DRUG HYPERSENSITIVITY – POSSIBILITIES OF LABORATORY DIAGNOSTIC

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SUMMARY

Approximately 17% of adverse drug reactions are caused by hypersensitivity to drugs. In this study, we utilised the lymphocyte transformation test (LTT) to detect drug hypersensitivity. The main purpose of the work was to perform analysis of results of 314 patients recommended from immunology-allergology outpatient clinics and examined for suspected drug hypersensitivity.

Significantly more females (77%) were sent by doctors for examination. In both genders, half population of patients was more than 60 years old. Altogether, 958 drugs were tested. Stimulation index (SI) ≥ 2.8 was considered as positive response to drug. Mean positivity to drugs was almost double in female population (12.6%) than in male patients (6.8%).

Among antibiotics, the most frequently positive response was seen in macrolides, linkosamides (20%), beta lactams and penicillins (18.9%), imidazoles (16.7%) and cephalosporins (15.8%). In group of cardiovascular drugs, vasodilatans, vasoprotectives, betablockers, renin-angiotensin blockers and cardiacs had positive response in more than 20% of the tested drugs. Occurrence of positivity among the most frequently tested group of drugs: local anaesthetics (3.7%), analgetics/antipyretics (4.6%), non-steroid anti-inflammatory drugs (NSAID) (5.5%), anticoagulants/antithrombotics (15.4%). In females, one third of positive responses to drugs had very high magnitude of response with Stimulation index > 5.

In conclusion, within our setting, the LTT has proven to be a useful test for the diagnosis of drug hypersensitivity reactions. Drug hypersensitivity diagnosis needs to rely on combination of history and variety of laboratory tests. Positive result in LTT test helps to define the suspect drug, but negative tests cannot rule out drug hypersensitivity.

INTRODUCTION

Approximately 17% of adverse drug reactions are caused by hypersensitivity to drugs. Multiple factors are involved in these outcomes and they lead to several unique clinical states which include: anaphylaxis, exanthemas of different calibre, Steven-Johnsons Syndrome, toxic epidermal necrolysis, interstitial kidney and interstitial lung disease, hepatitis, pancreatitis, blood cell dyscrasias and auto-immune diseases. It is worth noting that the exact pathological mechanism behind the majority of the above mentioned states is yet to be specified. T cells play a protagonistic role in the operational capabilities of immune mediated protection; two exact capacities have been studied. Firstly, they may act as the conductors choreographing the class of immune response and secondly, as effectors themselves. T cells achieve the second task by secreting cytokines; IL- 4 and IL- 13 in IgE mediated reactions, IL-5 in eosinophilic inflammation, IL- 8 in neutrophilic inflammation and IFN γ and TNF α in monocyte/macrophage inflammation. T cells have also the effect of cytotoxic killer cells as they damage and destroy cells e.g. keratinocytes and hepatocytes (1-2).

The diagnosis of drug hypersensitivity has proven to be challenging due to the fact that many drugs possess the ability to elicit immune – mediated morbidities. In this study, we utilised the **lymphocyte transformation test** (LTT) to detect the above-mentioned phenomenon. The main concept behind this test is the measurement of T cells in an *in vitro* environment. This hypothesis was confirmed by the generation of drug specific T cell clones and discovery of T cell receptors that directly interact with drugs. Drug specific T cells are involved in the vast majority of cases in hypersensitivity reactions, thus LTT is quite advantageous and may be applied to a wide spectrum of medication or drugs. LTT, however, is not without its disadvantages. LTT is reliant on the proliferation of T cells *in vitro* and therefore it is somewhat difficult to transfer this to the clinical setting. In addition to this, LTT is exemplary from the technical point of view and the sensitivity is also limited, further restricting its use. Hence, the diagnosis of drug hypersensitivity has to be a combination of patient history and a variety of laboratory tests (3).

The main purpose of my work was to get familiar with the LTT method in laboratory, to test several patients for drug hypersensitivity and finally to perform analysis of laboratory database of 314 patients analysed for drug hypersensitivity. Results of analysis will provide useful information to immunologists-allergologists on the most frequently positively tested groups of drugs, possibilities of LTT test to detect drug hypersensitivity and to find sub-populations at risk for drug hypersensitivity.

