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PETADOLEX®, A HERBAL EXTRACT FOR MIGRAINE PROPHYLAXIS WITH SPONTANEOUS CASE REPORTS OF DISPUTED LIVER INJURY: ROBUST CAUSALITY EVALUATION BY RUCAM, THE ROUSSEL UCLAF CAUSALITY ASSESSMENT METHOD

Rolf Teschke[1]*, Axel Eickhoff[1], Johannes Schulze[2], Albrecht Wolff[3], Christian Frenzel[4] and Dieter Melchart[5,6]

[1]Department of Internal Medicine II, Division of Gastroenterology and Hepatology, Klinikum Hanau, Teaching Hospital of the Goethe University Frankfurt/ Main, Germany.

[2]Institute of Occupational, Environmental and Social Medicine, Medical Faculty, Goethe University Frankfurt/Main, Germany.

[3]Department of Internal Medicine II, Division of Gastroenterology, Hepatology and Infectious Diseases, Friedrich Schiller University Jena, Germany.

[4]Department of Medicine I, University Medical Center Hamburg Eppendorf, Germany.

[5]Competence Centre for Complementary Medicine and Naturopathy (CoCoNat), Klinikum Rechts der Isar, Technische Universität München, Munich, Germany.

[6]Institute for Complementary and Integrative Medicine, University Hospital Zurich and University of Zurich, Zurich, Switzerland.

***Corresponding Author: Dr. Rolf Teschke**
Department of Internal Medicine II, Division of Gastroenterology and Hepatology, Klinikum Hanau, Teaching Hospital of the Goethe University Frankfurt/ Main, Germany. **Mail ID:** rolf.teschke@gmx.de

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ABSTRACT

Migraine is a major global health and economic burden, calling for efficient prophylactic medicines in patients with frequent attacks. Several synthetic drugs are in use but their low prophylactic efficacy of 20% to 40% is disappointing. Instead, the proprietary herbal medicine Petadolex® (PE), an analytically and pharmacologically well-defined, processed extract from *Petasites hybridus* (PH), reduces the frequency of migraine attacks by 48% to 60%, but claims of adverse liver reactions emerged that discourage its broader use. We analyzed 10 spontaneous case reports of suspected liver injury in assumed connection with the use of PE that were presented by hospital physicians to the manufacturer, who provided all case details upon our request. Using the robust, quantitative, structured and liver specific causality assessment method of RUCAM (Roussel Uclaf Causality Assessment Method), causality attribution was performed for PE. In none of the 10 liver cases, causality gradings of probable or highly probable were obtained for PE, indicating that these liver diseases cannot be attributed to PE with a high degree of probability; in detail, causality for PE was excluded in 3 patients, graded as unlikely in 5 patients, and possible in 2 patients without clinical relevance. In most of the 10 patient reports, alternative diagnoses, abundant comedications, and incomplete case data were major confounding variables, which complicated causality assessment. In conclusion, this clinical analysis and robust causality assessment by RUCAM failed to substantiate any potential liver injury by Petadolex® in the 10 spontaneous reports, opposing views to the contrary.

KEYWORDS: Migraine; migraine treatment; Petasites hybridus; Petadolex; Pyrrolizidine alkaloids; suspected herbal hepatotoxicity; herb induced liver injury.

1. INTRODUCTION

Migraine is a disease with a significant health burden and economic impact throughout the world, as outlined by the WHO[1] and several key publications focusing on clinical efficiency and socio-economic importance.[2-4] In particular, affected patients with frequent attacks suffer from impaired quality of life, disability and absence from work. Actually, 51% of migraine patients reported reduced work or school productivity by at least 50%.[3] The prevalence of migraine is high with variable figures from country to country.[1] In 1993, an analysis of population-based studies estimated the prevalence of migraine at approximately 6% among men and 15% to 17% among women, whereby prevalence varies by age, increases to ages of 40 years and then declines thereafter in both men and women.[2] These data were confirmed in 2001 by the American Migraine Study II with 6.5% in men and 18.2% in women.[3] The number of patients with migraine has increased from 23.6 million in 1989 to 27.9 million in 1999 in parallel with the growth of the population, while migraine-associated disability remained substantial and pervasive.

Effective prophylactic treatment to minimize migraine attack frequency and severity is a particular challenge for general practitioners and neurologists. Predominant prophylactic therapy is based on numerous synthetic and herbal medications, their cost, benefit and risk ratio is controversially discussed in national guidelines, statements of expert groups and numerous other publications.[5-20] Although a wide range of chemically defined drugs are recommended by guidelines, many of these have low efficacy with response rates ranging from 20% to 40%.[11] Instead, these compare to a reduction of migraine attacks by 48% to 60% with the proprietary herbal medicine Petadolex® (PE), as reported after analysis of two studies by the Subcommittee of the American Academy of Neurology and the American Headache Society, resulting in a level A evidence recommendation and establishing PE as an effective migraine prophylaxis.[8] Even better efficacies were reported after analysis of three clinical studies: PE reduced the attack frequency by 59% to 63%, associated with responder rates of 45% to 77%.[11] It therefore appears that PE has a fairly good efficacy profile for migraine prophylaxis.

Nevertheless, such percentage comparisons must be considered as tentative, since clinical studies were performed against placebo and not against other commonly recommended drugs or herbs. In view of the high prevalence of migraine, comparative clinical trials should be no problem and be performed to establish the best prophylactic treatment regimen. These studies may also reduce self-treatment and drug escalation by migraine patients with ineffective prophylaxis and the risk of drug overuse headache.[10] Even worse, patients may use additional drugs with a high risk of severe adverse reactions due to drug-drug interactions.

Although most migraine medications are well tolerated by the majority of patients, rare adverse reactions were reported and partially limited their use.[10] In addition, speculations and vague claims of adverse reactions of the liver by unsaturated pyrrolizidine alkaloids (PAs) in herbal products discouraged a broader use of PE.[11] Unquestionably, PAs can cause intrinsic liver injury, which by definition is dose dependent[19-23] and are present in variable amounts as ingredients of some unprocessed extracts from *Petasites hybridus* (PH)[20] but are lacking in PE. This is a processed extract from the rhizome of PH, also called butterbur, from which PAs are removed since 1988 by high supercritical liquid carbon dioxide extraction in a standardized and patented procedure.[11,18,20] Despite the absence of PAs in Petadolex, some physicians considered these as hepatotoxic in a few unpublished spontaneous liver case reports and assumed PAs as culprits.

In this study, we analyzed 10 spontaneous case reports of suspected liver injury in assumed connection with the use of PE. These cases were reported by hospital physicians to the manufacturer, who provided all case details upon our request. Clinical analysis and robust causality assessment by RUCAM, the Roussel Uclaf Causality Assessment Method[24], were used to substantiate or dismiss an intrinsic and PA related liver injury or an idiosyncratic unpredictable and dose independent liver injury by Petadolex® in these 10 spontaneous reports. However, these evaluations failed to establish a causal link and suggest a much more favourable benefit-risk ratio, not substantiating previous critical and opposing assumptions on PE safety.

2. METHODS

2.1 Internet search

PubMed database was searched for the following items: liver injury; Petadolex OR *Petasites hybridus*; Butterbur; Migraine; Migraine treatment; Migraine drugs; Migraine medications; adverse reactions of migraine medications. Only English language articles were included and retrieved.

2.2 Study cohort

Suspected adverse reactions in connection with the use of chemical drugs, herbal drugs, and dietary supplements, which are marketed in Germany, are commonly presented as spontaneous reports to the German regulatory agency BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) and/or the manufacturer, with the understanding to ensure data exchange among both. In the past, it was hard or impossible to obtain redacted case files of suspected liver injury by herbs as requested from BfArM. Consequently, our previous requests were directed to the respective manufacturers, who provided all case details in redacted form[25-27] The latter approach was used again for the present study, which was intended to clarify the controversial issue of the hepatotoxic risks from the use of Petadolex®.

Analyzed were redacted documents of spontaneous reports as provided by the manufacturer of Petadolex®, Weber & Weber International, Inning, Germany. The documents contained case details of 10 patients with severe liver diseases, which initially were assumed to be related to the use of the herbal medication Petadolex® because of a temporal and/or suspected causal relation. These patients are referred to as the study cohort. Of note, none of these spontaneous reports were published before as case report in a peer-reviewed scientific journal. In addition to the study cohort, the files of the manufacturer contained additional 49 spontaneous reports with abnormalities of liver enzymes not considered clinically relevant, since they were all transient, only minimally elevated and not meeting hepatotoxicity criteria; for some patients, preexisting increased liver tests were described suggestive of chronic liver disease; in one of these patients, preexisting increased liver enzymes vanished following initiation treatment with PE. The documentation of these cases also was mostly incomplete with respect to alternative causes such as the various hepatitis types; confounding

variables prevailed and included missing accurate liver enzyme values, isolated increases of γ-glutamyltranspeptidase, substantial comedication with potentially hepatotoxic drugs or herbs, intercurrent infections, and well documented alternative causes including non-alcoholic fatty liver disease, Gilbert syndrome as evidenced by isolated bilirubin increases, gall bladder stones and autoimmune hepatitis. These cases were not further considered in the study cohort analysis.

