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A New Poisson and Bayesian-Based Method to Assign Risk and Causality in Patients with Suspected Hepatic Adverse Drug Reactions

A Report of Two New Cases of Ticlopidine-Induced Hepatotoxicity

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Abstract

Objective: The diagnosis of drug-induced hepatotoxicity is based on circumstantial evidence and is often inaccurate. We have designed a method based on published data to assign causality to suspected cases of drug-induced hepatotoxicity.
Design: Forty-seven published cases of ticlopidine-induced hepatotoxicity were identified by a Medline-based literature search. Data regarding abnormal liver function in patients receiving ticlopidine were obtained from the only published placebo-ticlopidine clinical trial (the Canadian American Ticlopidine Study; CATS). Thus, we calculated the maximum number of expected hepatotoxicity cases in patients exposed to ticlopidine and those not exposed to the drug by means of the Poisson distribution. The calculated odds ratio was used as a prior odd for subsequent quantification, using a Bayesian-based approach, of individual ticlopidine-induced hepatotoxicity likelihood. Concretely, the prior odd is modified by several separate likelihood ratios: age; sex; AST level; ALT level; alkaline phosphatase level; total bilirubin level; latent period of adverse reaction appearance; and period of remission of adverse reaction. This methodology was applied to two new cases of suspected ticlopidine-induced hepatotoxicity.
Results: The prior probability of ticlopidine-induced hepatotoxicity derived from CATS data is 61.29%. This is in contrast with the 28.83% incidence rate of drug-induced liver alterations in the general population. Alkaline phosphatase levels and total bilirubin levels were six times the normal values among individuals with ticlopidine-induced hepatotoxicity than in the general population. They were the most relevant likelihood ratios of the Bayesian model to establish a high level of causality relationship between a hepatotoxicity event and ticlopidine use.
Conclusions: The proposed method, which links information from clinical trials with the profile of clinical hepatotoxicity of a drug defined from published cases reported after a drug is marketed, can be a useful tool for drug postmarketing surveillance research.

Background

Information about drug safety is frequently limited to clinical trials and spontaneous voluntary adverse drug reaction reporting systems. The reduced size and special characteristics of the patient population tested in clinical trials limit the possibility of discovering uncommon adverse events. Risks that are medically important but delayed in time or less frequent than 1 in 1000 administrations may not be revealed prior to marketing.[1]

Underreporting, selective reporting and misinterpretation of adverse clinical experiences as possible adverse reactions probably render the spontaneous voluntary adverse drug reaction reporting system an inappropriate source of data from which to draw conclusions about true rates of adverse experiences in general clinical practice, much less to compare such rates.[2,3] Numerous attribution algorithms for evaluation of drug-related liver abnormalities are available in the literature. However, since most drug reactions may be indistinguishable of naturally occurring liver diseases, the attributed causality may be difficult or even impossible to prove. Thus, in order to ascertain causality, the physician must take into account the patient's symptoms, the plausibility of a drug cause, and the likelihood of alternative explanations.[4,5]

Available data suggest that adverse reactions to drugs are responsible for a greater proportion of cases of hepatic disease than previously appreciated.[6] Recently, Duh et al.[7] described an incidence of liver enzyme abnormalities in the general population of central Massachusetts of 0.0017, and almost 23% these patients had a drug-induced hepatic illness.[7]

We have recently assisted in the management of two in-patients with suspected ticlopidine induced-hepatotoxicity. Ticlopidine is an antiplatelet agent used for prevention of thrombosis in cerebral vascular and coronary artery disease.[8] The development of abnormal liver function in ticlopidine premarketing trials was rarely reported. In the Canadian American Ticlopidine Study (CATS), the incidence of abnormal liver function tests in patients receiving ticlopidine was 0.4%.[9] Both ticlopidine and placebo administered to patients with intermittent claudication in the Swedish Ticlopidine Multicentre Study (STIMS) showed a 1.8% incidence of suspected liver-related symptoms, but no information was reported regarding the number of patients with abnormal liver function tests.[10] Finally, the Ticlopidine Aspirin Stroke Study (TASS), a comparative clinical trial of ticlopidine and aspirin (acetylsalicylic acid) in the prevention of transient ischaemic attacks (TIAs) and minor stroke, did not report any hepatotoxicity event among recipients of both treatments.[11]

In Spain, there have been 47 spontaneously notified ticlopidine-induced suspected hepatotoxicity cases, by means of the 'yellow card' method. Among them, ten showed a clear-cut relationship between the reported adverse reaction and the use of ticlopidine.[12]

Since most of the cases of suspected drug-induced hepatotoxicity are difficult to ascertain, we herein present a new method, based on data published in the medical literature, to help physicians in the diagnosis and evaluation of patients who, in association with taking a drug, develop clinical or analytical liver dysfunction.

Patients and Methods

A Medline database search was accomplished from 1982, the year of the first ticlopidine-induced hepatotoxicity cases report,[13] to 1999, looking for 'ticlopidine-toxicity' case reports. A similar search with Spanish and French-equivalent terms was completed. The case reports that were found were revised in order to select those with sufficient information regarding hepatic damage. Each one of the case reports found was compared with cases described in review articles about ticlopidine-induced hepatotoxicity.[14-17] Moreover, we looked for the number of patients with liver alterations in the only published placebo-controlled clinical trial with ticlopidine that detailed those patients with abnormal liver function, CATS.[9]

Ticlopidine-Induced Hepatotoxicity Risk Estimation with Data from Clinical Trials Using a Poisson Distribution-Based Method

