From the Department of Laboratory Medicine
Division of Clinical Pharmacology, Karolinska Institutet,
Stockholm, Sweden

The Institute for Environmental Medicine
Karolinska Institutet, Stockholm, Sweden

The Medical Products Agency, Uppsala, Sweden

Methods in Pharmacoepidemiology: Four Studies, Four Settings

by

Anders Sundström

Karolinska
Institutet

Stockholm 2010

All previously published papers were reproduced with permission from the publisher.

Dedicated to Sveriges Radio P1

Abstract

In the present thesis, four studies using four different settings and designs are presented in which associations between pharmacological treatments and possible adverse drug reactions are investigated; some methodological considerations identified in them are discussed.

In a population-based case-control study, using information from telephone interviews, the association between use of acid-suppressing drugs and the development of acute pancreatitis was investigated. Statistically significant odds ratios were found, associating use with a duration of less than six months of proton-pump inhibitors (OR 3.2; 95% CI 1.4-7.4) or H2-antagonists (OR 4.9; 95% CI 1.6-15) with the outcome. However, confounding by indication was present to some extent, but the association was interpreted as probably causal.

Using the UK General Practice Research Database (GPRD), a case-control study nested in a population of women with a diagnosis of menorrhagia, the relationship between use of tranexamic acid, mefenamic acid or norethisterone and the development of venous thromboembolism (VTE) was evaluated. Use of tranexamic acid was too scarce to allow for certain conclusions; 3 cases and 4 controls exposed to the drug yielded an OR of 3.2 (95% CI 0.6-15.8). Use of mefenamic acid was associated with the outcome with an OR of 5.5 (95% CI 2.1-5.8), as was use of norethisterone, OR 2.4 (95 % CI 1.0-5.8). A diagnosis of anemia, indicating a more severe menorrhagia, was also associated with the outcome, OR 2.2 (95% CI 1.0-4.9); a fact which may indicate that confounding by indication was at hand, and that menorrhagia itself may constitute a risk-factor for VTE.

The safety of antiretroviral treatment (ART) for HIV was analysed in a Swedish cohort-study of 1,072 patients. An association between ART and the development of midline- and inguinal hernias was identified. The hazard ratios (HR) were different depending on duration of ART containing a protease inhibitor (PI). The highest HRs were observed during the second to third year of treatment: HR 10.7 (95%CI 1.3-85.7) for midline hernia, and HR 4.4 (95% CI 1.1-16.6) for inguinal hernia.

By linking a cohort of 5,756 patients treated with isotretinoin for severe acne with the Swedish national patient register of hospitalizations, the association between treatment with isotretinoin and hospitalizations for suicide attempt was analyzed. The incidence and Standardized Incidence Ratios (SIR) of suicide attempts before, during and after treatment was estimated by use of a cohort cross-over approach. The SIR for suicide attempts started to rise above one already two years before treatment. It was highest within six months after treatment, SIR 1.9 (95% CI 1.1-3.2) for first attempts; within three years after treatment, the observed number of suicide attempts was very close to the expected. It was concluded that a strong association between severe acne and suicide attempt was present, and that a significantly increased risk associated with use of isotretinoin could not be detected on the population level. However, the possibility could not be excluded that certain patients, presumably sensitive to isotretinoin, made suicide attempts as a result of the treatment.

A number of methodological aspects identified in the included studies are discussed in the thesis: confounding by indication was identified, challenged and estimated; the importance of dose-response and duration-response relationships in assessing causality was demonstrated; reversed causality was observed and handled; assessing the presence of recall bias in an interview setting was described; and the effect of a presumed under-estimation of an important confounder was demonstrated using a simulation. Advantages and disadvantages of using the different data-sources, depending on type of treatment and character of adverse events, are discussed.

List of publications

I   **Sundström A**, Blomgren K, Alfredsson L, Wiholm BE. Acid-suppressing drugs and gastroesophageal reflux disease as risk factors for acute pancreatitis--results from a Swedish Case-Control Study. Pharmacoepidemiol Drug Saf 2006; 15(3): 141-9.

II   **Sundström A**, Seaman H, Kieler H, Alfredsson L. The risk of venous thromboembolism associated with the use of tranexamic acid and other drugs used to treat menorrhagia: a case-control study using the General Practice Research Database. BJOG 2009;116(1):91-7.

III   **Sundström A**, Mortimer O, Åkerlund B, Karlsson A, Flamholc L, Håkangard C, Granholm H, Persson I, Morfeldt L. Increased risk of abdominal wall hernia associated with combination anti-retroviral therapy in HIV-infected patients-results from a Swedish cohort-study. Pharmacoepidemiol Drug Saf 2010; Feb 24 (e-pub ahead of print).

IV   **Sundström A**, Alfredsson L, Sjölin-Forsberg G, Gerdén B, Bergman U, Jokinen J. Suicide attempts are associated with both acne and treatment with isotretinoin. A retrospective Swedish cohort study.

Under review

Table of contents

List of abbreviations

ACE	Angiotensin Converting Enzyme
ADR	Adverse Drug Reaction
AERS	Adverse Event Reporting System (of FDA - USA)
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Treatment
BMI	Body Mass Index (kg/cm^2)
CI	Confidence Interval
Cl	centilitres
DDD	Defined Daily Dose
DVT	Deep Vein Thrombosis
EMEA/EMA	European Medicines Agency
ERCP	Endoscopic Retrograde Cholangiopancreaticography
EU	European Union
FDA	Food and Drug Administration (USA)
GERD	Gastro-esophageal Reflux Disease
GI	Gastro-Intestinal
GP	General Practitioner
GPRD	General Practice Research Database (United Kingdom)
H2	Histamin-2 Receptor
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IQR	Inter-Quartile Range
MPA	Medical Products Agency
NHS	National Health Service (United Kingdom)
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NNTH	Number Needed To Harm
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NSAID	Non-Steroidal Anti-Inflammatory Drug
OC	Oral Contraceptive
OR	Odds Ratio
OTC	Over The Counter (Non-Prescription Drug)
PI	Protease Inhibitor
PPI	Proton-Pump Inhibitor
SIR	Standardized Incidence Ratio
SMR	Standardized Mortality Ratio
VTE	Venous Thromboembolism

Introduction

Ever since the thalidomide disaster (McBride 1961), pharmacoepidemiology and -vigilance have been central in the monitoring of potential side effects of pharmacological treatment. Virtually no drug is entirely without side-effects, and since an increasing number of potent and specifically targeting drugs are developed, with which follows that society's cost for supplying them increases rapidly, the need for proper assessments of any given treatment's benefit-harm balance is of great political, economical and personal - for the part of the patients - interest. The need for appropriate methods in the study of drug utilization and benefit-harm balance can probably not be emphasized enough.

