugs and environmental pollutants. The clinical manifestations of IDRs are probably primarily mediated by the immune system although in some cases a direct toxic effect of the reactive species generated from the drug during metabolism may be manifested clinically [28, 59]. It has been established for several decades that PBMCs from hypersensitive patients are more susceptible to *in vitro* toxicity from the reactive metabolite(s) of the suspected drug than are cells from healthy individuals (controls) who have tolerated the drug [60, 62–69]. T-cells are key mediators of any reaction that involve the immune system and T-cells that specifically recognize culprit drugs and their metabolites have long been cloned from patient blood samples and characterized [70–73]. *In vitro* detection of these drug-specific T-cells is considered indicative of the occurrence of immune-mediated reactions. This is achieved by measuring T-cell proliferation in short term primary cultures as a response to incubation with the suspected drug.

The lymphocyte transformation test (LTT) The LTT is the most extensively studied and widely used *in vitro* diagnostic test for drug, food and environmental allergies. The method was first used back in the early 1960s to evaluate hypersensitivity reactions to phenytoin [74] and sulfa drugs [75]. The test involved tedious visual counting of mitotic figures until Vischer [76] adopted measuring radiolabelled thymidine incorporation into cellular DNA as a reflection of cell division rate. Drug specific T-cell clones can be isolated and cloned *in vitro* and they respond to incubation with the culprit drug with proliferation and expression of certain surface markers [58, 71, 77].

Technical and mechanistic aspects First, the anticoagulated blood sample is obtained from the patient and the PBMCs are separated over density gradient (Ficoll®). Cells are then cultured in RPMI 1640 medium supplemented with fetal bovine serum (FBS) for 5 days at 37°C in the presence of the concentration range of the suspected drug (preferably in pure form). Tritium-labelled thymidine (^{3}H-thymidine) is then added to the culture and cells are harvested on day 6 to count incorporated radioactivity as a measure of cell proliferation. The increase in cell proliferation is expressed as a ratio called the stimulation index (SI = ^{3}H-thymidine in the presence of the drug/^{3}H-thymidine uptake in the absence of the drug) [3, 18]. Other markers of T-cell stimulation have also been used including secretion of mediators (IL-5, IL-10) and expression of specific antigens (e.g. CD 69) [78, 79].

Probably one of the most convincing factors for the involvement of the immune system in IDRs is the possibility to isolate drug-specific T-cell clones from blood samples of affected patients. This represents the mechanistic basis of the LTT as a diagnostic tool for IDRs.

The sensitivity of the LTT for the diagnosis of (drug hypersensitivity syndrome) has been estimated to be from 56% to 78% and its specificity to range from 85% to 93% [3, 80]. Factors that affect the performance of the LTT include