To estimate the risk of ticlopidine-induced hepatotoxicity we modified the method published in 1994 by Begaud et al.[18] to determine how likely coincidental associations may occur in the postmarketing surveillance setting. Using the data from CATS, we calculated the number of patients treated with ticlopidine for a length of time (mean in weeks; Dt) who showed abnormal liver function tests (Mt). Similarly, the number of patients with abnormal liver function tests that were not associated with ticlopidine (Mp) who would be expected in this sample of patients was calculated using the probability of appearance of these alterations among patients treated with placebo (Pp) for a length of time (mean in weeks; Dp) in the CATS trial (equation 1):

$$Mp = Nt \times Pp \times \left(\frac{Dt}{Dp}\right)$$

In the postmarketing surveillance experience with ticlopidine the number of patients treated with the drug (Nt) has been large whereas the number of patients with abnormal liver function tests (Mt) is expected to be small. Assuming the same probability of appearance for cases with abnormal liver function tests in ticlopidine and placebo-treated patients included in clinical trials and in patients treated out of clinical trials, we calculated the probability P(K) to find K cases of abnormal liver function tests (equation 2):

$$P(K) = \frac{(e^{-Mp} \times Mp^{K})}{K!}$$

and the cumulative probability P(≥K) of at least K cases using the Poisson distribution as has been described previously (equation 3):[18]

$$P(\geq K) = 1 - (P_0 + P_1 + ... + P_{K-1})$$

Considering an alpha error of 5%, it is then possible, for a given value of Mp, to calculate the critical value of K (Kp) for which P(≥K) becomes smaller than 0.05. Kp is the maximum number of expected cases of abnormal liver function tests among placebo-treated patients or patients not treated with ticlopidine. Likewise, we calculated a critical value of K (Kt) using Mt. Kt is the maximum number of cases of abnormal liver function tests expected among ticlopidine-treated patients. Then, we calculated the maximum expected incidence of ticlopidine-induced hepatotoxicity as Kt/Nt and the maximum expected incidence of background or placebo hepatotoxicity as Kp/Nt.

Quantification of Ticlopidine-Induced Hepatotoxicity and Drug Relationship

First, we calculated prior odds (PrO) of ticlopidine-induced hepatotoxicity. This prior odds is the probability odds of the drug causing the event versus any other cause, given the incidence in the CATS trial of abnormal liver function in ticlopidine-treated individuals (Kt/Nt in our method) and placebo-treated patients (Kp/Nt) [equation 4]:[19]

$$Pr\,O = \frac{[(Kt/Nt) - (Kp/Nt)]}{(Kp/Nt)} = \frac{(Kt - Kp)}{Kp}$$

Also, a prior probability (PrP) of ticlopidine-induced hepatotoxicity or probability of the risk among exposed patients[20] was calculated (equation 5):

$$Pr\,P = \frac{Pr\,O}{(1 + Pr\,O)}$$

Second, we calculated the posterior odds, which represents the probability of any drug-induced hepatotoxicity in an individual patient. The prior odds is modified by the likelihood ratio (LR), which is made up of the features of the individual case history.[20] This may be split into several separate LR. Each LR is the probability of observing a trait if the drug was responsible divided by the

probability of having this trait under the alternative hypothesis of non-responsibility of the drug.[20] If no data are available, the likelihood ratio is equal to 1 and the posterior odds are equal to the prior odds.

We calculated LR for the following traits: age, sex, AST level, ALT level, alkaline phosphatase level, total bilirubin level, time after initiating ticlopidine therapy and adverse reaction appearance (latent period or LP) and time elapsed between drug discontinuation and normalisation of blood tests (period of remission or PR). These traits are usually reported in published case reports. No patient with suspected ticlopidine-induced hepatotoxicity was rechallenged.

LR numerators were estimated from published case reports by dividing the number of patients with a trait by the total number of published case reports. LR denominators were estimated from recently published data of acute liver enzyme abnormalities in the general population of central Massachusetts.[7] In this study liver enzyme abnormalities were defined as an increase of over twice the upper limit of normal range in ALT, or a combined increase in AST, alkaline phosphatase and total bilirubin provided one of them was more than twice the upper limit of normal range. The reported mean incidence rates for every aetiology were added to sex (male and female) and age (25 to 44; 45 to 64; >65 years of age) in order to calculate the categories and trait's incidence rates.

Analytical data were divided in several intervals or categories assuming an upper limit of normal range values (θ) of 35 IU/L for AST and ALT, 120 IU/L for alkaline phosphatase and 1 mg/dl for total bilirubin.[21] We calculated, using mean and standard deviation (SD) values (assuming a normal distribution) and the number of patients identified by Duh et al.[7] for each trait considered, the probability and number of patients distributed in the intervals: [$\leq\theta$], [$>\theta$, $\leq 3\theta$] (limit considered by many authors as significant in terms of clinical relevance[22]), [$>3\theta$, $\leq 6\theta$], [$>6\theta$]. The sum of patients estimated to be included in each interval provides us a global distribution into four intervals of AST, ALT, alkaline phosphatase and total bilirubin values, as described above, that were further divided by the total number of patients with abnormal liver function tests in general population to calculate the LR denominator. A sensitivity analysis was carried out for variables used to calculate the LR: age, sex, AST, ALT, alkaline phosphatase (alk phos) and total bilirubin (tot bil) considering different incidence rates for aetiologies of liver enzyme abnormalities considered by Duh et al.[7]

LP, a time-dependent variable, was calculated by dividing 28 weeks (maximum LP in published cases of ticlopidine-induced hepatotoxicity was 26 weeks) in four 7 weeks intervals: [≤7], [8 to 14], [15 to 21] and [22 to 28] and assuming a random appearance in the time of hepatotoxicity cases each interval probability was 0.25. Similarly, PR was calculated by dividing 52 weeks (maximum PR in published cases of ticlopidine-induced hepatotoxicity was 52 weeks) in four 13-weeks intervals: [≤13], [14 to 26], [27 to 39] and [40 to 52] weeks. Similarly to LP, the probability of each PR interval was 0.25.

Finally, we calculated a posterior odds ratio (PsO) [equation 6]:

$$\begin{aligned}\mathrm{PsO} = {} & \mathrm{Pr\,O} \times \mathrm{LR(Age)} \times \mathrm{LR(Sex)} \times \mathrm{LR(AST)} \times \\ & \mathrm{LR(ALT)} \times \mathrm{LR(alk\ phos)} \times \mathrm{LR(tot\ bil)} \times \\ & \mathrm{LR(LP)} \times \mathrm{LR(PR)}\end{aligned}$$

and a posterior probability (PsP), which represents the probability in favour of the ticlopidine cause of a particular adverse drug reaction (equation 7):

$$\mathrm{PsP} = \frac{\mathrm{PsO}}{(1+\mathrm{PsO})}$$

If there is more than one drug administered to the patient, the posterior probability of each drug (D_i) can be calculated using the method proposed by Naranjo et al. (equation 8):[23]

$$\mathrm{PsP}(D_i) = \frac{[\mathrm{PsO}(D_i)]}{[1+\sum_{i-1}^{n} \mathrm{PsO}(D_i)]}$$

where n is the number of drugs administered to the patient.