In the early days, the monitoring and assessment of safety of drugs were mainly relying on spontaneous reporting systems. In time, the methods of performing formal pharmacoepidemiological studies began to emerge, using the study subjects as data-source by means of interviews or questionnaires, or by manual extraction of medical records. Then came the possibility to use computerized databases from which individual data on events and use of prescribed drugs could be extracted and analysed. Electronic medical records, claims databases and linkage between health-care registers are today by far the most commonly used data-sources when observational pharmacoepidemiological studies are undertaken; maybe too often: for some exposures and outcomes, the use of register-based data is far from optimal.

In recent years, pharmacogenetic covariates, not only in controlled, experimental trials, but also in observational pharmacoepidemiological studies on both benefits and harms of drug treatments have been increasingly used, and this will hopefully lead to better possibilities to identify patients at risk for specific and severe adverse reactions even before treatment is started. (Kimmel 2005, Becquemont 2009)

The present thesis includes four pharmacoepidemiological studies, using different sources of data, different study designs and various approaches to identify and evaluate some of the common sources of error in pharmacoepidemiology, sources of error with the common feature of having been identified and discussed in these studies.

Aims

<u>Overall aim:</u> The overall aim of this thesis was to present and discuss various methodological issues in pharmacoepidemiology, identified and exemplified in the four included papers; one population based case-control study using personal interviews; one nested case-control study using a database of computerized medical records; one cohort study

based on review of medical charts in specialist care; and one record-linkage cohort study with a crossover approach.

Confounding by indication is practically always a potential source of error in observatonal pharmacoepidemiological studies. It is present when the indication for use of the drug under study is or can be suspected in itself to be a risk-factor for the adverse reaction that is suspected to be caused by the treatment. Ways to identify, to challenge or to actually estimate the degree of confounding by indication will be presented.

Three different aspects of *time* – in a broad sense – will then be discussed. First, the importance of **dose-response and duration-response** relationship between exposure and outcome in assuming causality will be illustrated and discussed. Secondly, the problem of **reversed causality** (also known as "Protopathic Bias") is addressed, *i.e.* when exposure status is misclassified because the adverse reaction under study in fact precedes the start of treatment. Conditions for identifying and correcting such misclassification will be discussed. Thirdly, **recall bias** is an issue when study subjects are the source of information on drug use, and this information is collected after the event under study. Individuals who have undergone or developed a more or less serious disease may be more prone to correctly remember drug use preceding the event than "healthy" controls may be, resulting in a systematic error in exposure classification. Suggestions as how to identify signs of recall bias is presented.

Important confounders to the association between a drug exposure and an outcome may often be suspected to be underestimated in *e.g.* an interview setting where study subjects, dependant on their case status, underreports the use of or exposure to the confounder in question. Underestimation may also be at hand when information on the confounder in *e.g.* electronic medical records to a large extent is missing. The possibility to use simulations in order to estimate the effect of this underestimation is presented in a concrete example.

Finally, the different data sources used in the included studies are discussed with regard to suitability for different types of studies and research questions.

Specific aims: Beside the overall aim of presenting and discussing methodological issues, each of the four studies included carries its own aim: a specific association or suspected association between pharmacological treatment and one or more adverse drug reactions were investigated.

In Paper I, the association between use of acid-suppressing drugs (proton pump inhibitors and H2-antagonists) and the development of acute pancreatitis was investigated. It was a

population-base case-control study based on personal telephone interviews with cases and randomly selected population controls.

In paper II, the association between use of tranexamic acid and other drugs used in the treatment of menorrhagia, and subsequent development of venous thromboembolism (deep vein thrombosis and pulmonary embolism) was the aim of the study. It was a data-base case-control study, nested in a population of women with a diagnosis of menor-rhagia, conducted in the UK General Practice Research Database.

In paper III, the association between antiretroviral treatment in HIV-infected patients and the development of hernia of the abdominal wall (inguinal and midline hernias) was in-vestigated. It was a cohort study using mainly information extracted from medical charts of the study subjects.

Finally, in paper IV, the complicated association between severe acne, its treatment with isotretinoin and suicide attempts was investigated. It was a record-linkage cohort study of a crossover type, comparing incidence rates and standardized incidence ratios of hospi-talizations for suicide attempt before during and after treatment with isotretinoin.

Background to and brief presentations of articles

Paper I

Acid-suppressing drugs and gastroesophageal reflux disease as risk factors for acute pancreatitis—results from a Swedish Case-Control Study

Acute pancreatitis is a condition with varying incidence geographically and temporally. In several countries, the incidence was observed to increase during the 1980ies and 1990ies. (Corfield 1985, Jaakkola 1993, Tran 1994, McKay 1999) Temporal and geographical differences in the incidence of pancreatitis are attributed to such variations in alcohol use and prevalence of gall-stone diseases.

In a region of Sweden, also part of the study-base in the current study, the incidence of first episodes of acute pancreatitis during a 10-years' period immediately preceding our study was estimated to 23.4 per 100,000 inhabitants and year with a small (<10%) increase when comparing the first and second five-year period. (Appelros 1999)

The most common etiology of acute pancreatitis is excessive alcohol use and cholelithiasis. In the present study, some 70% of the cases were attributed to these reasons. (Blomgren 2002: 1)

As regards drug-induced acute pancreatitis, more than 500 drugs have been reported as suspected cause (Nitsche 2010), but most information originates from individual case reports. One early epidemiological study of drug induced acute pancreatitis was published in 1978, in which use of diuretics was twice as common among 100 cases with acute pancreatitis compared to 100 matched controls (Bourke 1978). One of the main aims with the present study was in fact to elucidate the relation between diuretics and acute pancreatitis.

The Medical Products Agency's regional pharmacovigilance centres for collection and evaluation of spontaneous reporting of adverse drug reactions (ADRs) was the logistic base for the present study on acute pancreatitis. The study was set up as a population based case control-study identifying cases of acute pancreatitis at eight hospitals in four cities in Sweden with a catchment population of approximately 2.2 million people.

The study population was restricted to those aged between 20 and 85, who had been resident of the area for a minimum of six months, had a publicly listed telephone number and spoke Swedish. The final study population was estimated to 1.4 million people, and the study period was from January 1995 through May 1998, leading to a study base of approximately 4.7 million person-years.