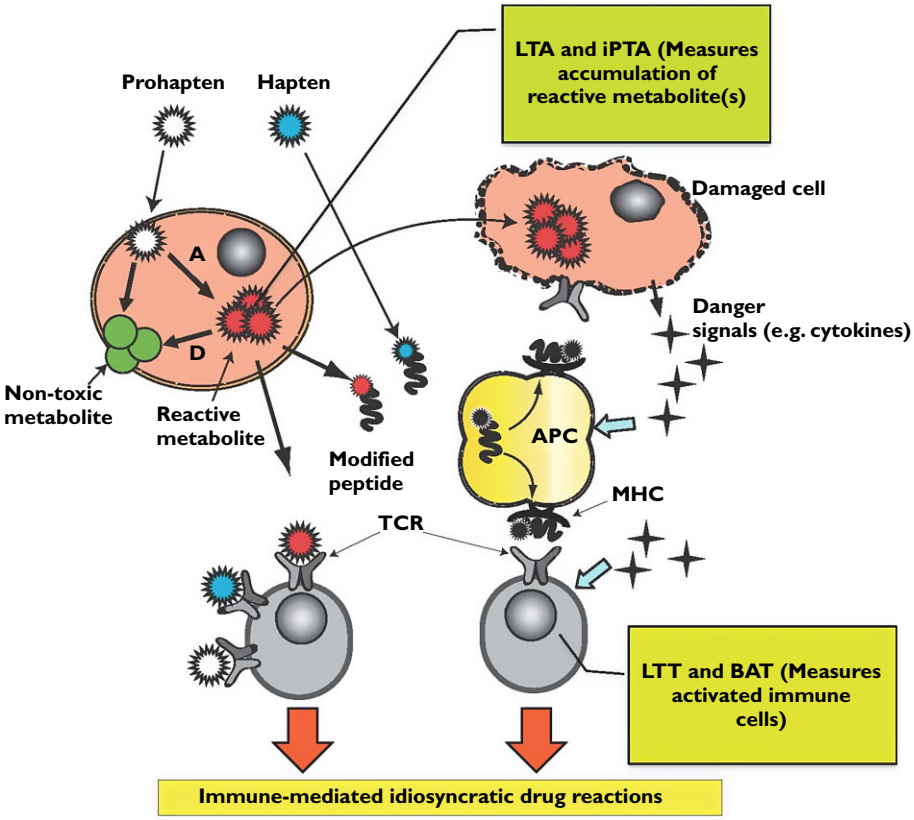

Figure 2

Molecular mechanisms of immune mediated idiosyncratic drug reactions demonstrating the principle of the lymphocyte toxicity assay (LTA), the *in vitro* platelet toxicity assay (iPTA), the basophil activation test (BAT) and the lymphocyte transformation test (LTT). (A = Activation); (D = detoxication); non-reactive parent drug (○); reactive parent drug (●); reactive metabolite (●)

(i) timing of the test with respect to the initial reaction, (ii) the clinical picture of the reactions, (iii) the type of drug involved and (iv) the test procedure and read-out systems used [3, 80]. The use of LTT in the diagnosis of drug hypersensitivity reactions has recently been systematically reviewed [3].

The lymphocyte toxicity assay (LTA) The LTA is similar to the LTT in using isolated PBMCs, but the principle of the assay is quite different [81]. The test is based on the observation that cells from hypersensitive patients express a higher degree of cell death when incubated with the culprit drug metabolite(s) than cells from healthy (drug tolerant) controls. Clinical and practical data and theoretical explanations exist to support this hypothesis but the validity of *in vitro* cell death as a marker for *in vivo* drug-induced IDR has long been questioned [67, 82]. Our three decade clinical and laboratory experience with the use of the LTA in the diagnosis of drug-induced hypersensitivity reactions have proven the test as a very useful diagnostic tool for IDRs to many drug classes including aromatic anticonvulsants, sulfonamides and β-lactam antibiotics [34].

Technical and mechanistic aspects The test includes incubation of Ficoll gradient isolated PBMCs from patients and controls with the drug in the presence of a metabolic activation system (usually phenobarbital-induced rat liver microsomes, RLM) or with the synthesized drug metabolite (if known and available) [81]. Following incubation with different concentrations of the tested drug for 2 h at 37°C, cells are incubated for recovery for 16 h and cell death is then determined using different methods (e.g. trypan blue exclusion, tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl) 2, 5 diohenyl-tetrazolium bromide (MTT)). Degrees of cell death are expressed as percentage of the control (cells incubated with vehicle without drug) and compared with percentage of cell death in cells from healthy individuals who did not experience an ADR with the same drug (controls). A cut off value of the percentage of increase in cell death of incubated patient cells (*vs.* controls) is considered as an indication of patient susceptibility. The predictive value of the LTA remains difficult to define due to lack of a 'gold standard' test for comparison and the technical complexity of the test [3, 34].

We have recently performed a population survey on a cohort of pre-tested patients to evaluate the predictive values of the LTA for diagnosis of IDRs to different classes of drugs including β-lactam antibiotics, sulfonamides and aromatic anticonvulsants [34]. In this study we included 147 patients who developed an IDR and searched for cases of accidental or purposeful re-exposure of the patient to the suspected drug. Among these patients we identified 26 cases of re-exposure in 22 patients. It is clear from our evaluation that the performance of the LTA test is affected by factors including timing of the test with respect to the initial reaction, type of reaction and class of drugs involved.

On the other hand, the metabolic activation system of the LTA test lacks standardization. There are many pharmacokinetic and pharmacodynamic factors that do not enter the equation including lack of the absence of any functional detoxification pathways. One important observation is that use of a synthetic reactive metabolite (e.g. as in case of sulfonamide drugs) resulted in increased test sensitivity and its positive predictive value [34, 62, 63]. Another factor limiting the more routine use of the LTA is the requirement for careful isolation of PBMCs.

Recent developments Recent research in our laboratory focused on developing and validating the use of peripheral blood platelets (PBPs) as a surrogate cell model for *in vitro* toxicity testing [35, 83]. Due to their small size and low density, PBPs are readily collectable from blood using differential centrifugation [84]. In addition to blood homeostasis, the role of platelets in inflammation, allergy and hypersensitivity reactions has recently been recognized [85–88]. Platelets are metabolically active and contain a full apoptotic system, which make them a good model to study drug toxicity *in vitro*. Furthermore, they do not proliferate which adds another advantage to the use of platelets as a cell model to evaluate the degree of cell death. Cell proliferation may mask part of cell death in the PBMCs model. Platelets from hypersensitive patients respond to *in vitro* chemical insult in a similar fashion to PBMCs and the degree of cell death is higher and easier to detect [83]. We also attributed this phenomenon to the lower capacity of platelets for detoxication of reactive metabolites.