MATERIAL AND METHODS

Lymphocyte transformation test

Proliferative activity of lymphocytes was assessed by measurement of ^{3}H-thymidine incorporation into DNA of proliferating cells using a Microbeta 2 liquid scintillation counter (PerkinElmer). Human heparinized whole blood (150 μL) diluted 1:15 in complete RPMI 1640 medium containing 10% FCS, L-glutamine, and gentamycin was dispensed in triplicates in wells of a 96-well microtiter culture plate under sterile conditions. Drugs dissolved in saline and diluted in medium were added (50 μl). Mitogen, phytohemagglutinin (PHA; 25 μg/mL) was added as positive control. The plates were incubated at 37 °C for 5 days in 5 % CO_2 atmosphere; the wells were then pulsed with 1 μCi [^{3}H]-thymidine diluted in medium (20 μL) and incubated at 37 °C for additional 24 h. After the 6-day incubation, cell cultures were harvested onto the glass filter paper, which was placed into a scintillation fluid, and radioactivity was measured as counts per minute (cpm)/per culture in triplicates for each variable. Stimulation index (SI) was calculated by dividing the mean dpm in response to stimulation by the mean dpm of the cells cultured alone.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 software (Chicago, IL, USA) and GraphpadPrism 6.01 (La Jolla, CA, USA). Normality was tested by Shapiro-Wilk's test. A Student T-test was used for normally distributed data and the Mann-Whitney test was used for non-normally distributed data to compare significant differences between the groups. Data were expressed as the mean values with a standard deviation (SD). Differences at $p < 0.05$ were considered to be statistically significant. Categorical variables were indicated as a number (%). Distributions of qualitative variables between subgroups were compared using the chi-square test. Statistical significance was considered for all tests as $p < 0.05$.

RESULTS AND DISCUSSION

The scientific background for the LTT has been well-established in the last years, and its usefulness has been demonstrated in various diseases and with many different drugs (4).

In Laboratory of Immunotoxicology, Slovak Medical University, LTT test has been introduced into diagnostic procedures approx. 20 years ago. During years 2015-2018, population of 314 adult people living in Slovakia, mostly from region Bratislava and West

Slovakia were investigated. Patients mainly from immunology-allergology outpatient clinics with suspected drug hypersensitivity were recommended by doctors for testing.

Patient characteristics are summarized in Table 1. Significantly more females than males were examined. We suppose that predisposition to drug hypersensitivity is age-specific because in children population data analysis showed a slight male predominance (5). Both sexes were of similar mean age. In our population, age groups for ten years were created and subsequently analysed (Figure 1). In males, half population of patients (54%) was more than 60 years old. Similarly to males, dramatic increase in percentage of older people was shown in female population and half population of patients (48%) was more than 60 years old. In summary, analysis of age groups showed age-dependent increase in percentage of people tested for drug hypersensitivity. Our results might indicate that human population over 60 years of age is population at risk for drug hypersensitivity.

Altogether, 958 drugs were tested; more than ¾ of drugs in female group (Table 1). Regardless the gender, three drugs per patient were analysed in average. Eighty percent of tested drugs came from the 5 drug groups: central nervous system (CNS) drugs, antibiotics, cardiovascular, musculoskeletal drugs and blood and hematopoietic drugs (Figure 2). Analysis of drug subgroups showed that among CNS groups, mainly analgetics antipyretics and local anesthetics were tested. Among antibiotics, beta lactams and penicillins, macrolides, linkosamides, quinolones, cephalosporins and tetracyclines created 90% of analyzed antibiotic molecules. In group of cardiovascular drugs, betablockers, antihypertensives, hypolipidemics, diuretics, cardiacs and Ca-canal blockers were examined. In group of musculosceletal drugs, mainly non-steroidal anti-inflammatory drugs, myorelaxans and anti-gout drugs were tested. Blood and hematopoietic drugs included in 80% anticoagulants/antithrombotics.