Microsoft Excel 97® was used for all mathematical calculations.

Results

Ticlopidine-Induced Hepatotoxicity Risk Calculated from CATS Trial Using a Poisson Distribution-Based Method

CATS was a randomised, double blind, placebo-controlled trial designed to assess the effect of ticlopidine (250mg twice daily) in reducing the rate of subsequent occurrence of stroke, myocardial infarction, or vascular death in patients who had had a recent thromboembolic stroke. Twenty-five centres entered 1053 patients into the study between 1 week and 4 months after their qualifying stroke. Patients were treated (528 with placebo and 525 with ticlopidine) and followed for an average period of 19 months (placebo) and 17 months (ticlopidine). The study cohort was 62% male with a mean age of 65 years and substantial cardiovascular co-morbidity (70% of patients required assistance in their activities of daily living). Eight patients treated with placebo (8 of 528 = 0.0151) and 23 with ticlopidine (23 of 525 = 0.0438) developed abnormal liver function and in 1 patient with placebo and 12 with ticlopidine the adverse experience was enough severe or troublesome to lead to discontinuation of drug. Following the described method: Mp = 525 × 0.0151 × (17/19) = 7; Mt = 23; and Kp and Kt critical values are Kp = 12 and Kt = 31. Then, PrO = 31 – 12/12 = 1.5833 and PrP = 1.5833/(1 + 1.5833) = 0.6129 = 61.29%.

Quantification of Ticlopidine-Induced Hepatotoxicity

From 1982, the year of the first ticlopidine-induced hepatotoxicity cases report,[13] to 1999, 47 reports of ticlopidine-induced hepatotoxicity cases have been indexed on Medline. The demographic and clinical information from these 47 cases is described in table I. Table II details the sum of incidence rates (SIr) and the global sum of incidence rates (GSIr) described in Massachusetts's population by Duh et al.[7] The frequency of appearance of each trait (freq), calculated by means of SIr and GSIr values, and the frequency of appearance of the same trait among ticlopidine-induced hepatotoxicity published case reports (freq cases), are described in the same table. Then, the trait value of LR (LR) is calculated by dividing freq cases by freq. (table II).

Estimation of Posterior Probability (PsP) in Two Patients with Probable Ticlopidine-Induced Hepatotoxicity

Two cases of possible ticlopidine-induced hepatotoxicity were identified in our hospital.

Case 1

A 62-year-old man was admitted to our hospital in April 1999, for investigation of jaundice. Four weeks prior to admission he had became markedly jaundiced and had experienced pruritus, choluria, weakness and abdominal pain. His past history included type 1 diabetes mellitus for 12 years, lipoprotein abnormalities controlled by diet, microalbuminuria and peripheral vascular disease. There was no previous history of liver disease or alcohol abuse. Two months prior to the current admission he was admitted to hospital due to necrosis of the first finger of his left foot that was finally amputated. After this episode he was prescribed enalapril 20mg twice daily, pentoxifylline 400mg three times daily and ticlopidine 250mg twice daily. On admission the following laboratory parameters were recorded: AST level 598 IU/L (laboratory range: 0 to 37 IU/L); ALT level 1243 IU/L (0 to 41 IU/L); alkaline phosphatase level 3955 IU/L (91 to 258 IU/L); total bilirubin 7.2 mg/dl (0.01 to 1.3 mg/dl); direct bilirubin 7 mg/dl; γ-glutamyl transferase level 2468 IU/L (0 to 30 IU/L), erythrocyte count $4.26 \times 10^6/mm^3$; haemoglobin level 11.9 g/dl; haematocrit 35%; platelet count $233000/mm^3$; and leucocytes $2.9 \times 10^3/mm^3$ (8% neutrophils, 48% lymphocytes). Hepatitis B sur-

Table I. Demographic and clinical information of 47 published ticlopidine-induced hepatotoxicity case reports[13,15-17,24-57]

Variable	Mean ± SD	Minimum	Maximum	Categories (no. of patients)			
Age (y)	69.3 ± 13.2	29	92	**25 to 44**	**45 to 64**	**>64**	
				1	13	33	
Sex (M/F)				**M**	**F**		
				22	23		
Dose (mg/day)				**250**	**500**		
				9	31		
Total bilirubin (mg/dl)	10.2 ± 8.0	0.5	32.5	**≤1**	**>1 to ≤3**	**>3 to ≤6**	**>6**
				3	3	11	26
Alkaline phosphatase (IU/L)	783.5 ± 410.3	180	2130	**≤120**	**>120 to ≤360**	**>360 to ≤720**	**>720**
				0	6	15	21
AST (IU/L)	228.9 ± 244.1	41	1400	**≤35**	**>35 to ≤105**	**>105 to ≤210**	**>210**
				0	11	13	15
ALT (IU/L)	364.3 ± 449.1	42	2661	**≤35**	**>35 to ≤105**	**>105 to ≤210**	**>210**
				0	7	11	23
LP (wk)	6.2 ± 4.9	2	26	**≤7**	**8 to 14**	**15 to 21**	**22 to 28**
				31	11	2	1
PR (wk)	4.4 ± 3.5	0.5	12	**≤13**	**14 to 26**	**27 to 39**	**40 to 52**
				21	8	3	6

F = female; **LP** = latent period; **M** = male; **PR** = period of remission; **SD** = standard deviation.

face antigen, antihepatitis C virus antibodies, and hepatitis A immunoglobulin M antibody test results, together with antinuclear (ANA), antismooth muscle (SMA), and antimitochondrial autoantibodies (AMA), were all negative. Copper and iron studies were negative. Abdominal ultrasound and computed tomography (CT) scan showed no evidence of biliary obstruction. A diagnosis of probable ticlopidine-induced severe neutropenia associated to hepatotoxicity was made. The patient was afebrile and a peripheral blood (except leucocytes and neutrophils) and bone marrow were both normal. Ticlopidine was then discontinued but enalapril and pentoxifylline were continued. Five and 10 days after ticlopidine discontinuation his leucocyte count was $4.1 \times 10^3/mm^3$ (33% neutrophils) and $6.1 \times 10^3/mm^3$ (50% neutrophils), respectively. Ten days after ticlopidine discontinuation serum liver analytical values were: AST 93 IU/L; ALT 329 IU/L; alkaline phosphatase 2461 IU/L; total bilirubin 10.8 mg/dl; and γ-glutamyl transferase 2461 IU/L. Analytical liver values returned towards normal in the subsequent 10 months.