In a validation study of the *in vitro* plasma toxicity assay (iPTA) using rigorous inclusion criteria of identified IDRs cases to sulfa drugs, there was 85% agreement (11 out of 13) between the LTA and the iPTA results in the 13 cases we tested. In the two clinically confirmed cases where the two tests did not agree the LTA was negative and the iPTA was positive. This disagreement between the LTA and the iPTA is probably due to the higher sensitivity of the platelet test to detect patient susceptibility.

In conclusion, the iPTA offers a simplified procedure for *in vitro* toxicity testing for IDRs with higher sensitivity than the LTA. We believe that the iPTA is more suitable as a diagnostic procedure for IDRs for wider clinical use.

Other in vitro *tests for IDRs*

In addition to the aforementioned specific tests, these are other *in vitro* tests, which play a major role in management of IDRs. However, most authors do not list them in their reviews as *in vitro* tests for IDRs. Because management of such a complex disease requires quick decisions based on evidence based medicine, a global approach is the most successful and the least costly in terms of patient wellbeing and healthcare resources. Valuable data from these tests can guide the treatment journey to a safe port.

Tissue biopsy Microscopic examination of tissue biopsies can be very valuable in the diagnosis of IDRs involving the skin, the liver, lymph nodes and other tissues [89–91]. In severe cases of skin lesions such as exanthematous pustulosis (AGEP), DRESS and SJS/TEN skin biopsy is an important part of disease management and may affect the course of therapy [92]. Early detection of severe cutaneous IDRs (CIRDs) can save lives and it is important to differentiate SJS/TEN from erythema multiforme (EM) early on because they have different courses of therapy. Hosaka *et al*. used frozen skin sections in 35 patients to differentiate TEN from EM in their early stages. From the 35 patients nine had signs of TEN, of them six were later diagnosed with TEN/SJS and three had EM and none of the rest developed TEN/SJS [93]. Drug-induced skin rashes have many distinct clinical and histopathological characteristics and features that range in severity from mild self-resolving simple rash to life threatening bullous reactions (SJS/TEN), and in delay time from hours to days. Each has characteristic histopathology features that can be determined in skin biopsy samples.

Measurement of serum markers for IDRs Analysis of peripheral blood samples of immune-mediated IDRs and studying circulating immune cell subpopulations can help determine the type of reaction and the underlying pathophysiology. Immediate and delayed hypersensitivity reactions have distinct circulating affector cells. Delayed reactions are characterized by Th_1 pattern with expression of interferon γ (IFN γ), interleukin-12 (IL-12) and tumour necrosis factor α (TNFα) and down regulation of IL-4 expression. IgE-mediated immediate reactions, on the other hand, are characterized by Th_2 pattern with production of IL-4 and downregulation of IFN γ. Fujita *et al*. developed a rapid immunichromatographic strip test to detect serum granulysin for early prediction of SJS/TEN. Although the sample size was not large enough to validate the test, the test seems very promising as a diagnostic tool for these severe types of reactions [94]. Soluble Fas (sFas) levels were also found to increase significantly in sera of SJS/TEN patients before skin detachment develops, which opens the possibility of using this marker for early diagnosis of SJS/TEN [95, 96]. Caproni *et al*. have also described that SJS/TEN patients with wide spread skin detachment have a high serum expression of soluble CD40L [97]. Serum

markers for different types of IDRs is a fast growing field which is useful for both diagnosis and understanding the pathophysiology of the disease [98].