2.3 Differentiation of intrinsic and idiosyncratic liver injury type

In each suspected case of herb induced liver injury (HILI), the type of liver injury was determined[24] as based on accepted criteria if possible (Figure 1).[21,28] Since PAs from herbal extracts usually cause intrinsic liver injury and affect most or all individuals who consume these herbs, differentiation of intrinsic versus idiosyncratic liver injury is necessary in the cases of the study cohort. Nearly all other common herbs usually cause idiosyncratic liver injury in only a few susceptible persons.[21,22,28]

2.4 Case classification using liver injury criteria

Causality assessment by RUCAM requires prior evaluation of liver injury criteria and its pattern.[24] These criteria are readily collected by initial measurement of ALT (alanine aminotransferase) and ALP (alkaline phosphatase). Liver injury is defined by increased serum activities of ALT of at least 5N and/or of ALP of at least 2N with N as upper limit of normal and should be assessed by simultaneous measurements of these enzyme activities on the first presentation. These thresholds increase the specificity of the hepatotoxicity causality assessment, since it eliminates false positive cases and substantiates hepatotoxicity causality at a high level of certainty. They are also in line with a recent consensus on drug induced liver injury (DILI).[29] However, when ALT is within the normal range, ALP increases should be paralleled by increased γ-glutamyltranspeptidase or preferably 5' nucleosidase to rule out isolated increases of ALP activities due to bone or other disorders rather than hepatobiliary disease.[24]

2.5 Liver injury pattern

RUCAM takes into account different laboratory constellations of the liver injury and provides two subscales[24], one for the hepatocellular type of injury and the other one for the cholestatic or mixed type of injury. These subtypes can be separated using the ratio R, calculated as the initial ALT/ ALP activity at the time liver injury is suspected, with both activities expressed as multiples of N. Hepatocellular injury is likely if ALT > 5N and ALP ≤ N, or if both ALT and ALP are elevated with R ≥ 5; cholestatic injury should be suspected if ALP > 2N and ALT ≤ N, or if both ALT and ALP are elevated with R ≤ 2; in all other constellations, the liver injury is mixed, i.e. ALT > 5N and ALP > N, with 2 < R < 5. This classification of liver injury pattern clearly assigns each DILI or HILI case in the RUCAM system, either to the hepatocellular injury or the cholestatic and mixed liver injury type.[24]

2.6 Diagnostic biomarkers

No specific diagnostic biomarkers are available in suspected idiosyncratic liver injury; its diagnosis relies on the exclusion of alternative causes as idiosyncratic HILI is defined as exclusion of other diseases.[21,28] For intrinsic liver injury by PAs, blood pyrrole-protein adducts are used as specific and diagnostic biomarkers to firmly establish the diagnosis of PA-caused HILI[21,22], which may have merits for the study cohort.

2.7 Diagnostic liver histology

As an invasive approach, liver biopsy for describing features of liver histology is not routinely indicated in patients with suspected HILI or DILI.[21,22,28] However, in the study cohort under consideration, liver histology may be helpful in retrospect, as PAs cause typical diagnostically valuable histological changes in the liver that can assist to confirm or exclude PA-associated liver injury.[21,22] Patients with liver injury due to PAs suffer from a specific type of liver disease, known as the hepatic sinusoidal obstruction syndrome (HSOS)[21,22,30] with characteristic clinical features.[21,22]

2.8 Sequential case data analysis

Redacted files of the patient's medical records were analyzed for data consistency, completeness of alternative cause exclusion, temporal association, clinical confounders, applied causality assessment methods and co-medication by synthetic drugs, herbal drugs, herbal dietary supplements, or other dietary supplements. The approach was similar to those applied in previous case assessments of HILI and DILI[24,31,22], as they were highly appreciated and proposed as well founded causality assessments by others.[33] Accordingly, the present approach included case narratives, thorough clinical assessment and causality assessment using RUCAM, as outlined previously.[24,34]

2.9 Case narratives

Narratives are extremely important for case assessment and reevaluation by other scientists.[34] They provide transparency and understanding of the disease course. Case narratives are therefore also presented in this study. Special care is needed for each case of HILI as this is a diagnosis of exclusion that can be established only when competing diagnosis have been validly ruled out, as defined and published previously.[21,28,34]

2.10 RUCAM

RUCAM is used worldwide and the preferred tool to evaluate causality in suspected cases of both HILI and DILI.[24] In the present study, it allows separate causality assessments for PE as well as each comedicated synthetic drug, herbal drug, or herbal dietary supplement.

2.10.1 RUCAM with its key elements and scoring system

RUCAM comprises seven domains of liver related and hepatotoxicity specific core elements, is well structured, user-friendly and clearly quantitative rather than qualitative as its scoring system considers all relevant elements.[24] Core elements of RUCAM include: challenge features as time period from beginning until cessation of herb intake in relation to disease course or from the cessation of herb use to the onset of the liver injury; dechallenge characteristics with course of serum aminotransferases after cessation or continuation of the herb use; risk factors such as alcohol use, age and pregnancy; co-medication with synthetic drugs or other herbs; search for alternative causes with special focus on all hepatitis virus types including infections of hepatitis E virus (HEV); available information on previous hepatotoxicity by the herb under consideration; and response to unintentional reexposure if available, as intentional reexposures for diagnostic purposes are obsolete and unethical due to high risks associated with this test.

2.10.2 Final RUCAM scores

Each item of RUCAM carries an individual score and the sum of the individual scores provides the final score for the patient.[24] From +14 down to -9 points, the final scores are translated to the following causality levels: ≤ 0 points, causality excluded; 1-2, unlikely; 3-5, possible; 6-8, probable; ≥ 9, highly probable.

2.10.3 Sensitivity, specificity, and predictive values of RUCAM

RUCAM[24,35] was validated by cases with known positive reexposure as gold standard.[36] Indeed, it was recognized that expert's opinion is too variable between observers to validate a causality assessment method against an expert panel and positive reexposure test case results were used to validate RUCAM. RUCAM-based assessment has shown high sensitivity (86%), specificity (89%), positive predictive value (93%) and negative predictive value (78%).[36]

3. RESULTS

3.1 Internet search data

Our search in the PubMed database provided numerous reports on migraine associated with therapeutic proposals. The search was negative for English language cases of liver injury by PE or *Petasites hybridus* assessed by RUCAM in peer-reviewed scientific journals. In addition, the Liver Tox database provided no or no substantial information of liver injury by Petadolex®, *Petasites hybridus*, or butterbur. The extended search revealed a few internet-based sources, which vaguely described anecdotal liver injury information without product identification, RUCAM evaluation, or scientific validity.

3.2 Narratives with case details of all 10 patients

Table 1 compiles the narratives of each case detail and attempts to establish uniform cohort characteristics, also called the signature of the initially suspected PE hepatotoxicity. Among the 10 cases, 8 patients resided in Germany, where PE was available as the original herbal drug, whereas one patient lived in the UK and another one in Austria; both used the herbal drug with the identical processed extract in the finished product under another brand. For reasons of simplicity, subsequently all patients were considered as PE cases with uniform PE use. In all 10 cases, the use of PE was sufficiently documented and all used PE for prophylaxis of their migraine attacks. Details of the narratives indicated that all patients using PE were hospitalized for their liver disease and were thereby under the professional care of hospital physicians; they were not treated by non-professional healthcare providers (Table 1). However, despite professional hospital care and case analyses, major flaws and confounding variables in the case documentation were evident (Table 1).

For instance, one patient (case 1) used PE intermittently, without any further details on intake frequency given (Table 1). This intermittent use of the drug commonly excludes a valid causality assessment of the case since no clear temporal association as prerequisite for an attribution can be established. Nevertheless, this case 1 is further included in the study cohort. Intermittent use of PE may also be present in other patients, who reported vomiting (cases 5 and 6) or nausea (cases 1, 5, 6 and 10) (Table 1). Some patients stopped PE use at first symptoms or after detection of increased liver tests, whereas others continued its use despite symptoms (Table 1). In another patient (case 3), symptoms appeared 6 weeks after PE use was stopped, lacking a clear temporal and thereby any causal association. Half of the patients were also medicated with synthetic drugs or other herbal products, most but not all of these are known for their hepatotoxic potential (Table 1), e.g. ibuprofen, paracetamol, estrogens, aspirin and the cytochrome P450 modulator St John's wort extract in patient 1 (Table 1).

Despite these and other data problems including alternative causes, the caring physicians suspected that the liver diseases were due to PE intake; little if any supportive evidence for these claims or attempts to substantiate causality was provided.