Stimulation index (SI) $\geq$ 2.8 was considered as positive response to drug. In total population, response of lymphocytes to the tested drug was positive in 11% of drug samples (Table 1). Positivity was almost double in female population (12.6%) than in male patients (6.8%). Analysis of subgroups of drugs revealed the highest positivity in vasodilatans and vasoprotectives (50%), analgetics/antispasmodics (33%) and plasma (33%); however findings are affected by error of small sized populations tested (n=6,3,9; data not displayed). Among widely used group of drugs - antibiotics, frequently positive subgroups were macrolides, linkosamides, beta lactams, penicillins, imidazoles and cephalosporins (Table 2). Concerning the penicillins and macrolides, one has to be careful when evaluating the reaction. Published data exhibited that those groups of drugs are typical haptens which can modify proteins.

Proteins might be stimulatory also for some T cells of non-sensitized donors. Thus, for those reactions SI > 3 to be judged as positive (4).

In group of cardiovascular drugs, vasodilatans, vasoprotectives, beta-blockers, renin-angiotensin blockers and cardiacs were positively tested in more than 20% of tested drug subgroups (Table 3). On the other hand, none of drug was tested as positive in group of sulfamethoxazoles and quinolones (ATB, n=6, n=22), Ca-canal blockers (n=14) and hypolipidemics (n=23).

From practical point of view, positivity of the most frequently used drugs is interesting. Occurrence of positivity among local anaesthetics or analgetics/antipyretics is quite low (3.7%, n=134 or 4.6%, n=65, respectively). Published data described that non-steroidal anti-inflammatory drugs (NSAIDs) are responsible for 21% to 25% of reported adverse drug reaction including immunological and non-immunological reactions (6). Fortunately, in our study, only 5.5% of truly positive allergic reactions in those group of drugs were recorded, when tested by LTT (n=73). Incidence of positivity in group of anticoagulants/antithrombotics is almost three times higher (15.4%, n=65).

European multicenter study suggests that at least 50% of the drug hypersensitive reactions to non-ionic radiocontrast media are caused by an immunological mechanism (6). In our study, it was not the case; positivity to contrast agents reached 7.4%.

Magnitude of response is summarized in Table 4. In females, one third of positive responses to drugs had very high magnitude with Stimulation index > 5. The biggest response SI=72 was observed in 76-year old female to gastrointestinal drug omeprazole. We were surprised by the number, but Pichler WJ and Tilch J (4) published that SI of > 60 were found quite frequently in response to penicillin G, lidocain, carbamazepin, phenytoin and sulfonamide.

Within our setting, the LTT has proven to be a useful test for the diagnosis of drug hypersensitivity reactions. LTT has many advantages: it is possible to perform the assay with many drugs and as an *in vitro test*, it is safe to the patient. Moreover, the assay is positive in drug reactions with different pathomechanism. Diseases, in which the lymphocyte transformation test (LTT) has been found to be frequently positive (>50%) are: generalized maculopapular exanthema, bullous exanthema, acute generalized pustulous exanthema, drug hypersensitivity syndrome with eosinophilia and systemic symptoms, and anaphylaxis (4).

The sensitivity of LTT was estimated to 58.4%, specificity 69.9%, positive predictive value 95.8 and negative predictive value 93.3% (7). Data based mainly on the analysis of beta-lactams showed similar findings (4). LTT has good specificity but low sensitivity for the

diagnosis of drug hypersensitivity reaction. Although sensitivity of LTT is limited, it is higher than of other tests for drug hypersensitivity diagnosis (4). Consequently, drug hypersensitivity diagnosis needs to rely on a combination of history and different tests, as none of the single tests available has per se a sufficiently good sensitivity. In conclusion, positive result in LTT test helps to define the suspect drug, but negative tests cannot rule out a drug hypersensitivity.

CONCLUSION

Our results might indicate that human population over 60 years of age is population at risk for drug hypersensitivity. Female gender has higher probability to be examined with suspect drug hypersensitivity. Women have almost twice the chance of positivity in LTT test in comparison with men. Moreover, in females, one third of positive responses to drugs had very high magnitude of response with Stimulation index > 5.