Cases were identified by regular screening of laboratory values of amylase-values at least twice the upper reference limit of the hospital. Research nurses scrutinized the medical charts of the patients with raised values and who had presented at emergency room with acute abdominal pain, and who were subsequently admitted to a surgery ward. Patients with chronic or previous episodes of acute pancreatitis, a history of biliary disease, malignancy in the GI-tract and/or pancreas, pancreatitis induced by endoscopic retrograde cholangiopancreaticography (ERCP), abdominal trauma, perforated GI ulcer, or those infected by HIV were not considered for inclusion. The remaining patients were eligible for interview, and informed written consent was sought. Patients who had a hospital-stay of more than 30 days were not eligible in order to minimize the risk for memory loss, and also patients staying at nursing homes – assumed as not taking care of their own medication – were excluded.

All potential cases were reviewed by two gastroenterologists taking into account summarized data from medical records, including results of diagnostic and surgical procedures and autopsies, the narrative of events leading to admission and the alcohol and GI-disease histories as obtained in the interview. The cases were then classified as being certainly or probably related to concurrent gallstone disease, high alcohol consumption, or other causes. They finally established an index-date, the first date of symptoms of the current episode of pancreatitis.

Controls were identified at regular intervals in the official population register, selected at random amongst those aged 20 to 85 years from the same geographical areas as the hospitals' catchment areas; no individual matching took place. The same exclusion criteria were applied to potential controls as for cases. Telephone interviews were conducted with cases and controls; some controls were excluded only after the interview since information on prior and current diseases was not known beforehand. A structured computer-based questionnaire was used, specially designed for the study. It contained questions on previous, life-time history of hospital-care, recent infections, life-time history of diseases diagnosed by a physician, use of drugs in the six months preceding the hospitalization for pancreatitis and the interview, respectively for cases and controls. Information on use of medications both prescribed by a doctor and purchased Over the Counter (OTC) as well as herbal remedies was requested. Finally, information on work and life-style, including use of alcohol and tobacco was collected.

In the final analyses, 462 cases and 1781 controls were included. Three articles based on this study were published before the one presented here. The first publication covered the over-all setting, study-design and clinical characteristics; adjusted odds-ratios for some

drug-groups and concurrent diseases were also presented. (Blomgren 2002:1) Statistically significant odds ratios were observed for acute myocardial infarction, disorders of gastro-intestinal tract, including regional enteritis and ulcerative cholitis. The drug groups H2-antagonists, proton-pump inhibitors, acetic acid derivatives (mainly diclofenac) and anti-bacterials for systemic use were associated with the outcome with statistically significant odds ratios. However, the time-window for etiologically relevant drug-exposure was 30 days preceding the hospital admission and interview for cases and controls respectively. The index-date of the cases, *i.e.* the first date of symptoms of the current disease was not taken into consideration; hence use of drugs that started *after* the occurrence of the first symptoms was possibly included as relevant exposures.

In another analysis, use of the oral anti-diabetic glibenclamide (glyburide in the U.S.) and obesity (BMI > 30 kg/m^2) was identified as risk factors for developing acute pancreatitis. (Blomgren 2002:2)

In paper I, the association between acute pancreatitis and acid suppressing drugs was more thoroughly investigated, taking into account the time between the index-date and the date of hospital admission for acute pancreatitis, and the role of indication for use of these drugs, *i.e.* peptic ulcer and gastritis or gastroesophageal reflux disease (GERD).

The time-window classifying exposure to drugs was set to the period within five days **prior to index-date**, *i.e.* the date of first symptoms of the disease for cases, and through to the index-date. Use of acid-suppressing drugs: the H2-antagonists cimetidine, raniti-dine and famotidine; the proton-pump inhibitors omperazol and lansoprazole; and in some cases, treatment with locally acting antacids had started in very near to the index-date. This may be an indication of use of these drugs for very early signs of the coming acute pancreatitis. For that reason, the index-date was moved backwards, in steps of one day at a time, assuming the *true* first day of signs or symptoms had occurred before the index-date defined by the expert-group. For each day the index-date was moved, the ORs associating antacids, proton-pump inhibitors and H2-antagonists with the outcome were recalculated and evaluated. When the index-date was thus assumed to have occurred three days *before* the one previously established, the unlikely association between antacids and pancreatitis had disappeared. For all groups of acid-suppressing drugs, the unadjusted odds ratio (95% CI) was decreased from 4.3 (1.8-10) to 1.0 (0.3-3.8) for antacids; from 4.0 (2.4-6.4) to 3.2 (1.9-5.4) for proton pump inhibitors and from 4.8 (2.4-9.5) to 3.4 (1.5-7.3) for H2-antagonists. (Table 1)

Table 1. The unadjusted odds ratio (OR) together with 95 % confidence interval (95 % C.I.) to develop acute pancreatitis associated with different drugs, by different definitions of the 5-day exposure window

	H2-antagonists			PPI			Antacids (no PPI/H2)		
Start of Exposure Window at	Exposed Cases/ Controls	OR $_{unadj}$	95% CI	Exposed Cases/ Controls	OR $_{unadj}$	95% CI	Exposed Cases/ Controls	OR $_{unadj}$	95% CI
Date of admission/interview	18/15	4.8	2.4-9.5	33/34	4.0	2.4-6.4	11/10	4.3	1.8-10
Indexdate	14/15	3.7	1.8-7.7	30/34	3.6	2.2-5.9	14/10	5.5	2.4-13
Indexdate minus 1 day	13/15	3.4	1.6_7.2	29/35	3.3	2.0-5.5	9/12	2.9	1.2-7.0
minus 2 days	13/15	3.4	1.6-7.2	27/35	3.1	1.8-5.2	4/12	1.3	0.4-4.0
minus 3 days	12/14	3.4	1.5-7.3	28/35	3.2	1.9-5.4	3/11	1.0	0.3-3.8

Use of drugs was further analyzed by duration of use and by dosage in number of defined daily doses (DDD).

In the multivariate logistic regression, current use (within three days prior to index-date) of proton-pump inhibitors and H2-antagonists that had started during the past six months were statistically significantly associated with the outcome: OR (95% CI) for proton-pump inhibitors 3.2 (1.4-7.4), and 4.9 (1.6-15) for H2-antagonists. Such current use with duration of **more** than six months was lowered for proton-pump inhibitors to 2.0 (0.9-4.2) and to 0.6 (0.1-3.2) for H2-antagonists. Prior use, finally, *i.e.* any use during six months prior to the index-date but that had stopped before, was not statistically significantly associated with the outcome: OR 1.3 (0.2-7.8) for proton-pump inhibitors and OR 1.8 (0.7-4.6) for H2-antagonists. (Table 2)

Table 2. The odds ratio (OR) together with 95 % confidence interval (95 % C.I.) to develop acute pancreatitis associated with different exposures resulting from multivariate unconditional logistic regression analysis.