3.3 RUCAM based causality grading for PE and comedication

In the study cohort patients, the hepatocellular type of injury was present and evaluated by the RUCAM subscale for this type of injury (Table 2). Each RUCAM core item was listed and individually scored and for each case a final score was provided for PE as well as any comedication if applicable (Table 2). Using RUCAM, the causality level for PE was low and lacked any clinical relevance (Tables 1 and 2). For instance, with a

score of 0, causality for PE was excluded in cases 6, 8 and 10, with a score of 1 or 2 unlikely for cases 1, 3, 4, 7 and 9 and possible in cases 2 and 5 with a score of 3. For none of the cases, a score of >3 points was obtained, for probable (6-8) or highly probable (>8 points) causality. This clearly indicated that the liver disease cannot be attributed to PE in any of these 10 cases; in essence, the existence of HILI by PE is highly questionable, also in view of the numerous alternative diagnoses which have clearly been established (Table 1).

Although multiple comedicated drugs were documented, details on their intake were rarely provided. RUCAM based causality was assessed in some cases also for the comedication (cases 2, 3, 4, 5 and 7) and provided little evidence that comedication may have caused the liver disease either (Tables 1 and 2). If assessable at all, the causality grading for comedications was low due to poor data for treatment duration and a questionable temporal association. Interestingly, virtually all patients had some comedication including intake of drugs with published risks of idiosyncratic hepatotoxicity (Tables 1 and 2). However, the treating physicians did not consider the causality for any of these alternative causes, which may represent a major clinical flaw; not a single physician did apply any structured causality assessment method such as RUCAM, which is in common use since 1993.

The listing of the key items of RUCAM shows gaps in the data presentation, including missing diagnostic parameters essential to rule out alternative causes (Table 2). Verified alternative causes would lead to a reduction of the total score by -3 points (Table 2), whereas possible and tentative alternative diagnoses (Table 1) remained unscored and are only mentioned.

3.4 Serology data

Among the core items necessary for the RUCAM assessment, serology data to exclude infectious diseases are very important. As seen in Tables 2 and 3, even these basic exclusion data were fragmentary; and the lack of virus serology does not support the exclusion diagnosis of HILI. As an example, in case 10 not a single type of virus hepatitis was excluded by the hospital physicians, at least data are lacking in the available documents (Table 3). For other cases, only global information was provided such as "hepatitis was excluded", but details on the excluded infections and applied parameters were not provided, leaving uncertainty and speculation. The most annoying clinical shortcoming may relate to hepatitis E, as this infection was not excluded in any of the study cohort patients (Tables 2 and 3).

3.5 Clinical case characteristics and outcome

Diagnostic hepatotoxicity criteria – sufficiently high liver values – were fulfilled in 9 out of the 10 patients of the study cohort (Tables 1, 4 and 5). However, based on serological criteria, clinical characteristics and low RUCAM scores (Tables 1 and 2), none of the 10 patients suffered from idiosyncratic or intrinsic HILI (Fig. 1), not substantiating claims of the hospital physicians. Indeed, there was not only a lack of HILI by PE but also missing evidence that the liver disease might have been caused by PAs as claimed by some of the physicians. No patient of the study cohort showed clinical features of HSOS as is typically caused by PAs (Table 6). In particular, patients of the study cohort showed no ascites, a key clinical sign of HSOS (Table 6). Additional evidence against PAs as culprits was provided by liver histology as reported in 8 patients, with none of the histology pattern (Table 1) showing the typical histology of HSOS (Tables 4 and 6) like fibrosis, sinusoidal dilatation and perivenous loss of hepatocytes.[30] Finally, no approach is documented in the clinical files having used a specific diagnostic biomarker in order to establish or exclude the role of PAs for the liver disease, as specific pyrrole-protein adducts in the blood were not assessed in the study cohort (Table 6).

The abundance of confounding variables, data gaps and overlooked alternative causes is remarkable in the setting of hospital physicians, who analyzed the case details of their patients (Table 5). In the study cohort, case features are not homogeneous and indicate differing mechanisms of the liver diseases; this is not suggestive for one single herb or toxin causing one single disease with homogeneous clinical characteristics (Tables 1 and 4). Examples of incongruence are the variable PE use from 2 to 130 weeks, the variable latency period from 3 days to 127 weeks, the variable symptoms of nausea, vomiting and jaundice, the variable range of ALT from 75 U/L (< 2N) to 4,458 U/L, of AST from 41/L (<N) to 3,101 U/L and of total bilirubin ranging from 0.7 mg/dL to 18.6 mg/dL (Table 4). The laboratory values show that in some cases ALT or AST were not or only marginally increased, while in other cases the increases in these parameters are substantial and highly suggestive of a hepatitis flare, a possible alternative explanation often not excluded. This inhomogeneity of case characteristics and the lack of causality for PE by RUCAM (Table 2) again do not warrant the assumption of HILI by PE; this disease likely does not exist in the study cohort.

Most patients experienced jaundice corroborated by increased bilirubin values, substantiating that their liver disease was severe (Table 4). Clinical outcome was good in 8 patients, but two patients (cases 3 and 9) required a liver transplant (Table 1). RUCAM based causality for PE was unlikely in both cases, leading to the diagnosis of acute liver failure (ALF) of undetermined etiology (Tables 1 and 2). With respect to case 3, symptoms were first noticed six weeks after PE cessation, lacking thereby a temporal association. Transition to liver cirrhosis as assessed by liver histology suggests a long lasting disease rather than an acute one (Table 1). The second ALT peak also favors an alternative cause, which could not be specified due to limited case data; it might be related to a disease acquired in Egypt. In case 9, the second ALT increase during the dechallenge phase is suggestive of an alternative cause since it cannot be

related to the use of PE (Table 1). Due to lack of serological data, HEV, CMV, HSV and VZV were possible viral hepatitis causes.

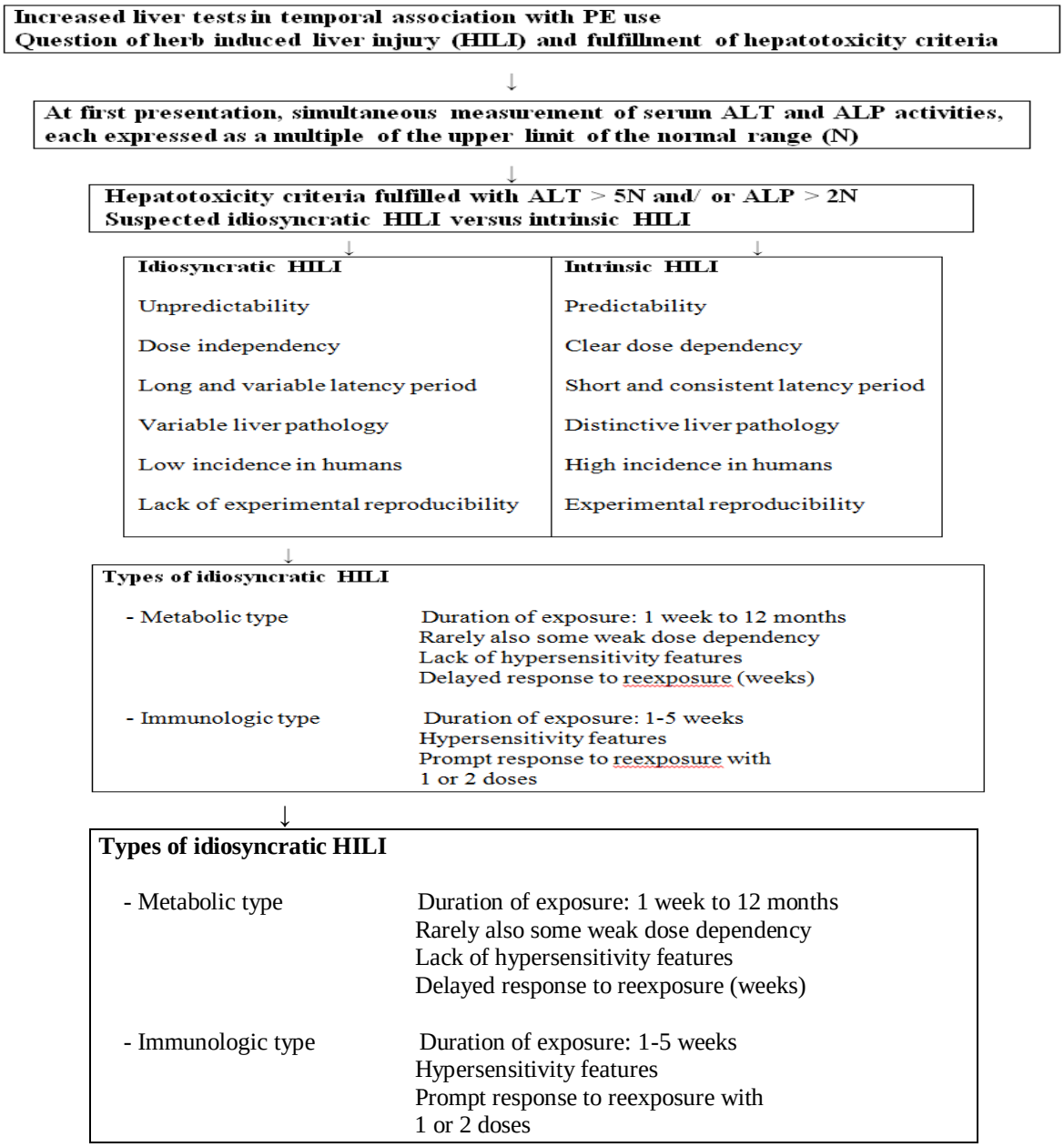

Figure 1: Diagnostic classification of liver injury required for the causality assessment of suspected HILI cases by RUCAM