Within our setting, the LTT has proven to be a useful test for the diagnosis of drug hypersensitivity reactions. Drug hypersensitivity diagnosis needs to rely on a combination of history and different tests, as none of the single tests available has per se a sufficiently good sensitivity.

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References:

1. CAROLINO, F., RIBEIRO, L., CERNADAS, J.R. et al.: Overview of Drug Allergy: From Immunogenetic Basis to Practice. *Acta Med Port*, 31, 2018, č. 10, s. 581-588.

2. FERNER, R., MCGETTIGAN, P.: The patient who reports a drug allergy. *BMJ*, 2020, č. 368, s. l6791.

3. BROCKOW, K., PRZYBILLA, B., ABERER, W. et al.: Guideline for the diagnosis of drug hypersensitivity reactions S2K-Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) and the German Dermatological Society (DDG) in collaboration with the Association of German Allergologists (AeDA), the German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Swiss Society for Allergy and Immunology (SGAI), the Austrian Society for Allergology and Immunology (ÖGAI), the German Academy of Allergology and Environmental Medicine (DAAU), the German Center for Documentation of Severe Skin Reactions and the German Federal Institute for Drugs and Medical Products (BfArM). *Allergo J Int,* 2015, č. 24, s. 94–105.

4. PICHLER, W.J., TILCH, J.: The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy*, 2004, č. 59, s. 809–820.

5. PICCOROSSI, A., LICCIOLI, G., BARNI, S. et al.: Epidemiology and drug allergy results in children investigated in allergy unit of a tertiary-care paediatric hospital setting. *Italian Journal of Pediatrics*, 2020, č. 46, s. 5.

6. DIAS DE CASTRO, D.E., FABRÍCIA, C., RIBEIRO, L. et al.: Overview of Drug Allergy: From Immunogenetic Basis to Practice. *Acta Med Port,* 31, 2018, č. 10, s. 581-588.

7. KARAMI, Z., MESDAGHI, M., KARIMZADEH, P. et al.: Evaluation of Lymphocyte Transformation Test Results in Patients with Delayed Hypersensitivity Reactions following the Use of Anticonvulsant Drugs. *Int Arch Allergy Immunol* 2016, č. 170, s. 158–162.

Tables:

Table 1. Patients characteristics and number of tested drugs

Drugs	**Total population**	**Males**	**Females**
Number of patients	314 (100%)	71 (22.6%)	243 (77.4%)
Mean age (years) (mean ± SD)	56.12 ± 16.17	56.92 ± 16.58	55.89 ±16.07
Tested drugs	958 (100%)	219 (22.9%)	739 (77.1%)
Tested drugs/per patient	3.05	3.08	3.04
Positive drugs/per tested drugs	108 (11.3%)	15 (6.8%)	93 (12.6%)
Positive drugs/per patient	34.4%	21.1%	38.3%

Table 2. Percentage of positive responses to antibiotics

Subgroup of antibiotics		**Positive**	**Negative**	**Total**
Macrolides, linkosamides	Count	10	40	50
	%	**20.0%**	80.0%	100%
Beta lactams, penicillins	Count	10	43	53
	%	**18.9%**	81.1%	100%
Imidazoles	Count	1	5	6
	%	**16.7%**	83.3%	100%
Cephalosporins	Count	3	16	19
	%	**15.8%**	84.2%	100%
Tetracyclines	Count	1	15	16
	%	6.3%	93.8%	100%
Aminoglycosides	Count	0	1	1
	%	0%	100%	100%
Fosfomycin	Count	0	1	1
	%	0%	100%	100%
Quinolones	Count	0	22	22

	%	0%	100%	100%
Sufamethoxazole, trimethoprim	Count	0	6	6
	%	0%	100%	100%
Total	Count	25	149	174
Average	%	14.4%	85.6%	100%