In vitro *test for IDILI* In addition to skin, the liver is the organ most affected by IDRs, mainly because it is the main site of drug metabolism. IDILI is a major cause of post marketing drug withdrawal and black box warnings. IDILI are estimated to have an incidence of 1 in 1000 to 1 in 10 000 patients with variable latency periods that ranges from days to months and are estimated to make up 20% of cases of severe liver injury requiring hospitalization [99]. It has been found that paracetamol (acetaminophen) overdose and idiosyncratic reactions are the most frequent cause of acute liver failure [100]. Prediction and prevention of IDILI have been difficult because of the lack of a reliable screening test and the lack of understanding of the underlying pathophysiology [101]. DILI can manifest as a main ADR or present as part of a full drug hypersensitivity syndrome (DRESS) [25]. IDILI were responsible for 13% of acute liver failure (ALF) in the USA between the years 1997 and 2001 [99]. DILI should be considered in any patient with liver dysfunction. A serologic test should be used to rule out any possibility of viral infection. Detailed history of prescription and over the counter medications should be taken as well as alternative and herbal products and foods including alcohol consumption. Time of exposure should be assessed very carefully as DILI most often occur within 6 months of drug exposure but can also occur within days or after a year. Causality assessment can be a challenge especially in cases of multiple medications. *In vitro* testing for DILI includes evaluation of liver function using liver enzymes as biological markers. Serum concentrations of alkaline phosphatase (ALP), alanine transaminase (ATA) and bilirubin are indicative of the degree of liver injury. Also hepatocellular liver injury can be differentiated from cholestatic liver injury by measuring liver enzymes. The latter is characterized by higher increase in alkaline phosphatase and bilirubin relative to alanine transaminase. DILI is often characterized by the presence of anti-drug antibodies and autoantibodies but tests to measure them are not always available [25]. Liver histology studies of biopsies or explant samples can confirm the mechanism of liver injury, as immune cell infiltrates are indicative of immune-mediated reaction.

Genetic testing Genetic testing for predisposing alleles for IDRs has recently increased exponentially. Genetic analysis has linked a few specific genetic polymorphisms with certain IDRs to some drugs in specific ethnic groups (e.g. HLA B*-1502 for carbamazepine-induced severe bullous reactions in the Han Chinese and HLA B*-5701 and abacavir hypersensitivity) [102]. Genetic testing has proven to be very useful in cases such as abacavir hypersensitivity and carbamazepine SJS/TEN reactions in Han Chinese populations. In fact, after implementing mandatory genetic testing for abacavir prescription, abacavir hypersensitivity cases have dropped dramatically in the last few years. Unfortunately, given the incidence of these haplotypes and the fact that the haplotypes are not clearly linked to mechanism, it is likely that using HLA typing to predict risk of adverse drug effects will deny therapy to many patients who would have tolerated the drug. These studies have also made it clear that much more work is required in both basic and clinical science to enable us to predict better, manage and prevent these type of ADRs. Further research is required to elucidate the pathophysiology of drug hypersensitivity syndrome as well as rigorous

Table 2

Pros and cons of *in vitro* tests available for idiosyncratic drug reactions

	Detection of drug-specific IgEs	LTT	LTA	BAT	iPTA
Pros	• The test has high positive predictive value. • Positive results are highly suggestive of type I immune-mediated reaction.	• The test has been used for a long time and has accumulated a reasonable amount of clinical experience. • Positive result demonstrates the involvement of the immune system in the reaction pathophysiology.	• A reasonable experience with clinical use is available. • In principle the test can be used to predict potential risk to develop a reaction prior to exposure as it detects phenotypic predisposition.	• Recent adaptations of flow cytometric methods have increased its sensitivity. • New techniques allow the use of whole blood samples.	• The test procedure is simplified and more reproducible. • Smaller volume of blood samples is needed. • Has been shown to have a higher sensitivity compared with the LTA.
Cones	• Low sensitivity and negative predictive value. • Traditionally the technique involves the use of radioactive reagents.	• Expensive and requires highly skilled personnel and sophisticated equipment. • Predictive value is not well defined.	• Procedure is time consuming and demands special skills, resources and reagents. • Has very limited use in few centres.	• Can detect limited types of reactions, which involve basophil activation. • Has low sensitivity. • Available only for a limited number of drugs.	• The test has not been validated by independent group.

BAT, basophil activation test; iPTA, *in vitro* platelet toxicity assay; LTA, lymphocyte toxicity assay; LTT, lymphocyte transformation test.

trials to determine which of the available *in vitro* evaluations is most suitable for the assessment of patients or research subjects with possible DHRs.

Conclusion

Evaluation and management of IDRs require a great deal of clinical and laboratory experience [103]. Incomplete understanding of the underlying pathophysiology of these complex ADRs has always been an obstacle to develop reliable *in vitro* diagnostic test for this disease. Available tests have proven to be useful tools for IDR management but they are not always available to clinicians and are still confined to well equipped research laboratories (Table 2). Lack of a 'gold standard test' for IDRs has made accurate determination of *in vitro* testing predictive values quite a challenge. Attempts should be made to simplify and standardize *in vitro* testing procedure, if wider clinical use is to be achieved. Clinicians should be aware that safe alternative *in vitro* tests for severe IDRs are available in order to avoid unnecessarily risky DPT. With a robust plan and a multidisciplinary approach, *in vitro* testing can play an important role in IDR management.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work.

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