Note: If ALT is within the normal range and ALP is increased, this should then be paralleled by increased γ-glutamyltranspeptidase or better 5'-nucleosidase to rule out isolated increases of ALP activities due to bone or another origin rather than hepatobiliary disease. Abbreviations: ALP, alkaline phosphatase; ALT, Alanine aminotransferase; N, Upper limit of normal. Details are described in previous reports.[21,24,28]

Table 1: Narratives of the study cohort (cases 1 –10)

Patient	Narratives
Case 1 Female, 34 years Germany	**Narrative** The patient used 2 capsules of PE daily for around 2 months from 07/11/1998 to 09/04/1998 intermittently, corresponding to a dose of 50 mg/d. At the end of 08/1998 and thereby around 7 weeks after starting PE use, first symptoms of nausea and pruritus emerged. Jaundice and myalgia led to first presentation on 09/05/1998.

	Consequently, PE use was not discontinued from end of 08/1998 until 09/04/1998, despite initial symptoms. PMH with amenorrhea since 1 year of unknown etiology, starting prior to PE use and possibly related to a pre-existing liver disease. Amenorrhea started at an age of around 34 years, an unusual early time point. Amenorrhea is a typical early but not specific symptom of liver disease in females. Use of estrogens for unknown indication and for unknown duration. Other comedication included intermittent and rare use of some other, potentially hepatotoxic drugs such as ibuprofen, Aktren (ibuprofen), paracetamol, aspirin and hyperforat, all of unknown daily dose and duration. At admission: ALT 676 U/L with a second peak of 917 U/L (suggesting alternative cause other than PE); 203 U/L; AST 251 U/L; ALP 203 U/L; total bilirubin 5.7 mg/dL. Normal aminotransferase on 09/11/1998. Anti-HBs >25 U/L, suggesting previous HBV immunization or resolving acute hepatitis HBV infection. HBs Antigen negative. Lymphocyte stimulation test for PE with index of 3.0 (normal <2) slightly stimulated; with a borderline reaction also for hyperforat and lacking reactions for paracetamol, aspirin and Aktren (ibuprofen). Fatty liver. Liver histology with striking necrotizing cholestatic hepatitis, fat, incipient fibrosis. Pathologist: Compatible with DILI. **RUCAM** Causality for PE unlikely (score +2), for the other drugs such as paracetamol, ibuprofen, aspirin and hyperforat excluded (score 0). **Final diagnoses** **1. Liver disease with unlikely causality for PE.** **2. Undetermined pre-existing or co-existing liver disease.** **Alternative and other relevant diagnoses** **1. Possible pre-existing liver disease with amenorrhea** Amenorrhea of unknown etiology, possibly related to a pre-existing liver disease unrelated to PE use. This raises the question whether menstruation recurred along with improvement of the liver disease. **2. Possible resolving acute hepatitis by HBV infection, CMV infection, or EBV infection** Positive anti-HBs. Positive titers, mostly IgG but without further evaluation for titer changes in the further course. **3. Fatty liver** **Final commentary** Good outcome, Hy's law criteria fulfilled. Alternative diagnoses were not strictly excluded, such as HSV and VZV. Intermittent use of PE and other comedicated drugs, some of these with hepatotoxic potential. As ALT showed a second peak despite PE cessation, this finding strongly suggests some other cause such as a yet undetermined virus infection with a flare-up. Liver histology with beginning fibrosis, suggestive of a prolonged injurious process.
Case 2 Male, 58 years Germany	**Narrative** This patient took PE for around 4 months from 05/18/2001 to 09/14/2001. PE was used at a dose of 75 mg/d. After around 3 months of PE use and in the middle of 08/2001, first symptoms of fatigue emerged. Therefore, PE use was continued despite symptoms. For several years, medication with the potentially hepatotoxic Ascotop (zolmitripan) of unknown daily dose. At first presentation on 09/14/2001 jaundice. Before on 09/12/2001 ALT 1134 U/L, AST 384 U/L, ALP 218 U/L, total bilirubin 7.1 mg/dL. Normalization of aminotransferases on 01/15/2002. Anti-EBV IgG and IgM positive, but titers not documented, also not in the further course. Hepatitis serology described as without pathological changes, but details not communicated. Liver histology with intralobular necrotizing hepatitis and some cholangitis. **RUCAM** Causality for PE low-graded possible (score +3), for Ascotop unlikely (score +2). **Final diagnosis** **Acute EBV virus infection with rapid initial ALT resolution.** Diagnosis verified by positive anti-EBV IgM. **Alternative and other relevant diagnoses** As EBV is the primary diagnosis, DILI by PE is unlikely despite RUCAM score of +3, which is the lowest level

	of a possible causality with a range of 3 – 5 scores. **Final commentary** Good outcome, Hy's law criteria fulfilled. Serology results strongly suggest acute EBV virus infection with initial rapid and later undulating ALT resolution, providing much more evidence for EBV as causative as compared to PE.
Case 3 Female, 37 years Germany	**Narrative** This female patient took PE for approximately 6 months from 04/01/2001 to 09/30/2001; the dose was 75 mg/d. First symptoms of malaise were noticed around middle of 11/2001, thereby around 6 weeks after PE use had been discontinued. She also noticed weight loss and hepatitis-like symptoms. Jaundice. Comedication with the potentially hepatotoxic Gestamestrol N since 10 years until mid of 11/2001. Since 01/02/2002 in hospital, on the following day ALT 220 U/, AST 266 U/L, ALP 180 U/L, total bilirubin 6.64 mg/dL. On 01/14/2002 ALT 143 U/L, AST 131 U/L, ALP 198 U/L and total bilirubin 25.4 mg/dL. On 01/22/2002: ALT 318 U/L (second peak), AST 518 U/L. Liver histology with massive necroses, fibrosis and transition to liver cirrhosis. Negative hepatitis serology. Anti-EBV IgG 85 U/L. Negative results of autoimmune parameters. ALF. On 02/08/2002 liver transplantation. Patient was in Egypt somewhere in 2001, but no search was done for any tropical disease. **RUCAM** Causality for PE unlikely (score +1), for Gestamestrol N unlikely (score +1). **Final diagnoses** **1. Unlikely hepatotoxicity by PE.** **2. Liver cirrhosis of undetermined cause.** **Alternative and other relevant diagnoses** Undetermined alternative diagnosis, possible tropical disease after stay in Egypt. **Final commentary** Good outcome after liver transplantation. Hy's law criteria fulfilled. Lacking evidence of PE hepatotoxicity, alternative causes not evident. Histology with fibrosis and transition to liver cirrhosis suggests a prolonged liver disease and not an acute event. Clinical symptoms emerged long after product cessation, providing further evidence against a causal relationship regarding PE.
Case 4 Female, 45 years Germany	**Narrative** The patient working in the agriculture (contact to animals infected by HEV?) and was treated with PE 100 mg/d for 5 weeks from 09/15/2002 to 10/21/2002. On 20/10/2002 symptoms of dull pains and also colicky pains in the epigastric area, jaundice, dark colored urine and light color of stool appeared on 10/20/2002 and PE use was stopped the other day. Comedication with Biovol (contraceptive drug) since several years and used until 10/20/2002. On 10/7/2002 and thereby 18 days prior to admission, ALT 136 U/L. At hospital admission on 10/25/2002, ALT 990 U/L, AST 540 U/L, ALP 170 U/L and total bilirubin 14 mg /dL. Improvements of LTs in the further course with normalization on 12/18/2002. Antibodies reported as negative for hepatitis A-C without details of used parameters. HCV RNS negative. CMV DNS negative, anti-CMV IgG <120 U/L, IgM negative. HSV IgG 1:34.000 without further titer evaluation, IgM negative. ANA 1:128 positive, other autoantibodies negative. Imaging data with gallbladder wall of 10mm with several layers, gallbladder sludge and 2 polyps. Liver histology: Hepatitis with necroses, no veno-occlusive disease. **RUCAM** Causality for PE unlikely (score +2) and for Biovol unlikely (score +1). **Final diagnoses** **1. Resolving acute hepatitis by HSV infection** **2. Suspected cholecystitis** **3. Unlikely hepatotoxicity by PE**