The system proposed by Danan[58] indicates a suggestive causal relationship between ticlopidine and abnormal liver function in this patient. When we apply the proposed method the PsO was: PsO = PrO × LR(Age) × LR(Sex) × LR(AST) × LR(ALT) × LR(alk phos) × LR(tot bil) × LR(LP) × LR(PR) = 1.58 × 1.14 × 0.88 × 2.28 × 2.51 × 109.50 × 13.24 × 0.98 × 0.63 = 8119.36 and PsP = 8119.36 / (1 + 8119.36) = 0.9999.

Case 2

A 76-year-old man with a medical history of type 2 diabetes mellitus controlled with glibenclamide (glyburide) 2.5mg twice daily, hiatal hernia, arthrosis, cholecystectomy and prostatectomy for prostatic hyperplasia was admitted to the liver

Table II. Sum of incidence rates, frequency and LR considering several traits estimated using published data of acute liver enzyme abnormalities in a general population[7]

Variable	Categories	SIr	N(calc)	Freq	Freq cases	LR	Freq cases#	LR#
Sex	Males	181.2		0.553	22/45 = 0.489	0.88	24/47 = 0.511	0.92
	Females	146.2		0.447	23/45 = 0.511	1.14	23/47 = 0.489	1.10
	GSIr	327.4						
Age (y)	25-44	120.9		0.201	1/47 = 0.021	0.11	1/49 = 0.020	0.10
	45-64	146.8		0.244	13/47 = 0.277	1.14	14/49 = 0.286	1.17
	>64	334.7		0.556	33/47 = 0.702	1.26	34/49 = 0.694	1.25
	GSIr	602.4						
AST (IU/L)	≤35		67	0.306	0/39 = 0.000	1.00	0/41 = 0.000	1.00
	36-105		57	0.260	11/39 = 0.282	1.08	12/41 = 0.293	1.12
	106-210		58	0.265	13/39 = 0.333	1.26	13/41 = 0.317	1.20
	>210		37	0.169	15/39 = 0.385	2.28	16/41 = 0.390	2.31
	GSIr		219					
ALT (IU/L)	≤35		58	0.265	0/41 = 0.000	1.00	0/43 = 0.000	1.00
	36-105		57	0.260	7/41 = 0.171	0.66	7/43 = 0.163	0.63
	106-210		55	0.251	11/41 = 0.268	1.07	12/43 = 0.279	1.11
	>210		49	0.224	23/41 = 0.561	2.51	24/43 = 0.558	2.49
	GSIr		219					
ALP	≤120		95	0.434	0/42 = 0.000	1.00	0/44 = 0.000	1.00
(IU/L)	121-360		106	0.484	6/42 = 0.143	0.30	6/44 = 0.136	0.28
	361-720		17	0.078	15/42 = 0.357	4.60	15/44 = 0.341	4.39
	>720		1	0.005	21/42 = 0.500	109.50	23/44 = 0.523	114.48
	GSIr		219					
Total bilirubin	≤1		90	0.411	3/43 = 0.070	0.17	3/45 = 0.067	0.16
(mg/dl)	1.1-3		80	0.365	3/43 = 0.070	0.19	4/45 = 0.089	0.24
	3.1-6		39	0.178	11/43 = 0.256	1.44	11/45 = 0.244	1.37
	>6		10	0.046	26/43 = 0.605	13.24	27/45 = 0.600	13.14
	GSIr		219					
LP (wk)	≤ 7			0.250	31/45 = 0.688	2.75	31/47 = 0.659	2.64
	8-14			0.250	11/45 = 0.244	0.98	12/47 = 0.255	1.02
	15-21			0.250	2/45 = 0.044	0.18	3/47 = 0.064	0.26
	22-28			0.250	1/45 = 0.022	0.09	1/47 = 0.021	0.08
PR (wk)	≤ 13			0.250	21/38 = 0.553	2.21	21/40 = 0.525	2.10
	14-26			0.250	8/38 = 0.211	0.84	8/40 = 0.200	0.80
	27-39			0.250	3/38 = 0.079	0.32	3/40 = 0.075	0.30
	40-52			0.250	6/38 = 0.158	0.63	8/40 = 0.200	0.80

ALP = alkaline phosphatase; **Freq** = SIr/GSIr for each category; **Freq cases** = number of induced-ticlopidine published cases with the condition / total number of ticlopidine-induced published cases; **Freq cases#** = number of induced-ticlopidine published cases (including the two new cases described by authors) with the condition/total number of induced-ticlopidine cases; **GSIr** = global sum of incidence rates; **LP** = latent period; **LR** = freq cases/freq (when a frequency is 0, LR is 1); **LR#** = freq cases#/freq (when a frequency is 0 LR is 1); **N (calc)** = number of cases in each category, assuming a normal distribution, calculated from mean and standard deviation data in 219 liver alterations cases described by Duh et al.;[7] **PR** = period of remission; **SIr** = sum of incidence rates. Incidence rate unit is number of cases per 100 000 persons per year.