	Cases/ Controls	OR adj (sex & age)	95% C.I.	OR$_{adj}$[a]	95% C.I.
Age, *increment 10 y*	na			1.1	1.0 - 1.2
Male sex	260/865	1.4	1.1 - 1.7	1.3	1.0 - 1.6
BMI *<19*	14/51	1.4	0.7 - 2.5	1.0	0.5 - 2.0
19-24	219/990	*reference*		*reference*	
25-29	172/583	1.2	1.0 - 1.5	1.2	0.9 - 1.5
30-	53/130	1.7	1.2 - 2.5	1.6	1.1 - 2.3
Unknown	4/27	0.7	0.2 - 1.9	0.5	0.2 -1.7
Number of hospitalisations past year					
None	381/1629	*reference*		*reference*	
1-2	69/135	2.0	1.5 - 2.7	1.7	1.2 - 2.4
3 or more	12/17	2.8	1.3 - 6.1	2.0	0.8 - 4.6
Non-use of alcohol	69/226	1.0	0.6 - 1.4	0.8	0.5 - 1.2
> 0, < 20 G/week	66/213	*reference*		*reference*	
≥ 20, < 120 G/week	168/780	0.7	0.5 - 1.0	0.7	0.5 -1.1
≥ 120, < 220 G/week	52/286	0.6	0.4 - 1.0	0.7	0.4 - 1.0
≥ 220, < 320 G/week	31/85	1.3	0.8 - 2.1	1.2	0.7 - 2.0
≥ 320, < 420 G/week	12/31	1.5	0.7 - 3.2	1.1	0.5 - 2.4
≥ 420, < 520 G/week	7/16	1.6	0.6 - 4.1	1.1	0.4 - 3.2
≥ 520, < 720 G/week	9/3	11.4	2.9 - 44.8	8.7	2.2 - 36
≥ 720 G/week	13/3	17.0	4.6 - 62.4	9.1	2.3 - 36
amount unknown	35/138	0.9	0.6 - 1.4	0.8	0.5 - 1.4
non-smokers	310/1379	*reference*		*reference*	
1-10 cigarettes / day	45/196	1.2	0.8 - 1.7	1.1	0.8 - 1.6
11-20 cigarettes /day[b]	88/184	2.2	1.7 - 2.9	1.9	1.4 - 2.7
21 cigarettes or more / day	19/22	3.9	2.0 - 7.3	2.8	1.4 - 5.6
Peptic ulcer[c]	2/4	1.7	0.3 - 9.2	1.1	0.2 - 7.3
Gastritis/GERD[c]	34/76	1.9	1.2 - 2.9	1.9	1.2 - 3.0
IBD, untreated[e]	12/10	5.9	2.5 - 13.8	5.1	2.0 - 13
Diverticulae	8/19	1.4	0.6 - 3.3	1.0	0.4 - 2.6
Dysfunction of colon	6/21	1.0	0.4 - 2.4	0.7	0.3 - 1.9
Coeliac diasease	1/6	0.6	0.1 - 5.3	0.9	0.1 - 7.9
H2 antagonists					
Current use [f]*, ≤ 6 months*	10/6	6.7	2.4 - 19	4.9	1.6 - 15
Current use, > 6 months	2/8	0.8	0.2 - 3.8	0.6	0.1 - 3.2
Use prior to index	7/16	1.7	0.7 - 4.3	1.8	0.7 - 4.6
PPI					
Current use, ≤ 6 months	14/14	4.0	1.9 - 8.5	3.2	1.4 - 7.4
Current use, > 6 months	14/21	2.5	1.2 - 4.9	2.0	0.9 - 4.2
Use prior to index	12/36	1.4	0.7 - 2.7	1.3	0.6 - 2.6
Salazines, *exposed on index*	2/6	1.4	0.3 - 7.0	1.3	0.2 - 7.8
Prednisolone, *exposed on index*	7/18	1.3	0.5 - 3.1	1.0	0.4 - 2.6

a) Also included in the model: occupational status, history or presence of psychiatric disorders, sleep disturbances, hypertension, myocardial infarction, cardiac failure, renal disorders, prostate disorders, unspecified back-pain: absence of disease as reference. Use, of any duration, within five-days of adjusted index-date of: glibenclamide, fibrates, ace-inhibitors, diuretics, beta-blockers, calcium channel blockers, acetylsalicylic acid, benzodiazepines and selective serotonin re-uptake inhibitors: no use during preceding six months as reference

b) Among controls, 2 former and 5 current smokers stated unknown number of cigarettes per day. The average number of cigarettes smoked per day among smoking controls, 13 per day, was assigned to these 7 subjects.

c) No treatment with H2-antagonists or PPIs during the preceding six months (but including treatment with local antacid effect).

d) GERD: Gastroesophageal Reflux Disease; Gastritis, esophagitis, hiatus hernia, "stress-stomach", epigastralgia

e) No treatment with salazines, corticosteroids or azathioprine

f) Current use = exposed within five days of adjusted index-date

As regards number of DDDs taken per day, for both proton-pump inhibitors and H2-antagonists, the ORs (adjusted for sex and age) were significantly raised when the daily dose was more than or equal to 1 DDD compared to a daily dose of less than 1 DDD. OR (95% CI) for <1 DDD/day of proton-pump inhibitors: 2.0 (0.5-8.3); ≥ 1 DDD/day: 5.2 (2.1-13). OR (95% CI) for H2-antagonists, <1 DDD/day 2.8 (0.6-13); ≥ 1 DDD/day: 15 (3.1-72). (Paper I, Table 2)

Finally, the role of the indication for use of acid-suppressing drugs was assessed in order to address the possibility of confounding by indication. Gastric or duodenal ulcer, whether treated or not with the drugs in question, were not statistically significantly associated with pancreatitis. On the other hand, gastritis/GERD was associated with the outcome with OR (95% CI) 1.9 (1.4-2.6) whether treated or not with proton-pump inhibitors or H2-antagonists, and 1.9 (1.2-2.9) when treated with these drugs. (Paper I, Table 3)

Inflammatory bowel disease, a known risk-factor for acute pancreatitis, was also associated with statistically significant ORs: 4.7 (2.2-10) over-all, and 5.9 (2.5-14) for such disorders without treatment with salazines, corticosteroids or azathioprine during six months prior to index.

It cannot be established with certainty that the association between the outcome and use of proton-pump inhibitors and H2-antagonists was not due to the underlying disease, *i.e.* confounding by indication. The increased risk observed for higher daily doses of the drugs can in fact be due to more severe gastritis. However, the observation that more recent use and not long-term use of these drugs was associated with the outcome may be indicative of a causal relationship between exposure and outcome. If this relationship would also be due to confounding by indication, it follows that newly diagnosed gastritis would be a stronger risk-factor for acute pancreatitis than such disease with long duration.