	Alternative and other relevant diagnosis Possible HEV infection. **Final commentary** Good outcome. Hy's law criteria fulfilled. High anti-HSV IgG titers highly suggestive of resolving acute HSV infection where IgM has vanished. Clinical features compatible with HSV. Association to work in agriculture, also HEV infection should be considered, as animals often are infected by HEV. Colicky symptoms and imaging data in line with cholecystitis.
Case 5 Female, 24 years Germany	**Narrative** This patient was on PE 50 – 75 mg/d for 3.5 months from 05/2003 to 08/17/2003. When PE had been used for about 3 months, symptoms of nausea, vomiting, reduced appetite and discomfort in the epigastrium appeared on 08/08/2003, thereby 10 days prior to PE discontinuation. Unclear, whether PE was taken despite vomiting. Possible comedication by tetrazepam, but daily dose and duration of intake not documented. Within prior 10 months body weight reduction by 15 kg, possibly related to pre-existing or co-existing liver disease. Hospital admission on 08/18/2003: ALT 625 U/L with rapid decline, ALP 270 U/L. On 11/14/2003 normalization of LTs. Anti-HAV IgM negative, HBs antigen negative, anti-HBc negative, anti-HCV negative. Imaging data with splenomegaly 14 cm, but exclusion of EBV or CMV by serology not done despite age of 24 years; no hepatomegaly. **RUCAM** Causality for PE possible (score +3) and for tetrazepam not assessable. **Final diagnoses** **Clinically suspected hepatitis by EBV or CMV infection with splenomegaly.** By serology assessment, EBV was not excluded. Idiosyncratic DILI or HILI are commonly not associated with splenomegaly. Splenomegaly, lab tests, weight loss and clinical features fit best to EBV or CMV infection, EBV is commonly observed in this age group of around 24 years. **Alternative and other relevant diagnoses** None. **Final commentary** Good outcome. Hy's law criteria not assessable, since values of total bilirubin are missing. Insufficient data quality for exclusion of various virus infections.
Case 6 Female, 50 years United Kingdom	**Narrative** The patient used PE (under another brand) 50 mg/d for 2 weeks from around 01/28/2004 to 02/14/2004. First symptoms of epigastric pains, nausea, vomiting (with use of PE?), loss of appetite and adynamia were noticed after around 3 days of PE use on 01/31/2004. On around 02/07/2004 dark urine. Although not explicitly documented, jaundice was likely present in face of the increased values of total bilirubin. On 02/14/2004 hospital admission in London: ALT 1125 U/l, with values for the following days: 996/1326/1510/1491/1449/1601/178 1U/L, thereby a second peak and then decline; ALP 252 U/L, then decline and undulating 189-219 U/L; Bilirubin 138 (units unknown but likely umol/L, would then correspond to 8 mg/dL and signify jaundice) and increasing up to 287. Negative results of Monospot, HBsAg, anti-HBc IgM, anti-HCV, anti-hepatitis A IgM. Pending: HCV PCR, anti-EBV IgM, anti-CMV IgM, autoantibody serology. Ultrasound: Liver slightly coarse, hypoechoic; patent hepatic, portal veins and hepatic arteria with normal flow; no biliary dilatation; gallbladder shrunken and thick-walled, no gallstones. Documented is also the use of thuja, nettle and herbal tincture, garlic and horseradish winterformula, beta carotene, chromium, luffa, euphorbia, all consumed since 12/15/2 003 for several weeks with unknown daily dose and further product information. The indication for treatment is not mentioned, possibly for an incipient liver disease (?). PMH: Hysterectomy and appendectomy. Around 01/12-16/2004 in hospital for nasal operation (nasal polyp) with postoperative bleeding and need for 4 units blood transfusions. Liver histology: Perivenular and lobular portal tract inflammation, mixed inflammatory infiltrates with eosinophils and plasma cells; portal tract disarrayed and disorganized; balloon degeneration; focal bile duct proliferation and canalicular cholestasis.

	RUCAM Causality for PE excluded (score 0) and for comedication excluded (score 0). **Final diagnoses** **1. Unlikely hepatotoxicity by PE** **2. Possible post-transfusion hepatitis** **3. Undetermined** **Alternative and other relevant diagnoses** None. **Final commentary** Good outcome. Hy's law criteria fulfilled.
Case 7 Female, 58 years Germany	**Narrative** The patient was treated with PE at a maximum of 100 mg/d for around 8 months from 10/27/2003 to 06/2004; 75 mg/d from 10/27/2003 to end 4/2003, then 100 mg/d in 5 and 6/2003. From 10/27/2003 until end of 06/2004 comedication with Ascotop if needed, no daily dose or other details documented. In 06 and 07/2004 decrease of body weight by 4 – 5 kg. On 07/01/2004, by chance detection of increased LTs at the occasion of a planned blood donation, but actual values not presented. About 1 month after the last PE dose, hospital admission on 08/02/2004 with LT results of the following day: ALT 75 U/L, AST 41 U/L, ALP 88 U/L, total bilirubin 0.65 mg/dL, rapid improvement of initially slightly altered ALT. With respect to ALT, criteria of hepatotoxicity are not fulfilled. MCV 99.6 – 101.1 fl. Ferritin 608 ng/mL, 5-fold increase over upper range of normal. Normal values of transferrin with 2.3 g/L and of transferrin saturation with 42%. Hemochromatosis gentest (HFE) C282Y-allele heterocygot present, H63D-allele not present, S65C-allele not present, E168X-allele not present. ANA 1: 320 increased. Other autoimmune parameters normal. Immunoglobulins all normal. Results for Fe not documented, also reported as increased. Imaging data normal. Liver histology: Scattered centrolobular liver cell necroses, possibly alimentary or toxic etiology, and little fibrosis. Lacking exclusion diagnostic work up. Insufficient case data quality. **RUCAM** Causality for PE unlikely (score), but hepatotoxicity criteria not fulfilled. Causality for Ascotop not assessable due to insufficient data and sporadic use. **Final diagnoses** **1. Undetermined.** **2. Lacking criteria of hepatotoxicity due to insufficient ALT increase.** **3. Lacking evidence for hemochromatosis.** **4. ANA-positive autoimmune hepatitis (AIH) less likely and not compatible with liver histology results and normal immunoglobulins.** **Alternative and other relevant diagnosis** MCV increase of unknown etiology, possibly related to latent B_{12} deficiency or chronic-atrophic gastritis. **Final commentary** Good outcome, rapid decrease of LT but normalization not reported. No Hy's law criteria. No evidence of toxic liver injury, lack of required criteria. Genuine AIH unlikely, lacking ANA and LT results in the further course. Unproven drug AIH.
Case 8 Female, 40 years Germany	**Narrative** Patient was on PE 150 mg/d for 3 months from 08/2005 to 11/07/2005. Comedication consisted of ibuprofen, if needed without details of daily dose and duration of use. She noticed first symptoms of dark urine, jaundice, epigastric discomfort and loss of appetite on 11/01/2005 but she continued PE use until 11/07 40). At hospital admission on 11/07/2005: ALT 1432 U/L, AST 1567 U/L (with second AST peak of 1848 U/L on 11/11/2005, not accompanied by a second ALT peak at the same time as ALT was not assessed), ALP 107 U/L, total bilirubin 5.7 mg/dL with increase up to 23.3 mg/dL. Anti-HAV IgG positive, anti-HAV IgM negative, HBsAg negative, HBV-DNS-PCR negative, anti-HBc negative, anti-HCV negative, anti-CMV IgG >21.000 U/L, anti-