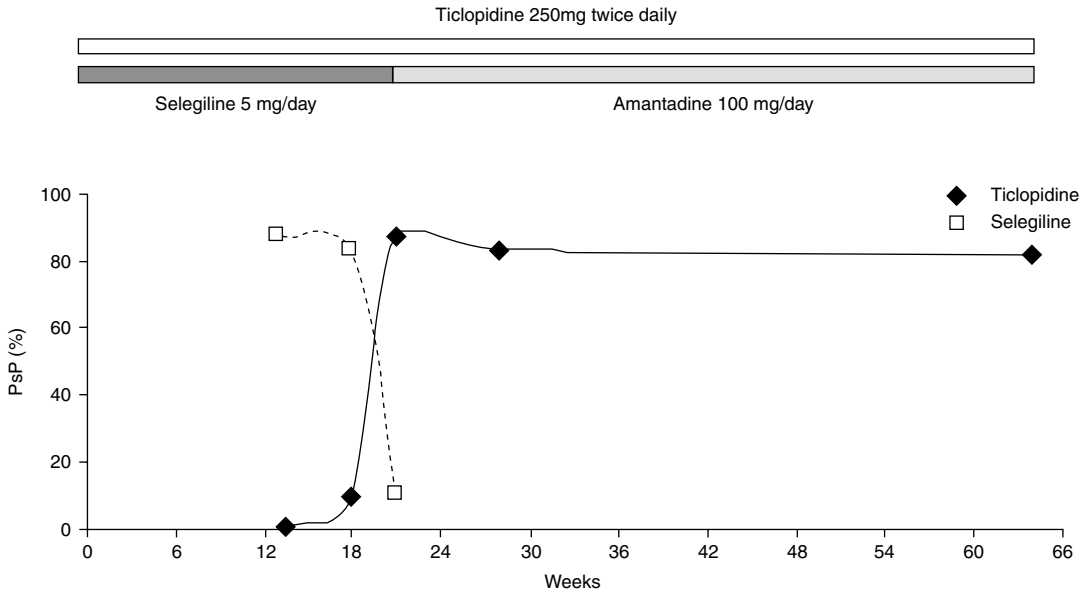
unit of our hospital for investigation of abnormal liver function tests. Five months before he had been diagnosed of multiple brain infarcts and Parkinson's disease and began treatment with ticlopidine 250mg twice daily and selegiline 5 mg/day. Routine follow-up blood tests in the preceding 5 months disclosed only slight elevations of liver enzymes without any clinical repercussion. However, 21 weeks after ticlopidine and selegiline were started, the liver enzymes showed severe alterations: AST level 91 IU/L (range: 0 to 37 IU/L); ALT level 134 IU/L (0 to 41 IU/L); γ-glutamyl transferase 1029 IU/L (0 to 30 IU/L); alkaline phosphatase level 754 IU/L (91 to 258 IU/L); and total bilirubin 1.7 mg/dl (0.01 to 1.3 mg/dl). The treatment of selegiline was discontinued and substituted by amantadine (100 mg/day) and the patient was readmitted to the liver unit for further evaluation. Hepatitis B surface antigen, antihepatitis C virus antibody, hepatitis A immunoglobulin M antibody tests, and ANA, SMA and AMA were all negative. Abdominal ultrasound and CT scan showed no evidence of biliary obstruction. Successive analytical controls showed an important elevation of total bilirubin (up to 11.5 mg/dl) simultaneously with an improvement of AST, ALT and alkaline phosphatase. After 64 weeks of treatment, ticlopidine was discontinued. Total bilirubin values after ticlopidine discontinuation were 7.1, 5.7, 4.0, 3.2, 2.2 and 1.0 mg/dl on 3, 6, 15, 30, 50 and 76 weeks, respectively. The system proposed by Danan[58] indicates a suggestive causal relationship between selegiline and abnormal liver function at week 21 and between ticlopidine and abnormal liver function at week 64 in this patient.

This is a complex case in that took a long time to identify ticlopidine as responsible for the elevated liver function tests. Initially it was suspected that the patient was experiencing selegiline-induced hepatotoxicity, and therefore this drug was changed by amantadine. Parkinsonism is not associated with an increased incidence of abnormal liver function.[59] In a clinical trial sponsored by The Parkinson Study Group, 800 subjects were randomly assigned in a 2 × 2 factorial design to receive selegiline 10 mg/day, tocopherol (vitamin E), a combination of both drugs, or placebo, and were followed up an average 12 months. A total of 401 subjects did not receive selegiline (group control) and 399 did receive this agent. Fifteen subjects in selegiline group (15 of 399 = 0.037), as compared with two subjects in control group (2 of 401 = 0.005), had serum ALT levels that exceeded 50 U/L. Only four subjects in selegiline group had ALT levels above 100 U/L. These increases occurred typically in the first 6 months of treatment and were unassociated with clinical evidence of hepatic dysfunction. In all but one subject, the values became normal with continued treatment.[60] Using this information the selegiline PrO was calculated: Mp = 399 × 0.005 × (12/12) = 2, Mt = 15, Kp = 5, and Kt = 22. Where Mp and Kp was referred to control group and Mt and Kt to selegiline group. Then PrO = 22 – 5/5 = 3.4 and PrP = 3.4/(1 + 3.4) = 0.7727 = 77.27%. A Bayesian-based estimation of PsP was made using analytical and demographic information from nine patients who had received selegiline during long periods of time and who had presented elevations in liver enzymes levels as described by Golbe.[61] To our knowledge no other cases of selegiline-induced hepatotoxicity have been published.

Figure 1 shows the temporary evolution of this patient's analytical data and ticlopidine and selegiline PsP.

Modification of PsP with Each New Case Analysed

One advantage of this method is that its accuracy may improve when adding information from new cases. In the example, data from cases 1 and 2 were used to modify freq. cases and LR values previously calculated (see table II; freq. cases # and LR#). The addition of information from cases 1 and 2 emphasised the importance of alkaline phosphatase in the diagnosis of ticlopidine-induced liver alterations and induces one to consider a much longer PR. Addition of future patients iden-



Weeks	Alkaline phosphatase	Total bilirubin	AST	ALT	Ticlopidine PsO	Ticlopidine PsP (%)	Selegiline PsO	Selegiline PsP (%)
13	307	0.8	56	88	0.0629	0.70	7.49	87.60
18	518	0.9	68	122	1.5948	10.40	12.71	83.00
21	754	1.7	91	134	42.7014	87.30	5.23	10.70
28	266	11.5	50	52	4.9001	83.05		
64	343	8.9	26	45	4.5217	81.89		

Fig. 1. Temporal evolution of analytical data for the patient described as 'case 2' and ticlopidine and selegiline posterior probability (PsP). In this case, the calculation of ticlopidine posterior odd ratio (PsO) was made assuming latent period (LP) and period of remission values of 1 because the LP in one of calculations was greater than 28 weeks (maximum LP in the published cases) and in most of estimations ticlopidine had not been removed. Ticlopidine PsP is corrected by selegiline PsP and selegiline PsP by ticlopidine PsP using equation 8.

tified to have ticlopidine-induced hepatotoxicity will permit a better model to be built.