Paper II

The risk of venous thromboembolism associated with the use of tranexamic acid and other drugs used to treat menorrhagia: a case–control study using the General Practice Research Database.

Tranexamic acid is an agent that stops bleeding by inhibiting the activation of plasminogen. The role of plasminogen is to decompose fibrin in clotted blood; thus the deactivation of this enzyme by tranexamic acid stops the dissolution of the clots, and the bleeding may stop or decrease. The indications for use of tranexamic acid include excessive menstrual bleeding (menorrhagia), haematuria, severe nose-bleeding and reduction of blood-loss during and after surgery.

Since its introduction in the 1960ies, it has been suspected to cause, or being part of the causal pathway leading to thrombosis, as is any drug that inhibits dissolution of thrombi. In spontaneous reporting systems and in a number of case reports, tranexamic acid has been more or less definitely been judged as causing VTEs. (For example: Woo 1989, Taparia 2002). At the time of performing this study and its analyses, only one observational study on the association had been published (Berntorp 2001), but no increased risk for VTE among users of tranexamic acid was observed.

The marketing authorisation holder of tranexamic acid (Cyklokapron) in 1999, Pharmacia UpJohn, was requested to present safety data on the association between use of tranexamic acid for menorrhagia and the development of venous thromboembolism as part of an application for approval to sell cyklokapron without prescription in the UK. For this reason, the GPRD was considered a suitable source of information on this possible association, and Pharmacia UpJohn sponsored a study undertaken by Karolinska Institutet in collaboration with the European Institute of Health and Medical Sciences at the University of Surrey, Guildford, England.

The General Practice Research Database, GPRD, is a UK database containing anonymised electronic medical records from general practices, including data on diseases and symptoms, prescribed drugs, examinations, lab-tests and life-style factors. At the time of performing this study, the GPRD held data on approximately 4% of the population in the UK (study period 1992 – 1998). The "old" GPRD was thus used in this study; since 1999, the "full featured GPRD", collects data from a larger number of general practices, and the number of patients registered now reflects approximately 7% of the population in the UK, or 4.4 million currently active research quality patients, and a total of 9 million usable number of researchable persons. (www.gprd.com; accessed 6 April 2010).

The objective of the present study was to investigate whether tranexamic acid used to treat menorrhagia was associated with an increased risk to develop venous thromboembolism (VTE), and to put those results into context by comparing the risk for this outcome associated with other treatments for mennorhagia (excluding oral contraceptives).

A nested case control study was designed in a cohort of women with menorrhagia. The study population was thus women aged 15-49 years with a diagnosis of mennorhagia at any time between 1992 and 1998 (N=122,237). They were considered at risk for venous thromboembolism until they left the practice, reached the age of 50, died or suffered a first episode of VTE (deep vein thrombosis or pulmonary embolism), whichever occurred first.

Cases were identified by a recorded diagnosis of deep vein thrombosis or pulmonary embolism and confirmation of the diagnosis by anticoagulant therapy, or death from a cause consistent with the diagnosis.

For each case, up to eight controls were randomly selected, individually matched to the case by year of birth, general practice and index-date (the case's first date of symptoms of the VTE).

Since there are many risk factors known to entail a very strong increase in risk to develop VTE, we chose to exclude cases with evidence of such risk factors, instead of attempting to make a large and thus possibly not very transparent multivariate model with many competing risk factors and confounders.

Cases and controls were therefore excluded if they had suffered a VTE before the study period, had records of a recent trauma or surgery within 42 days before the index-date, a diagnosis of cancer recorded within 90 days before and after the index-date, a diagnosis at any time of systemic lupus erythematosus, multiple sclerosis or Crohn's disease. Women with a record indicating pregnancy (e.g. antenatal care, termination of pregnancy, miscarriage or birth) within 42 days before the VTE event date were also excluded because pregnancy is a well-known risk factor for VTE and also because they were not at risk of menorrhagia during the time preceding the event. Finally, women who had a prescription for a combined oral contraceptive (OC) that covered the index-date were also excluded.

Two hundred and eighty six cases of VTE were first identified, and 1163 matched controls selected. After applying the exclusion criteria, 134 cases and 552 controls remained.

A diagnosis of pulmonary embolism was established for 52 cases (39%) of which 4 (7.7%) had a fatal outcome. The remaining 82 (61%) cases suffered a DVT, none of which had a fatal outcome.

The selection of cases from a population with menorrhagia did thus not entail a change in the distribution of cases between pulmonary embolisms and DVTs, compared with a rather contemporaneous study on VTE among women (Spitzer 1996). Of 471 cases of VTE 61% suffered a DVT, and 39% a pulmonary embolism (with four fatal events, 2.2%).

Prescriptions of drugs used to treat menorrhagia were extracted, i.e. tranexamic acid, mefenamic acid, norethisterone and ethamsylate. Cases and controls were considered exposed if a prescription to either of the drugs had been issued within 90 days prior to the index-date. The 90 days time window was established after reviewing prescription patterns for these drugs in the source cohort of women with menorrhagia. Other factors identified were fibroma, anaemia, varicose veins, high BMI, smoking habits and a number of chronic conditions: treated asthma, diabetes, or thyroid disease, as well as renal disorders.

Use of tranexamic acid was scarce in this study: only 3 cases and 4 controls had been prescribed the drug within 90 days before the index date. Mefenamic acid (10 cases, 12 controls) and norethisterone (10 cases 17 controls) were the most commonly prescribed treatments. Ethamsylate was only recorded for 1 case and 1 control, and was not included in the analysis. In the multivariate conditional logistic regression (including BMI, smoking and anaemia as covariates) the ORs (95% CI) were 3.2 (0.6-15. 8) for use of tranexamic acid; of mefenamic acid 5.5 (2.1-14.4); and of norethisterone 2.4 (1.0-5.8). (Table 3)

Table 3. Crude and adjusted odds ratios for VTE associated with different factors as derived by univariate and multivariate conditional logistic regression analysis (95% confidence interval in parentheses).

Prescription within 90 days of index date	n/ exposed cases	n/ exposed controls	CrudeOR(95% CI)	Adjusted OR[1] (95% CI)
Tranexamic acid	3	4	3.0 (0.6, 13.6)	3.2 (0.6, 15. 8)
Mefenamic acid	10	12	4.1 (1.7, 10.1)	5.5 (2.1, 14.4)
Norethisterone	10	17	2.7 (1.2, 6.2)	2.4 (1.0, 5.8)

1) Adjusted for BMI, smoking and anaemia/Hb < 11.5 g/dL

Because of the low number of users of tranexamic acid, the result is difficult to interpret, even if the point estimate above 3 is to be noted. Norethisterone is contraindicated for use in women at increased risk for VTE, and the observed OR may reflect a causal relationship between use of this hormone and the outcome. The strong and statistically significant association between mefenamic acid, a non-steroidal anti-inflammatory drug (NSAID) of the class anthranilic acid derivatives, is difficult to interpret. An association between NSAIDs and venous tromboembolisms is yet to be proposed in the literature. However, since mefenamic acid is also used as an analgesic and it may be that it has been used as a pain-killer in patients suffering from a condition with an increased risk for VTE.