	CMV IgM as borderline result described without actual result, anti-EBV IgM negative, anti-EBV IgG 250 IU/ml (normal <25). Reported negative results for HEV and HSV without details. All autoimmune parameters, ferritin, ceruloplasmin, and alpha-1-AT all with negative results. Monocytosis of 11.8%. Virtually normal LTs on 4/10/2006. Urinary copper 68 microg (normal <25). After a prolonged time with slow decline. Imaging data with hepatomegaly (around 15 cm) and splenomegaly (14 x 4.3 cm). Doppler with normal vessels, no Budd Chiari syndrome. Liver histology with subacute hepatitis with single cell necroses and central confluent necroses, no clear findings suggesting virus infection, by immunohistology lack of positive results for CMV (details not reported). **RUCAM** Causality for PE excluded (score 0), causality for ibuprofen not assessable due to intermittent use. **Final diagnoses** **1. Suspected resolving acute hepatitis by CMV infection with hepatosplenomegaly** **2. HILI by PE or DILI by ibuprofen excluded because hepatosplenomegaly is unknown in idiosyncratic HILI and DILI and thereby excludes both.** **Alternative and other relevant diagnoses** None, but HEV, HSV and VZV infections possibly not excluded. **Final commentary** Good outcome. The key clinical finding by imaging examination was a hepatosplenomegaly. This was associated with monocytosis, a borderline titer (actual result not presented) of anti-CMV IgM and an extremely high titer of anti-CMV IgG (> 21.000 U/L). Monocytosis and hepatosplenomegaly are known published features in CMV.
Case 9 Female, 24 years Germany	**Narrative** The patient used PE 50 mg/d for 4 months from 02/2006 to 05/2006 and noticed first symptoms of fatigue, loss of appetite and jaundice in early 05/2006. On 05/18/2006: ALT 4458 U/L (with increase up to 5502 U/L as a second ALT peak on 05/21/2006 and subsequent rapid fall), AST 3101 U/L, ALP 149 U/L, total bilirubin 9.4 mg/dL, monocytosis with 13.0% (normal 2 – 8). Negative serologies reported of anti-HAV IgM and IgG, HBs antigen, anti-HBs, anti-HBc, and anti-HCV. HCV RNA negative. Autoimmune parameters all negative. Exclusion of hemochromatosis, and alpha-1-antitrypsin deficiency. Wilson disease was reported as being excluded in 1997, now normal ceruloplasmin. Reported are normal results of CMV and rare hepatotropic viruses but details are missing. Of note, in a hospital summary dated of 11/20/2006 it is described that a tonsillectomy was done 2 months prior to the liver event of 2006 with a possible association regarding a narcotic drug such as halothane was discussed. However, earlier in this report of 11/20/2006, a tonsillectomy done on 09/09/1997 was described, a contradictory statement. The patient suffered from acute liver failure and received a liver transplant on 05/29/2006. The explanted liver showed a subtotal liver dystrophy with lymphocytic infiltrates and some fibrosis. Changes were considered compatible with toxic injury, such as by PE. The clinical report may be incomplete regarding exclusion of rare hepatitis virus infections. HEV, HSV, CMV, and VZV were not specifically mentioned as having been excluded. ALF. Recovery after successful liver transplantation. **RUCAM** Causality for PE unlikely (score +1) **Final diagnosis** **Acute liver failure (ALF) unlikely due to PE** **Alternative and other relevant diagnosis** Acute liver failure of undetermined cause **Final commentary** Good outcome after liver transplantation. Most common but not necessarily rare types of hepatitis are excluded; questions remain regarding HEV, CMV, HSV and VZV. Although Wilson disease was reported as having been excluded in 1997 without presenting actual data, it may have not been excluded in 2006 as a normal ceruloplasmin value does not exclude Wilson disease. Unclearly mentioned operation 2 months prior to the liver event 2006. The second ALT peak after PE cessation strongly supports an alternative cause.

Case 10 Male, 53 years Austria	**Narrative** The patient experienced pruritus since 3 months and was admitted to the hospital in Innsbruck on 09/28/2011 with colicky pains and jaundice. Relevant LT results at admission are poorly documented, restricted to total bilirubin with around 8 mg/dL according to a documented figure, with subsequent increases and falls under conditions of lacking documented drug or herb use including PE (under another brand) during that time, substantiating lack of a temporal and thereby causal association. Later on and as example on 10/19/2011, the following results are documented: ALT 52 U/L, AST 61 U/L, ALP 269 U/L and total bilirubin 18.59 mg/dL. The patient was initially admitted because of abdominal complaints, nausea and asthenia since one week and heavy pains in the right upper quadrant that started in the night before. The subsequent diagnostic work-up uncovered a complex biliary disease consisting of a common bile duct stone, which required an endoscopic papillotomy and subsequent extraction of the stone out of the common bile duct to ensure an appropriate bile flow. However, the further clinical course was complicated by bleeding at the site of the papillotomy with a blood clot which led to a complete occlusion of the distal common bile duct, requiring an endoscopic insertion of a stent into the common bile duct to establish a good bile flow. With a stent occlusion, another complication emerged that was treated by stent removal out of the distal common bile duct. Concomitantly, sludge was removed out of the common bile duct. The biliary disease included also multiple stones of the gall bladder and a cholecystitis as suspected by imaging, which commonly requires a cholecystectomy to surgically remove the gall bladder with its multiple stones as causes. It is not documented whether and when this was done, as the patient was discharged on 11/16/2011. There was also the clinical suspicion that the patient might have had a hepatotoxic reaction by PE, which he used since 2008 and thereby likely for more than 3.5 years but further details including time when PE use was stopped were not provided. He also used opioids and butylscopolaminiumbromid to cope his migraine symptoms, but details were not documented. A liver biopsy was done on 10/24/2011, but the indication for this invasive diagnostic procedure and it possible major risks remains unknown as the diagnosis of a complex biliary disease was established which does not require a diagnostic confirmation by liver histology. As expected, the liver histology result is vague. The pathologist suggests as diagnosis a drug-toxic cholestasis, if a mechanic cholestasis is excluded. As a mechanic cholestasis was clearly diagnosed and underwent treatment, the liver histology does finally not support any drug-induced liver injury, also not one possibly caused by Chinese tea mixtures which the patient consumed and had received from a TCM physician in Vienna, but again details were not provided. Overall, case data are insufficient, not in line with mainstream medicine. . **RUCAM** Causality for PE excluded (score 0) and for TCM tea also excluded (score -1). No assessment for the other comedicated drugs due to insufficient details. **Final diagnoses** **1. Verified complex biliary disease, consisting of common bile duct stone (removed), cholecystitis in connection with multiple stones in the gallbladder providing a clear indication for surgical minimal-invasive cholecystectomy in the interval.** **2. Lack of hepatotoxicity with excluded causality for PE and TCM tea.** **Alternative and other relevant diagnoses** None. **Final commentary** This patient has a well-established and clearly documented biliary disease, unrelated to the use of PE or TCM tea. The biliary disease was complex and consisted of a common bile duct stone of around 3.5 mm diameter, which was removed after endoscopic papillotomy since this stone did cause the symptoms, of a cholecystitis in the context of multiple stones in the gall bladder. In retrospect, one of the multiple stones residing in the lumen of the gall bladder likely left the gallbladder during any of the normal gall bladder contractions, passed the cystic duct and remained in the common bile duct as it could not pass the papilla of Vateri. Partial occlusion of the common bile duct by this stone caused the described symptoms, relieved by endoscopic stone removal.

Causality for all products was assessed using RUCAM.[24] Abbreviation: AIH: Autoimmune hepatitis; ALF: acute liver failure; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CMV: cytomegalovirus; DILI: drug induced liver injury; EBV: Epstein Barr virus; HAV: hepatitis A virus; HBc: hepatitis B core; HBs: hepatitis B surface; HBV: hepatitis B virus; HCV: hepatitis C virus; HEV: hepatitis E virus; HILI: Herb induced liver injury; HSV: herpes simplex virus; LTs: liver tests PCP: primary care provider; PCR: polymerase chain reaction; PE: Petadolex®; PMH: past medical history; RUCAM: Roussel Uclaf Causality Assessment Method: TCM: Traditional Chinese Medicine; VZV: varicella zoster virus.

Table 2: Causality assessments for PE and other products using RUCAM in the study cohort (cases 1-10)

		Case 1		Case 2		Case 3		Case 4		Case 5	Case 6		Case 7	Case 8	Case 9	Case 10
Items	**Score**	PE	Other drugs	PE	Other drug	PE	Other drug	PE	Other drug	PE	PE	Other drugs	PE	PE	PE	PE
1. Time to onset from the beginning of the drug/herb																
● 5–90 d, rechallenge: 1-15 d	+2	+2	?	+2		+1	+1	+2		+1	+1	+2				
● <5 or >90 d, rechall.: >15 d	+1				+1				+1				+1	+1	+1	+1
Alternative: Time to onset from cessation of the drug/herb																
● ≤15 d (except for slowly metabolized chemicals: >15 d)	+1															?
2. Course of ALT after cessation of the drug/herb. Percentage difference between ALT peak and N																
● Decrease ≥ 50 % within 8 d	+3			+3	+3					+3						
● Decrease ≥ 50 % within 30 d	+2							+2	+2					+2		
● No information or continued drug use	0										0	0	0			0
● Decrease ≥ 50 % after 30 d	0															
● Decrease < 50 % after the 30th day or recurrent increase	-2	-2	-2			-2	-2				-2	-2		-2	-2	
3. Risk factors ● Alcohol use (current drinks/d: > 2 for women, >3 for men)	+1															
● Alcohol use (current drinks/d: ≤ 2 for women, ≤ 3 for men)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
● Age ≥ 55 years	+1															
● Age < 55 years	0	0	0	0	0	0	0	0	0	0	0	0	+1	0	0	0
4. Concomitant drug/herb																
● None or no information	0									0				0	0	
● Concomitant drug/herb with incompatible time to onset	0	?	?			0	0					0	0?			0
● Concomitant drug/herb with compatible or suggestive time to onset	-1			-1	-1			-1	-1		-1					

● Concomitant drug/herb known as hepatotoxin and with compatible or suggestive time to onset	-2															
● Concomitant drug/herb with evidence for its role in this case (positive rechallenge or validated test)	-3															
5. Search for alternative causes Group I (7 causes)																
● HAV: Anti-HAV-IgM		-	-	-?	-?	-	-	-	-	-	-	-	n.a.	-	-	n.a.
● HBV: HBsAg, anti-HBc-IgM, HBV-DNA		+?	+?	-?	-?	-	-	-	-	-	-	-	n.a.	-	-	n.a.
● HCV: Anti-HCV, HCV-RNA		-	-	-?	-?	-	-	-	-	-	-	-	n.a.	-	-	n.a.
● HEV: Anti-HEV-IgM, anti-HEV-IgG, HEV-RNA		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.?	n.a.	n.a.
● Hepatobiliary sonography / colour Doppler sonography of liver vessels/ endosonography /CT/ MRC		-	-	-	-	-	-	+	+	+	-	-	-	+	-	+
● Alcoholism (AST/ ALT ≥ 2)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
● Acute recent hypotension history (particularly if underlying heart disease)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group II (6 causes)																
● Complications of underlying disease(s) such as sepsis, metastatic malignancy, autoimmune hepatitis, chronic hepatitis B or C, primary biliary cholangitis or sclerosing cholangitis, genetic liver diseases		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Infection suggested by titer changes and PCR:																
● CMV (anti-CMV-IgM, anti-CMV-IgG, CMV PCR)		-	-	?	?	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	n.a.	+?	n.a.?	n.a.
● EBV (anti-EBV-IgM, anti-EBV-IgG, EBV PCR)		-	-	+	+	(+)?	(+)?	n.a.	n.a.	n.a.	-	-	n.a.	-	?	n.a.
● HSV (anti-HSV-IgM, anti-HSV-IgG, HSV PCR)		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+?	+?	n.a.	n.a.	n.a.	n.a.	n.a.?	n.a.?	n.a.