Differential Analytical Profile of Ticlopidine-Induced Hepatotoxicity

The frequency of appearance of each trait (sex, age, AST, ALT, alkaline phosphatase, total bilirubin) in patients with abnormal liver function tests in a general population (table II) and in patients with various types of incident liver enzyme abnormalities described by Duh et al.[7] were calculated. Then a trait value of LR was calculated to each aetiological category by dividing the aetiological category frequency by the frequency calculated in all cases described in the general population (freq.; table II). Using this information and the PrO calculated for ticlopidine (1.58) the PsP for the 49 reports of ticlopidine-induced hepatotoxicity patients (the 47 described in table I and the two patients described in this paper) were simulated using

the LR of seven aetiological categories considered: alcohol, malignancy, viral hepatitis (A,B,C), biliary pathologies, mononucleosis and other presumptive viral infections, drug-associated and uncertain aetiology. LP and PR were assumed to be 1. Mean PSP and the 95% confidence intervals calculated are showed in figure 2. Only the analytical pattern for malignancy and biliary pathologies are not statistically different from ticlopidine. Alcohol, viral hepatitis, mononucleosis, drug-associated and uncertain aetiology PsP values are very different from ticlopidine PsP, suggesting that analytical traits, usually not considered in causality assignment, are very important in the ticlopidine Bayesian model.

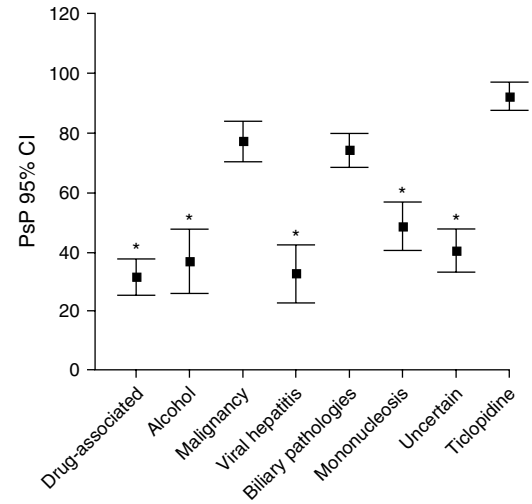


Fig. 2. Mean posterior probability (PsP) and 95% confidence intervals (CI) of the 49 reports of ticlopidine-induced hepatotoxicity patients (47 described in tab I and the two patients described in this paper) calculated using the likelihood ratio calculated for each of seven aetiological categories considered: alcohol, malignancy, viral hepatitis (A,B,C), biliary pathologies, mononucleosis and other presumptive viral infections, drug-associated and uncertain aetiology. Latent period and period of remission values were assumed to be 1. * $p < 0.05$ compared with ticlopidine. Means compared using a one-way ANOVA and *post hoc* Scheffé test.

Effects of Different Incidence Rates of the Various Liver Enzyme Abnormalities Not Related to Ticlopidine in Ticlopidine-Related PsP Calculations

To our knowledge there is no published information regarding possible differences in the aetiology of abnormal liver function in patients treated with ticlopidine compared with cases presented in a general population. To control for the possibility that PsP may be biased by a high incidence rate of a particular aetiology in these patients, we have made a sensitivity analysis considering populations with diverse incidence rates of the various categories of liver enzyme abnormalities. In the extreme situation in which all non-ticlopidine associated abnormal liver functions were caused by only one of these categories, the calculated PsP (mean ± SD) of the 49 reports of ticlopidine-induced hepatotoxicity were: 0.94 ± 0.15, 0.87 ± 0.25, 0.91 ± 0.17, 0.91 ± 0.20, 0.94 ± 0.13, 0.91 ± 0.19 for all causes grouped, alcohol, malignancy, viral hepatitis (A,B,C), biliary pathologies, and drug-associated, respectively. When mononucleosis and other presumptive viral infections (0.73 ± 0.23) and uncertain aetiology (0.81 ± 0.26) were considered, the calculated mean PsP was statistically inferior to PsP calculated using all causes grouped, but superior to 0.61, the prior odds of ticlopidine. Then, the sensitivity of the model is high independently of differences in the incidence rates of causes of abnormal liver function in the population of patients considered.

Differential Value of the Analytical Parameters in the Ticlopidine PsP Calculations in Function of Characteristics of the Population of Patients Considered

We have calculated the PsP of the 49 reports of ticlopidine-induced hepatotoxicity in three different situations: (i) without including analytical variables in the calculation; (ii) including only one of the variables: ALT, AST, alkaline phosphatase and total bilirubin; and (iii) including simultaneously the four analytical variables. Table III shows the calculated PsP, mean ± SD, PsP 95% confidence

Table III. Posterior probability (PsP) of the 49 reports of ticlopidine-induced hepatotoxicity without including analytical variables in the calculation, including only one of the variables: ALT, AST, alkaline phosphatase and total bilirubin, and simultaneously including the four analytical variables. Data in each cell are PsP mean ± standard deviation, PsP 95% confidence interval and the number of cases with a PsP inferior to 0.61 (the prior odds of ticlopidine).