Table 3. Percentage of positive responses to cardiovascular drugs

Subgroup of cardiovascular drugs		**Positive**	**Negative**	**Total**
Vasodilatans, vasoprotectives	Count	3	3	6
	%	**50.0%**	50.0%	100%
Betablockers	Count	11	31	42
	%	**26.2%**	73.8%	100%
Renin-angiotensin	Count	2	7	9
	%	**22.2%**	77.8%	100%
Cardiacs	Count	3	12	15
	%	**20.0%**	80.0%	100%
Diuretics	Count	2	16	18
	%	11.1%	88.9%	100%
Antihypertensives	Count	3	36	39
	%	7.7%	92.3%	100%
Ca canal blockers	Count	0	14	14
	%	0%	100%	100%
Hypolipidemics	Count	0	23	23
	%	0%	100%	100%
Total	Count	24	142	166
Average	%	14.5%	85.5%	100%

Table 4. Magnitude of positive response

	Total population	Males	Females
Average positive response (SI ± SD)	5.19 ± 6.89	4.40 ± 2.76	5.32 ± 7.35
Maximum response (SI)	72.00	13.96	72.00
Magnitude of response			
SI (2.80 - 3.00)	24 (22%)	2 (13%)	22 (24%)
SI (3.01 - 5.00)	53 (49%)	10 (67%)	43 (46%)
SI > 5.01	31 (29%)	3 (20%)	28 (30%)
Total No. of positive drugs	108 (100%)	15 (100%)	93 (100%)

Figure 1. Representation of different age groups in male and female population

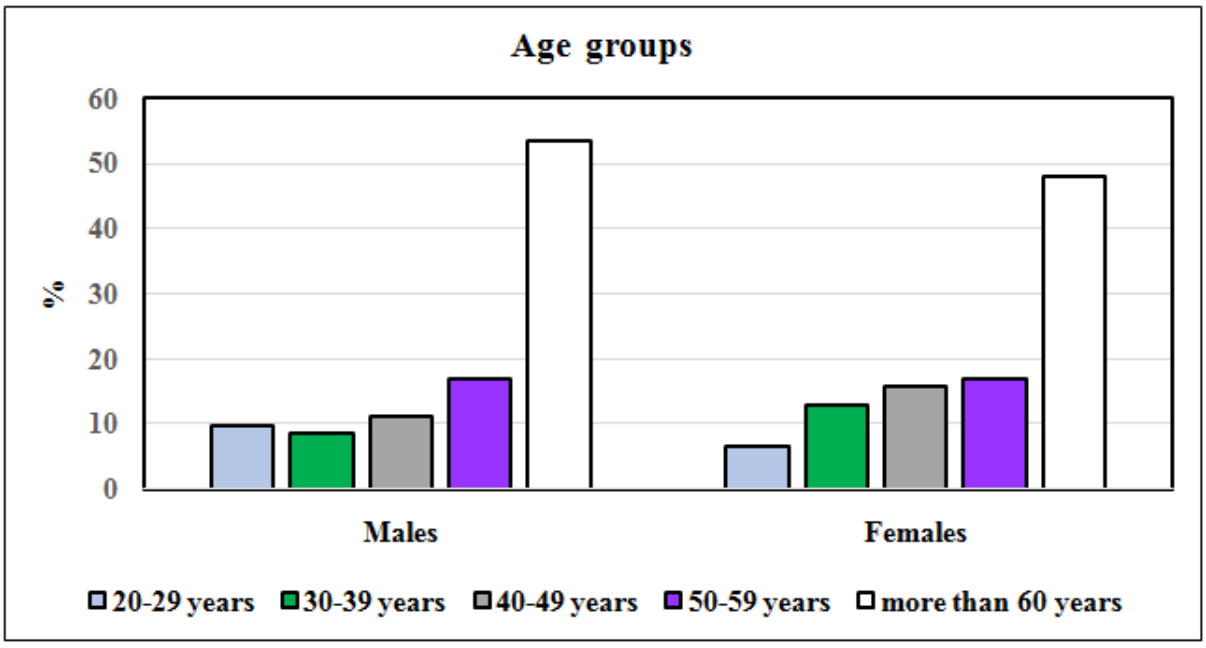

Figure 2. Representation of individual groups of tested drugs