● VZV (anti-VZV-IgM, anti-VZV-IgG, VZV PCR)		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.		n.a.	n.a.	n.a.	n.a.?	n.a.
Evaluation of groups I and II																
All causes-groups I and II – reasonably ruled out	+2															
● The 7 causes of group I ruled out	+1															
● 6 or 5 causes of group I ruled out	0	0	0			0	0				0	0			0	
● Less than 5 causes of group I ruled out	-2												-2			
● Alternative cause highly probable	-3			-3	-3			-3	-3	-3				-3		-3
6. Previous hepatotoxicity of the drug/herb																
● Reaction labelled in the product characteristics	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	?	+2	+2	+2	+2
● Reaction published but unlabelled	+1															
● Reaction unknown	0															
7. Response to unintentional reexposure																
● Doubling of ALT with the drug/herb alone, provided ALT below 5N before reexposure	+3															
● Doubling of ALT with the drug(s)/herb(s) already given at the time of first reaction	+1															
● Increase of ALT but less than N in the same conditions as for the first administration	-2															
● Other situations	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total score for patient		+2	0?	+3	+2	+1	+1	+2	+1	+3	0	0	+2	0	+1	0

Details of RUCAM are presented in a recent report[24], whereby for this cohort the RUCAM subscale was used that is specifically reserved for the hepatocellular type of injury. The symbol "+" indicates that an abnormal result was obtained, whereas "-"indicates a normal result. Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CMV, Cytomegalovirus; CT, Computer tomography; DILI, Drug induced liver injury; EBV, Epstein Barr virus; HAV, Hepatitis A virus; HBc, Hepatitis B core; HBsAg, Hepatitis B antigen; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HEV, Hepatitis E virus; HILI, Herb induced liver injury; HSV, Herpes simplex virus; MRC, Magnetic resonance cholangiography; N, upper limit of the normal range; n.a. not assessed or not available; PE, Petadolex®; RUCAM, Roussel Uclaf Causality Assessment Method; VZV, Varicella zoster virus. Total score and resulting causality grading: ≤ 0, excluded; 1-2, unlikely; 3-5, possible; 6-8, probable; ≥ 9, highly probable.

Table 3: Documented hepatitis serologies of the study cohort (cases 1 – 10)

Data	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
HAV	Anti-HAV IgM negative.	No details.	Negative result for anti-HAV.	Anti-HAV reported as negative without details.	Anti-HAV IgM negative.	Ant-HAV IgM negative.	Anti-HAV IgM and IgG n.a.	Anti-HAV IgM negative, anti-HAV IgG positive.	Anti-HAV IgM and IgG, both negative.	n.a.
HBV	Anti-HBs positive (> 25 U/L), HBV vaccination not documented in the files. HBsAg negative.	No details.	HBs antigen, HBV RNA, anti-HBs and anti-HBC were all negative.	HBV antibodies reported as negative, but lacking details.	HBs antigen, anti-HBc, all negative.	HBsAg, anti-HBc IgM, all negative.	Anti-HBc IgM, HBs antigen, anti-HBs, all n.a.	HBs Ag negative, HBV-DNS PCR negative, anti HBc negative.	HBs antigen, anti-HBs, anti-HBc, all negative.	n.a.
HCV	Excluded: HCV RNA negative.	No details.	Anti-HCV and HCV RNA negative.	HCV RNS negative, antibodies reported as negative without details.	Anti-HCV negative.	Anti-HCV negative.	n.a.	Anti-HCV negative.	Anti-HCV and HCV RNA negative.	n.a.
HEV	Anti-HEV IgM, anti-HEV-IgG, and HEV PCR, all n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.?	n.a.
CMV	Anti-CMV IgG positive (> 230 U/L) without assessed titer changes in the further course; anti-CMV negative.	n.a.	Likely excluded.	Excluded.	n.a.	n.a.	n.a.	Anti-CMV IgM with borderline but not quantified titer. Anti-CMV IgG > 21.000 U/L, but lacking titer changes in the further course.	n.a.?	n.a.
EBV	Anti-EBV negative, anti-EBV IgG positive (<170 U/L).	Anti-EBV IgG and IgM positive, initial titers and subsequent titer changes not evaluated.	Anti-EBV IgG 85 U/L.	n.a.	n.a.	Monospot negative.	n.a.	Anti-EBV IgM negative, anti-EBV 250 U/L.	n.a.?	n.a.
HSV	n.a.	n.a.	n.a	High anti-HSV IgG titers, 1: 34.000. Titer changes in the further course not assessed. Anti-HSV IgM negative.	n.a.	n.a.	n.a.	Reported as negative , but details n.a.	n.a.?	n.a.
VZV	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.?	n.a.

Abbreviations: CMV: cytomegalovirus; EBV: Epstein Barr virus; HAV: hepatitis A virus; HBc: hepatitis B core; HBs: hepatitis B surface; HBV: hepatitis B virus; HCV: hepatitis C virus; HEV: hepatitis E virus; HSV: herpes simplex virus; n.a.: not assessed/ not available; PE, Petadolex®; PCR: polymerase chain reaction; VZV: varicella zoster virus.

Table 4: Clinical characteristics of suspected but not confirmed liver injury in the study cohort (cases 1 – 10)

Items	Study cohort with individual 10 cases									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
● Duration of use (weeks)	8	16	24	5	14	2	32	12	16	182
● Latency period (weeks)	7	12	30	5	12	0.4	32	11	16	127
● Time to onset from PE cessation (weeks)	NA	NA	6	NA	NA	NA	4	NA	NA	NA
● Nausea	+	-	-	-	+	+	-	-	-	+
● Vomiting	-	-	-	-	+	+	-	+	-	-
● Jaundice	+	+	+	+	+	+	-	+	+	+
● ALT (U/L)	676	1134	220	990	625	1125	75	1432	4458	52
● AST (U/L)	251	384	266	540	NA	NA	41	1567	3101	61
● Total bilirubin (mg/dL)	5.7	7.1	6.64	14	NA	8	0.7	5.7	9.4	18.6
● Severe liver disease	+	+	+	+	NA	+	-	+	+	-
● Hepatocellular injury	+	+	+	+	+	+	+	+	+	-
● Cholestatic liver injury	-	-	-	-	-	-	-	-	-	+
● Mixed liver injury	-	-	-	-	-	-	-	-	-	-
● Ascites	-	-	-	-	-	-	-	-	-	-
● Hepatomegaly	-	-	-	-	-	-	-	+**	-	-
● Splenomegaly	-	-	-	-	+*	-	-	+**	-	-
● HSOS confirmed by liver histology	-	-	-	-	NA	-	-	-	-	-
● Blood pyrrole-protein adducts	-	-	-	-	-	-	-	-	-	-
● Comedication	+	+	+	+	+	+	+	+	-	+
● Confounding variables	+	+	+	+	+	+	+	+	+	+
● Alternative diagnoses	+	+	+	+	+	+	-	+	+	+

Details of HSOS regarding clinical signs, histology features and diagnostic biomarkers such as blood pyrrole-protein adducts can be found in the report of Gao et al., 2012[30] in reference to *Gynura segetum*, a plant which is rich in unsaturated PAs and leads to HSOS. This is the type of toxic liver disease typically caused by PA containing plants. Liver histology of HSOS is commonly described with fibrosis, sinusoidal dilatation and hepatocyte loss around the central vein.[30]
*Isolated splenomegaly in one single patient (case 5),

possibly due to hepatits by EBV or CMV infection but serology was not done in this 24 year old woman who had no hepatomegaly (Table 1); **Hepatosplenomegaly in one patient (case 8) with suspected acute hepatitis by CMV infection (Table 1).