Aetiological categories considered	Without analytical variables	Including ALT	Including AST	Including alkaline phosphatase	Including total bilirubin	Including all analytical variables
All	0.75 ± 0.20	0.79 ± 0.19	0.79 ± 0.19	0.90 ± 0.20	0.84 ± 0.22	0.94 ± 0.15
	[0.69-0.80]	[0.74-0.85]	[0.74-0.84]	[0.84-0.95]	[0.78-0.91]	[0.90-0.98]
	10	7	7	7	7	4
Alcohol	0.80 ± 0.22	0.84 ± 0.24	0.82 ± 0.23	0.81 ± 0.26	0.83 ± 0.22	0.87 ± 0.25
	[0.74-0.87]	[0.77-0.91]	[0.75-0.88]	[0.74-0.89]	[0.76-0.89]	[0.80-0.94]
	10	7	10	8	10	6
Malignancy	0.72 ± 0.21	0.74 ± 0.25	0.77 ± 0.20	0.83 ± 0.20	0.82 ± 0.23	0.91 ± 0.17
	[0.66-0.78]	[0.67-0.81]	[0.72-0.83]	[0.78-0.89]	[0.75-0.88]	[0.86-0.96]
	12	12	9	8	9	3
Viral hepatitis (A,B,C,)	0.73 ± 0.20	0.79 ± 0.19	0.81 ± 0.19	0.73 ± 0.26	0.84 ± 0.23	0.91 ± 0.20
	[0.67-0.79]	[0.74-0.85]	[0.76-0.86]	[0.66-0.81]	[0.77-0.90]	[0.85-0.96]
	11	7	7	15	6	6
Biliary pathologies	0.76 ± 0.19	0.80 ± 0.17	0.80 ± 0.17	0.88 ± 0.18	0.85 ± 0.21	0.94 ± 0.13
	[0.70-0.81]	[0.75-0.85]	[0.75-0.85]	[0.83-0.93]	[0.79-0.90]	[0.90-0.97]
	10	4	5	7	5	2
Mononucleosis and other presumptive viral infections	0.68 ± 0.23	0.72 ± 0.22	0.73 ± 0.22	0.65 ± 0.24	0.68 ± 0.24	0.73 ± 0.23
	[0.62-0.75]	[0.66-0.78]	[0.67-0.80]	[0.58-0.72]	[0.61-0.75]	[0.67-0.80]
	13	12	11	16	15	13
Drug-associated	0.75 ± 0.19	0.81 ± 0.19	0.80 ± 0.19	0.76 ± 0.24	0.85 ± 0.22	0.91 ± 0.19
	[0.70-0.81]	[0.76-0.86]	[0.75-0.86]	[0.69-0.83]	[0.79-0.92]	[0.86-0.97]
	9	8	7	13	7	5
Uncertain aetiology	0.76 ± 0.23	0.83 ± 0.22	0.81 ± 0.23	0.75 ± 0.24	0.72 ± 0.26	0.81 ± 0.26
	[0.70-0.83]	[0.77-0.89]	[0.75-0.88]	[0.68-0.82]	[0.65-0.79]	[0.73-0.88]
	11	6	8	11	14	9

interval and the number of cases of ticlopidine-induced hepatotoxicity with a PsP inferior to 0.61 (the prior odds of ticlopidine). This analysis showed that ALT is the most important analytical variable to differentiate cases with ticlopidine-induced hepatotoxicity from cases related to alcohol, biliary disease and those of uncertain aetiology. Further, alkaline phosphatase is the parameter that allows a differential diagnosis with malignancy cases. The inclusion of the four analytical variables in the model explains most ticlopidine-induced cases.

Discussion

The method detailed here is aimed to reflect, from a mathematical point of view, the clinical approach of physicians when evaluating a patient with liver enzyme abnormalities while receiving several drugs. The incidence of drug-induced liver alterations has been scarcely described in controlled epidemiological studies.[2] The most frequent situation is the existence of limited and imprecise information that forces to physicians: (i) to look for published information of clinical trials about

the number of liver alterations cases among drug-treated patients; and (ii) to apply this information to a particular patient.

To show how the proposed method works, we have chosen ticlopidine, which has been associated with various cases of hepatic alterations and for which no published accepted hepatotoxic risk exists.[8,15] First, we have evaluated the information obtained from clinical trials carried out with ticlopidine. Three major clinical trials have been published in which the effectiveness of ticlopidine has been tested for secondary prevention of thrombosis (CATS and TASS)[9,11] and treatment of intermittent claudication syndrome (STIMS).[10] Only the first quoted study indicated the number of patients with abnormal liver function tests. The description of results of STIMS trial includes the number of patients with liver dysfunction although the authors did not described that term. No cases of abnormal liver enzymes were described in the TASS study. This paucity of information about drug safety in the description of clinical trials results is common and represents a serious problem to physicians. We have used data from CATS trial assuming that this study fulfils the quality criteria usually accepted by the scientific community: randomised, double blind, controlled with placebo and including homogeneous groups of patients, to perform *a priori* estimation of the probability of ticlopidine-induced serum liver enzyme alterations (PrP). In spite of the inherent limitations to any experimental result, we believe that to use data from clinical trials could be a better methodological approach that other systems frequently used to calculated *a priori* risks, such as the consensus of experts or expert revisions of published information.

It is common that classical statistical approaches do not find significant differences in safety data from clinical trials (type II error). However, a Poisson distribution-based approach allows us to calculate the probability to find a number of patients with an uncommon adverse event when a large number of patients have been treated with the drug.[18] The incidence of adverse events in a clinical trial is usually, in the absence of epidemiological studies, the only data that makes it possible to calculate the maximum number of cases that would be reasonable to find when the drug is administered to many patients. Figure 3 shows the expected number of patients with liver alterations, calculated using a Poisson distribution-based approach, in a population of 525 patients with moderate-severe stroke treated with ticlopidine or placebo. It is obvious from figure 3 that ticlopidine-treated patients have a higher risk of developing liver alterations than placebo-treated patients.

Duh et al.[7] have estimated a 0.0017 incidence of abnormal liver function tests in a general population, similar to previously described incidences.[18] Figure 3 shows the Poisson distribution of the number of patients with liver alterations that would be expected to appear in a population of 525 patients assuming a 0.0017 incidence. The differences between the three groups here detailed are evident. It is likely that the higher risk of liver alterations in placebo-treated patients with moderate-severe stroke be explained by the characteristics of the

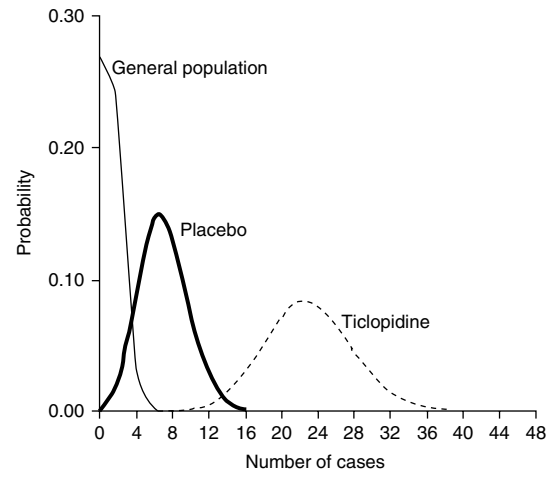


Fig. 3. Probability Poisson distributions of the expected number of patients with liver alterations in 525 patients with moderate-severe stroke treated with ticlopidine or placebo or in a 525 patient population assuming a 0.0017 incidence (general population).