Finally, confounding by indication can be present: there is a possibility that menorrhagia, and its severity, is a risk factor for development of VTE. The association between menorrhagia and laboratory abnormalities of hemostasis has for example been established. (Kouides 2007) All three substances, with different modes of actions and different spectrums of side-effects, present strong point estimates of an increased risk for developing VTE. Also, a diagnosis of anaemia (or an Hb < 11.5 dl/L) was associated with VTE in the multivariate model: OR 2.2 (95% CI 1.0-4.9). Anaemia may be indicative of a more severe menorrhagia, and thus confounding by indication may be at hand when considering an association between VTE and treatment for menorrhagia.

Paper III

Increased risk of abdominal wall hernia associated with combination anti-retroviral therapy in HIV-infected patients-results from a Swedish cohort-study.

Before the introduction of combination antiretroviral therapy (ART), including the drug class protease inhibitors (PI) in 1996, the prognosis of patients infected with the human immunodeficiency virus (HIV) was very poor. When combination ART, in the first years normally a PI with two non-nucleoside reverse transcriptase inhibitors (NRTI), was made available, HIV-infection in principle transformed from a mortal to chronic condition with much improved survival. However, even if combination ART drastically reduces the viral count, it is not eradicated. A life-long treatment with these drugs is thus necessary.

Several of the ART drugs were registered and marketed in an expedited manner because of the great need for these treatments, and no long-time (long in the context of randomized clinical trials) studies regarding safety of these drugs were required as is normally the case.

In 1987, the FDA (in USA) approved the first antiretroviral drug for treatment of HIV-infection, zidovudine, a non-nucleoside reverse transcriptase inhibitor (NRTI). (Nelson 2006)

The first PI to be approved under "accelerated approval" by the FDA, based mainly on laboratory markers, was saquinavir, in December 2005 (McGuire 1996). In Europe, the first PI to be approved was ritonavir. It was approved under "exceptional circumstances" in 1996. (EMEA 1996: http://www.ema.europa.eu/humandocs/PDFs/EPAR/Norvir/052796en7.pdf)

Therefore, even rather common adverse drug reactions (ADR) - usually identified in controlled studies - had to be detected in a post-marketing setting.

In the beginning of the era with combination ART, the benefits of this treatment were so obvious that concerns on potential ADRs was not an issue. However, as the years went by, it was recognized that adverse events associated with ART could threaten to outweigh the initially overwhelmingly positive risk-harm balance of the treatment, with some adverse events being considered as important as AIDS-events. (Nolan 2005, Reisler 2003)

Because of rising interest on ART-related ADRs of some physicians in Sweden, and not to forget also of the treated patients, a study was set up in Sweden in order to monitor patients infected by HIV, the HivBivUs-project.

The HivBivus-cohort was established in 1999 enrolling all eligible patients treated at the then four largest HIV-units in Sweden, and it was coordinated and partly financed by the Medical Products Agency. A total of 1,072 patients were ultimately included (79.5% men, median age 37), representing approximately 30% of all HIV-infected in Sweden at that period.

Prospectively from 1999 until early 2004, data on ART, stage of infection, AIDS-diagnoses, suspected ADRs and metabolic lab-tests were collected. In the same time, specially trained local physicians and nurses started a review of medical charts for the patients' disease history prior to the establishment of the cohort. All antiretroviral treatment since the first HIV-positive test, as well as all AIDS diagnoses were collected; from 1996 and onwards, information on all suspected adverse events and all viral (HIV-RNA) and CD4-counts were collected. (Paper III, Figure 1) At the time of establishing the cohort, all study subjects completed a questionnaire of 99 questions on *e.g.* experiences of adverse events and life-style factors.

One of the conditions that were noted in the review of possible side-effects was hernia of the abdominal wall, of inguinal or midline type.

The total number of person-years of follow-up in the study base was 7,242, of which 1,434 was therapy naïve person-time. The total person-time on different ART, 5,218 person-years, was classified in three categories: treatment with NRTI only; NRTI + a non-nucleoside reverse transcriptase inhibitor (NNRTI); and any PI-containing treatment regimen. The latter category was the dominating treatment with 3,561 person-years of treatment, or 63% of all ART person-time.

Sixty-three patients were identified in the medical charts with an abdominal wall hernia, 34 patients suffered a total of 40 inguinal hernias, and 29 had as many hernias of the midline type. The median age at diagnosis was 43 years (IQR 36-53), and 92% were male.

The overall incidence of inguinal hernia among the male study-population was compared, regardless of type of or duration on ART, with the population incidence among Swedish males according to the National Quality Register of Hernia Surgery (http://www.incanet.se/Svenskt-Brackregister/). Using the incidence in the quality register to calculate the expected number of inguinal hernias in the study-population given the age specific person-time of follow-up yielded an over-all standardized incidence ratio (SIR) of 2.0 (95% CI 1.4 – 2.8). The highest SIR was observed in the age interval 40-49 years, where 14 cases of inguinal hernia was observed, compared with 5.9 expected (SIR 2.4; 95% CI 1.3-4.0). In the highest age-interval (60-79), two cases of hernia were observed, with 1.9 expected. (Paper III, figure 2)

The role of different types of ART was assessed by use of multivariate Cox' proportional hazards models, one for the outcome midline, and one for inguinal hernias.

The majority of patients who developed a hernia were treated with a PI-containing ART at the time of the event. Such treatment was therefore possible to divide in categories of duration of treatment: less than one year; one to two years; and three years or more. Other treatment-combinations were defined as dichotomous variables.

For both types of hernia, the hazard ratios (HR) for PI-containing ART were highest, and statistically significant, during the second year of treatment. (Table 4) The same pattern was observed in the unadjusted incidence rates. (Table 5)

Table 4. Unadjusted and adjusted Hazard Ratio (HR), of developing midline and inguinal hernia as estimated by Cox' multivariable proportional hazard models.