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HSOS: Hepatic sinusoidal obstruction syndrome; PE: Petadolex®; RUCAM: Roussel Uclaf Causality Asssessment Method.

Table 5: Confounding variables in the study cohort (cases 1 – 10)

Confounding variables	Study cohort consisting of 10 cases									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
● Intermittent PE use	+	-	-	-	?	?	-	?	-	?
● Nausea	+	-	-	-	+	+	-	-	-	+
● Vomiting	-	-	-	-	+	+	-	+	-	-
● Liver histology with HSOS signs	-	-	-	-	NA	-	-	-	-	-
● Alternative diagnoses	+	+	-	+	+	+	-	+	-	+
● Comedication	+	-	+	+	-	+	+	+	-	+
● ALT/AST, second peak	+	-	+	-	-	+	-	+	+	?
● Disease onset long after PE cessation	-	-	+	-	-	-	+	-	-	-
● Hepatotoxicity criteria fulfilled	+	+	+	+	+	+	-	+	+	+
● Complete exclusion of alternative causes	-	-	-	-	-	-	-	-	-	-

Data from Gao et al., 2012.[30] *Gynura segetum* is a plant rich in unsaturated PAs which cause HSOS, the type of toxic liver disease typically caused by PA containing plants. Liver histology of HSOS is commonly described with fibrosis, sinusoidal dilatation and hepatocyte loss around the central vein.[30] Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HSOS: Hepatic sinusoidal obstruction syndrome; PAs: Pyrrolizidine alkaloids; PE, Petadolex®.

Table 6: Clinical characteristics of liver patients who consumed *Gynura segetum* as compared to the study cohort of patients who used PE

Items	*Gynura segetum* large study cohort (n)	*Gynura segetum* small study cohort (n)	Present study cohort (n)
	116	5	10
● **Ascites**	115/116	5/5	0/10
● **Hepatomegaly**	104/113	4/5	1/10*
● **Splenomegaly**	NA	2/5	1/10**
● **Jaundice**	95/113	5/5	9/10
● **ALT elevation**	47/60	NA	10/10
● **AST elevation**	50/58	NA	8/8
● **RUCAM**	NA	Done	Now done
● **RUCAM scores**	**NA**	Final scores of 5,5,6,7, and 7	Final scores of 2,3,1,2,3,0,2,0,1, and 0
● **Blood pyrrole-protein adducts**	**NA**	5/5	0/10
●**Liver histology**	Mostly done	Partially done	Done in 9/10
●**HSOS confirmed by liver histology**	Yes	Yes	Not confirmed in 9/9

Data of both the *Gynura segetum* large study cohort and the small study cohort are derived from the report of Gao et al., 2012.[30] *Gynura segetum* is a typical plant rich in unsaturated PAs which cause HSOS, the type of toxic liver disease typically caused by PA containing plants. Liver histology of HSOS is commonly described with fibrosis, sinusoidal dilatation and loss of hepatocyte loss around the central vein.[30] *Hepatomegaly plus splenomegaly in one patient (case 8). **Isolated splenomegaly in one single patient (case 5), possibly due to EBV or CMV infection but serology was not done in this 24 year old woman; no hepatomegaly (Table 1). Details of the PE study cohort are derived from Table 1. Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HSOS: Hepatic sinusoidal obstruction syndrome; NA: Not available or not assessed; PE: Petadolex®; RUCAM: Roussel Uclaf Causality Asssessment Method.

4. DISCUSSION

Herbal liver injuries attract general interest and are highlighted or discussed in many case series.[21,37-44] For some of these, causality is controversial and represents major clinical, scientific, regulatory and manufactural challenges, especially if the causality of herbal hepatotoxicity is not verified, not assessed, or alternative causes are not excluded. For instance, problems of lacking or unassessed causality refer to herbs or herbal dietary supplements such as black cohosh[45-47], kava[25,42,48,49], khat[42], kratom[42], *Pelargonium sidoides*[26,27], Greater Celandine[50], herbal Traditional Chinese Medicine[37-40], green tea extract[51], Herbalife[52,53] and OxyELITE Pro.[31,32,54] The discussions around herbal PH products including PE are much less intensive as validly documented and RUCAM-based case reports of liver injury by PE or PH were not published. Presently, liver injury by PE was claimed in 10 spontaneous case reports; these cases are now analyzed to provide more clarity and insight into the relevant case details. Based on individual narratives (Table 1), causality assessment by RUCAM (Table 2) including a search for alternative causes (Tables 2 and 3), the clinical assessment (Table 4), confounding variables (Table 5) and comparative evaluations (Table 6), there is no evidence of liver injury by PE – or PAs as suspected but not verified ingredients of PE – in the 10 study cohort patients; they also do not meet the

diagnostic criteria of idiosyncratic or intrinsic HILI by PAs such as HSOS (Figure 1, Table 6), invalidating contrary opinions.

Nevertheless, on the side of caution it appears to be prudent that the product information should have some kind of a cautionary label, mentioning that the product may cause extremely rare liver injury in susceptible individuals. We suggest this general recommendation for all drugs, herbs, herbal drugs, herbal dietary supplements and other supplements, which covers potential idiosyncratic reactions (Figure 1). Such information may contribute to patient safety and it also prevents unjustified claims of patients against the manufacturer and prescribing physician. Indeed, idiosyncratic liver injury is well described and a characteristic feature of drug and herbal use that is not easily recognized by clinical trials or case series due to its rarity. Despite the lack of hepatotoxicity in this study cohort, a careful, complete data collection and the use of RUCAM for causality assessment are recommended in a future case if idiosyncratic PE hepatotoxicity is suspected. This is in accordance with recent suggestions[34] and previous

causality assessment reports[31,32], which were highly appreciated as examples of a thorough case analysis.[33]

With a high efficiency for migraine prophylaxis[8,11], the present exclusion of liver injury in the study cohort (Tables 1 and 2) and the commonly good tolerance[15], PE has a favorable benefit-risk profile for migraine prophylaxis. PE has drug characteristics and contains petasin and isopetasin as the active chemicals, among other well-defined ingredients.[15] Due to its refined production technique, it lacks PAs and carries a negligible risk of plant misidentification, impurities and adulterants, all of which are not uncommon in other herbal products.[38,43,55]

Some confusion exists from the labelling of plants and products: Petasites and Petadolex® were seemingly used interchangeable or mixed without a clear plant and product definition. For instance, when Petasites is discussed in connection with migraine prophylaxis, it should be identified as the plant *Petasites hybridus* (PH), as opposed to *P. japonicus.* The PH extract should be labelled as unprocessed, PA-containing PH since it still contains PAs as natural ingredients, whereas the processed Petadolex® is free of PAs. Thus, PE is in line with official regulations of PA limits[23]; natural products including herbal medicines for human use should be free from unsaturated PAs[21], which are metabolized in the liver and toxic to sinusoidal cells, resulting in the HSOS (Table 6).[22,56]

Finally, the present analysis confirms previous publications on other herbal products that spontaneous reports of adverse liver reactions following herbal use as submitted to regulatory agencies often are of little clinical value.[25-27,47,48,50,52-54] As a consequence, many cases of initially suspected HILI have a low causality level upon careful reanalysis or are attributable to alternative causes overlooked in the first evaluation. For instance, among 573 cases of suspected herbal hepatotoxicity, in 278 patients (48.5%) and therefore in almost half of the cases, competing causes were found more likely.[57] Similar but less pronounced problems of incorrect diagnoses were reported for DILI in 417 out of 2,906 cases (14%).[58] These data were confirmed by a subsequent study, which revealed that many published DILI reports did not withstand to a critical review and lacked convincing evidence that some published hepatotoxic drugs indeed are hepatotoxic.[59] Notably, most of these misdiagnoses were discovered by RUCAM and unrecognized preexisting chronic liver diseases were then judged as confounding variables. Liver injury cases including HILI remain a diagnostic challenge, as also evidenced in the present study cohort.

5. CONCLUSIONS

In conclusion, our analysis disproved claims of adverse liver reactions by the use of Petadolex® in 10 spontaneous case reports. Using the robust, quantitative, structured and liver specific causality assessment method of RUCAM, the Roussel Uclaf Causality Assessment Method, causality attribution was evaluated for PE. In all 10 liver cases, causality gradings for PE are low and do not support classifying PE as a hepatotoxic herbal medicine; in detail, causality for PE was excluded in 3 patients, unlikely in 5 patients and possible without clinical relevance in 2 patients. In most of the 10 patients, alternative diagnoses, abundant comedications and incomplete case data prevailed as major confounding variables. In essence, our clinical analysis and robust causality assessment by RUCAM failed to substantiate any liver injury by Petadolex® in the 10 spontaneous report cases, opposing views to the contrary.

6. DISCLOSURES

Rolf Teschke received an honorarium by Merz Pharmaceuticals and Max Zeller Pharmaceuticals for lectures at scientific meetings and by Covington & Burling LLP Washington DC and by Weber & Weber Inc. for consultation. None of the other authors received honoraria or has a conflict of interest.

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