population studied, such as age, previous disease, lifestyle, and other. This data confirms the relevance of having a placebo-treated control group in clinical trials to accurately calculate a drug-induced hepatotoxicity risk. Our model is based on data from patients included in a clinical trial (CATS), reports of ticlopidine-induced hepatotoxicity and patients with liver enzyme abnormalities identified in the general population of central Massachusetts. The LRs used in our method are based in the population of Massachusetts and this population may not be representative of other regions and ethnic groups in the world. In fact, reports of ticlopidine-induced hepatotoxicity, patients included in the CATS trial, and cases 1 and 2 were from populations outside Massachusetts. Then, the use of these data is a limitation of our method and, ideally, the profile of liver enzyme abnormalities of nontreated post-stroke patients should be used if available. Nevertheless, the use of data obtained from the general population is useful in order to apply our diagnostic approach to patients treated with ticlopidine for non-stroke reasons. No validation of this method has been made because all the cases of ticlopidine-induced hepatotoxicity described to date have been used to build the model, that assigns a high probability to 92% of all published cases (table III). The comparison of the calculated PsP by means of this model, to the attribution of causality using algorithms for evaluation of drug-related liver abnormalities to future cases of suspected hepatotoxicity would give us the accuracy of this approach.

Two other limitations with this Bayesian model must be considered. First, we are not certain that some analytical parameters used to calculate LRs, such as AST and ALT, are really independent in which case false increased odds in favour of the drug would be possible. However, the inclusion of the four analytical variables in the model explains most ticlopidine-induced cases and, if different aetiological categories of liver enzyme abnormalities are considered, the influence of the different analytical variables may also change. For example, for the aetiological category 'alcohol', if no analytical variables are included in the model, ten published cases of ticlopidine-induced hepatotoxicity have a PsP inferior to 0.61 (the prior odds of ticlopidine). Inclusion of ALT in the model reduces this number to seven, an effect not found when AST is the variable included in the model. This is not a surprising effect given that alcoholic hepatitis is characterised by an AST/ALT ≥2. On the contrary, AST, and not ALT, change the number of cases with PsP inferior to 0.61 when malignancy is the aetiological category considered. Second, data inputs for the model were taken from different sources: clinical trials, pharmacoepidemiological studies and published case reports. It may be that each of these sources uses a different definition of an hepatic adverse drug reaction and further, that different events have been used to calculate prior odds and LRs; therefore, the results of the model may be inaccurate.

The PrP calculated for ticlopidine was 61.29% and therefore it should be considered that this drug is the cause of 61% of all abnormal liver function tests in moderate-severe stroke patients treated with ticlopidine. This in contrast with the 22.83% frequency rate of drug-induced liver alterations shown by Duh et al.[7] in the general population.

We calculated the patient PsO and PsP. In 70% of published cases of suspected ticlopidine-hepatotoxicity the patients involved are older than 64 years (table I), probably because ticlopidine is a drug that is commonly used in older people. Liver alterations are also more frequent in the general elderly population (table II), a fact that is rectified by the model adjusting with LR near to 1. Younger patients (aged 25 to 44 years) are different because the frequency of liver alterations due to any cause in this group of age in the general population is 10 times higher than published ticlopidine-induced cases. This situation results in a small LR. The sex distribution was similar in published cases and the general population, and then the LR was near 1. Analytical variables (AST, ALT, alkaline phosphatase and total bilirubin) are usually not con-

sidered in causality assessment, but they are all considered when defining the existence of 'liver injury', when defining the liver injury as 'hepatocellular', 'cholestatic' or 'alcohol-associated' and when establishing the severity and prognosis.[62] The analytical distribution pattern of ticlopidine-induced hepatotoxicity was different from analytical patterns of other liver diseases and drug-associated hepatotoxicity in a general population (figure 2), suggesting that the ticlopidine analytical pattern offers specific information worth considering in the diagnosis of ticlopidine-induced hepatotoxicity. Alkaline phosphatase and total bilirubin are 6 times the normal values among ticlopidine-induced hepatotoxicity cases than in general population, and alkaline phosphatase is probably the most relevant analytical measurement to establish ticlopidine causality. To our knowledge, no information is available to assume that cases of abnormal liver function in a population of post-stroke patients are caused by different aetiologies from those in a general population. As detailed above a sensitivity analysis has showed that the PsP calculated using this Bayesian method were not influenced by the different categories of aetiologies of abnormal liver function in each population.

Case 1 was a patient with a typical evolution of drug-induced hepatotoxicity. The patient recovered after discontinued the drug, confirming the initial suspicion, and the calculated PsP was in agreement with the clinical impression. In case 2, however, it was difficult to suspect early a relationship between ticlopidine and liver alteration because the delay in drug discontinuation and doubts about other causes. Figure 1 shows the values of different PsP calculated in several times during the evolution of case 2, corrected by selegiline PsP, which was the other suspected drug. *A priori*, selegiline is a more hepatotoxic drug than ticlopidine. However, after 21 weeks of ticlopidine treatment the analytical profile and the evolution of the patient were very suggestive of ticlopidine-induced hepatotoxicity (PsP = 87.30%), even taking into account the coadministration of selegiline. At this moment, the PsP for selegiline, considering ticlopidine, is only 10.7%. In this complex case it is not possible to absolutely rule out an association of two hepatotoxicities with different analytic expression and temporal sequence. The changes that took place before the week 21 (elevation of AST level and ALT level without variations in the values of total bilirubin) are equivalent to those observed in published cases of selegiline-associated hepatotoxicity,[60,61] but none of the published cases of selegiline-induced hepatotoxicity has been shown to be associated to abnormal values of total bilirubin. In this context, the withdrawal of selegiline would explain to the improvement of AST and ALT but not the later sustained elevation of total bilirubin. Total bilirubin showed a low tendency to normalisation after ticlopidine removal. In this case, the application of the proposed method might have permitted to discontinue promptly ticlopidine with the subsequent benefit to the patient.

A more systematic collection of clinical and epidemiological information from every new patient with drug-induced disease and the addition of information about new adverse events from clinical trials or epidemiological studies to this method makes it a powerful and useful tool for drug post-marketing surveillance research. Meanwhile, a method like this, that links information from clinical trials with the profile of clinical hepatotoxicity of a drug defined from published cases reported after a drug is marketed, can be a useful tool to help physicians to take adequate decisions in front of a particular patient.

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