Midline Hernia (N=29)	N/Cases	HR^{unadj}	95% C.I	HR^{adj}	95% C.I
Age (increments of 10 years)	-	2.26	1.57 - 3.26	1.94	1.30 - 2.90
Male sex	26	2.38	0.72 - 7.88	1.52	0.44 - 5.29
HIV mode of transmission: IV	4	1.30	0.45 - 3.73	2.30	0.73 - 7.21
Central accumulation of fat *or* fat athropy	10	2.05	0.69 - 6.08	1.04	0.31 - 3.58
Diabetes or impaired fasting glucose	8	6.44	2.60 - 15.96	3.85	1.45 - 10.23
Hypercholosterolaemia	17	4.49	1.50 - 13.44	2.78	0.86 - 8.97
ART up 3 months prior to event:					
NRTI only	2	4.88	0.44 - 53.94	3.54	0.32 - 39.44
NRTI + NNRTI [a]	6	10.61	1.25 - 90.21	6.59	0.74 - 58.79
any PI-containing regimen:					
1st year	3	8.34	0.84 - 82.59	6.55	0.66 - 65.42
2nd - 3rd year	12	13.82	1.75 - 108.94	10.66	1.33 - 85.71
4th+ year	5	5.50	0.63 - 48.02	3.63	0.40 - 32.66

Inguinal Hernia (N=34)	N/Cases	HR^{unadj}	95% C.I	HR^{adj}	95% C.I
Age (increments of 10 years)	-	1.71	1.21 - 2.42	1.60	1.10 - 2.31
Male sex	32	4.37	1.05 - 18.24	1.86	0.36 - 9.58
HIV mode of transmission: Homosexual	27	2.99	1.30 - 6.87	2.09	0.81 - 5.42
Diagnosis of AIDS	13	3.09	1.55 - 6.18	2.35	1.16 - 4.77
ART up 3 months prior to event:					
NRTI only	1	0.88	0.09 - 8.45	0.74	0.08 - 7.16
NRTI + NNRTI [a]	5	3.55	0.81 - 15.61	2.16	0.49 - 9.60
any PI-containing regimen:					
1st year	6	5.27	1.28 - 21.76	3.64	0.85 - 15.57
2nd - 3rd year	13	5.96	1.61 - 22.15	4.35	1.14 - 16.61
4th+ year	6	2.44	0.58 - 10.22	1.48	0.35 - 6.29

[a] 10 of 11 (6 midline, 5 inguinal) patients on NRTI+NNRTI at the event were previously (median = 1.5 years) exposed to PI-containing regimens for a duration of 1 – 3.5 years (median 2.7 years) . One patient with inguinal hernia, had never received any PI. BråckTabellerNov2006.xls

Table 5. Total number of person-years and number of cases of midline or inguinal hernia. Corresponding unadjusted incidence rates with 95% exact confidence intervals (CI) in the whole cohort, among treatment naives, among those on therapy interruption and those treated within three months before the event; specification of number of person-years of exposure according to ART regime and duration of PI-containing treatment.

Population	Number of patients contributing person-time	Person-years	Midline hernias		Inguinal hernias	
			Cases	Incidence per 1000 PY (95% C.I.)	Cases	Incidence per 1000 PY (95% C.I.)
Whole cohort	**1072**	**7242**	**29**	**4.0 (2.7 - 5.8)**	**34**	**4.7 (3.3 - 6.6)**
Naïve	674	1434	1	0.7 (0.02 - 3.9)	2	1.4 (0.2 - 5.0)
Therapy interruption	456	590	-		1	1.7 (0.04 - 9.4)
ART-exposed	949	5218	28	5.4 (3.6 - 7.8)	31	5.9 (4.0 - 8.4)
Specification of ART:						
NRTI only	656	892	2	2.2 (0.3 - 8.1)	1	1.1 (0.03 - 6.2)
NRTI + NNRTI	379	765	6	7.8 (2.9 - 17.1)	5	6.5 (2.1 - 15.3)
any PI-containing regimen:						
1st year	882	868	3	3.5 (0.7 - 10.1)	6	6.9 (2.5 - 15.0)
2nd - 3rd year	814	1565	12	7.7 (3.9 - 13.4)	13	8.3 (4.4 - 14.2)
4th+ year	532	1128	5	4.4 (1.4 - 10.3)	6	5.3 (2.0 - 11.6)

Other statistically significant HRs - beside age - were a diagnosis of AIDS (HR 2.4; 95% CI 1.2-4.8) in the analysis of inguinal hernias, and diabetes/impaired fasting glucose (HR 3.8; 95% CI 1.4-10.2) in the analysis of midline hernias.

It can be concluded that inguinal hernias, irrespective of treatment, occur twice as often in HIV-infected men compared to the general population. It can also be concluded with a relatively high degree of certainty that hernias occurred more often in treated patients than in non-treated also when taking into consideration the degree of the underlying disease as indicated by HIV-RNA- and CD4-counts. However, the association between treatment with PI-containing regimens and the development of hernia must be cautiously interpreted: the number of patients with hernia that was treated with non-PI containing regimens was too small to allow for definite assessments of their risks for developing this condition; further, all PI regimens in this study consisted of one or more NRTIs with or without an NNRTI; it was not possible to disentangle the role of the individual drug-classes in these complex treatments.

Paper IV

Suicide attempts are associated with both acne and treatment with isotretinoin. A retrospective Swedish cohort study

Isotretinoin, a retinoic acid, has been used for systemic treatment of severe recalcitrant acne since the early 1980ies. It is however a very teratogenic drug, with a relative risk of 25.6 (95% CI 11.4-57.5) to develop a major malformation after fetal exposure to isotretinoin according to an early study. (Lammer 1985) It has also been established that all therapeutic doses of isotretinoin, even with very short durations of use in the first trimester entail a substantial risk for malformations. (Dai 1992)

Therefore, in Sweden isotretinoin was - and still is in 2010 - only available within a compassionate-use program after special application by specialists in dermatology to the MPA. Prescription of isotretinoin was only granted if tetracyclines had been used for at least six months without improvement of the acne, and if female patients used acceptable methods for contraception.

For the first couple of years, from 1982 to 1986/1987, applications were made individually for each patient to be treated. Beginning in 1986/1987, however, dermatology clinics could apply for permission to prescribe a given quantity of isotretinoin under the condition that the requirements for individual applications were fulfilled. This clinic approval was held in one given pharmacy, the only one at which the patients could fill their prescriptions. Because of this, the MPA could request copies of the lists over patients filling these prescriptions from the pharmacies, and thus practically all treated patients could be identified, regardless of type of application.

In 1988, the pharmacovigilance department at the MPA started identifying all patients granted treatment with isotretinoin from the compassionate-use register, as well as all clinics granted the right to prescribe the drug and the corresponding dispensing pharmacies. The purpose was to evaluate the efficiency of the Swedish system of compassionate use in avoiding exposure in pregnant women and subsequent malformations of the infants. This was done by linking the female patients of the established cohort with the national medical birth registry. The results were encouraging, since only three women were identified as theoretically exposed to istotretinoin during pregnancy, and their infants were born without malformations. (Källén 1999)

Use of isotretinoin has been associated with a number of side-effects. In a recent review, cutaneous, ocular, neurological, musculoskeletal, and hepatic reactions are listed as known side-effects to isotretinoin treatment. (Brelsford 2008) As for psychiatric side-effects, the first report in the literature was published in 1983. (Hazen 1983) In the fol-

lowing years, depression, suicides, suicide attempts and –ideation, and also personality changes have been reported in the literature, mainly as case reports and to spontaneous reporting systems. In a review of the FDA's AERS-database in 2000, isotretinoin ranked in the top-ten of drugs reported as having caused depression, suicide-attempts and -ideation, and suicide. (Wysowski 2001) In a review of spontaneous reports submitted to the Swedish MPA, undertaken by Jokinen (not published) and encompassing the period 1983 – 2004, a total of 422 reports on isotretinoin-related adverse reactions were identified. Of these, 104 were psychiatric and this was the system organ class with the largest number of reports. In 1998, despite more evidence than case-reports, warnings for *e.g.* depression were added in the product information both in Europe and in the USA.

The MPA cohort of 5,756 patients treated with isotretinoin for severe acne was at that time, in the late 1990ies, considered suitable for studying possible psychiatric side-effects, and such a study was initiated by the department of Drug Safety of the MPA.

By use of the unique personal identity number, linkage to the national registers on cause of death and hospitalizations was possible. All deaths in the cohort, as well as all hospitalizations with psychiatric diagnoses were extracted. In the present analysis, deaths by suicide and hospitalizations for suicide attempts were analyzed; hospitalizations with other psychiatric diagnoses will be analyzed in a coming article.

Mortality rates by suicide were evaluated by calculating a standardized mortality ratio (SMR). The observed number of suicides was divided by the expected number, standardized by age, sex and calendar year according to the cause of death register.

The outcome measures regarding suicide attempts were two: one internal comparing the incidence rates of suicide attempts before treatment with the incidence rates during and after end of treatment (a "cohort crossover" approach); one external in which standardized incidence ratios (SIR) were calculated: the observed number of suicide attempts was divided by the expected number standardized by age, sex and calendar year according to the patient register of hospitalizations. The SIRs for suicide attempts were also calculated before, during and after treatment. In this way, no traditional unexposed control-group was identified; rather, a method of self-control was used. This method can be considered the cohort "exposed-only" approach mirroring the "case-only" case crossover study design. (Marcil 2001, Maclure 1991)

The total time of follow-up was 17,197 person-years up to three years before treatment, 2,905 person-years on treatment and 87,120 person-years up to 15 years thereafter.
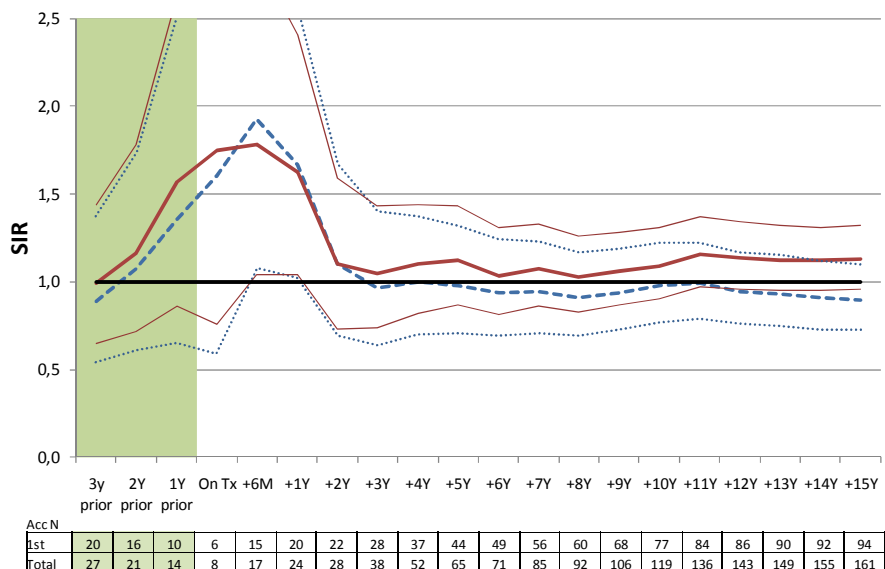
A total of 24 (0.4%) patients committed suicide, 17 (0.5%) men and 7 (0.3%) females during the entire follow-up period. However, it was only three men that committed suicide during or within one year after treatment, yielding an SMR of 1.9 (95% CI 0.4-5.4).

The remaining suicides were committed long after treatment; the first one by a female occurred five years after end of treatment.

During the entire observation period, a total of 128 individuals were hospitalized at 210 occasions for suicide attempt. It was more common among females (N=58, 2.7%) than in males (N=70, 1.9%). Calculations of SIRs and incidence rates were calculated both for first suicide attempts, at which occasion these patient were censored from follow-up, and for repeat events, where censoring only took place in the case of death or end of observation on 31 December 2001. This was of special interest since one of the strongest predictors of suicide attempt and suicide is a previous suicide attempt. (Spirito 2006)

The SIR was observed to be rising above one already during one year before treatment with isotretinoin, even if the risk was not statistically significant until six months after treatment: SIR for first attempts 1.93 (95% CI 1.08-2.18); for all attempts 1.78 (95% CI 1.04 – 2.85) . Within three years after treatment, the SIRs were close to one. (Figure 1)

Figure 1 Total, males + females: Standardized incidence ratios (SIR) for accumulated first attempt of suicides (dashed lines) and total number of attempts (solid lines) from up to three years prior to (shaded area), and up to 15 years after treatment (Tx)

Acc N	3y prior	2Y prior	1Y prior	On Tx	+6M	+1Y	+2Y	+3Y	+4Y	+5Y	+6Y	+7Y	+8Y	+9Y	+10Y	+11Y	+12Y	+13Y	+14Y	+15Y
1st	20	16	10	6	15	20	22	28	37	44	49	56	60	68	77	84	86	90	92	94
Total	27	21	14	8	17	24	28	38	52	65	71	85	92	106	119	136	143	149	155	161

In the internal analysis, the incidence rates of suicide attempts were also rising during the years before treatment, and peaked within six months after treatment. The incidence rate differences and ratios were however not statistically significant. (Figure 2)

Figure 2 Males + Females: incidence rates per 1,000 person-years of follow-up for first attempt of suicides (light grey bars) and total number of attempts (dark grey bars) from up to three years prior to, and up to two years after treatment (Tx). The figures inside the bars are the accumulated number of events.
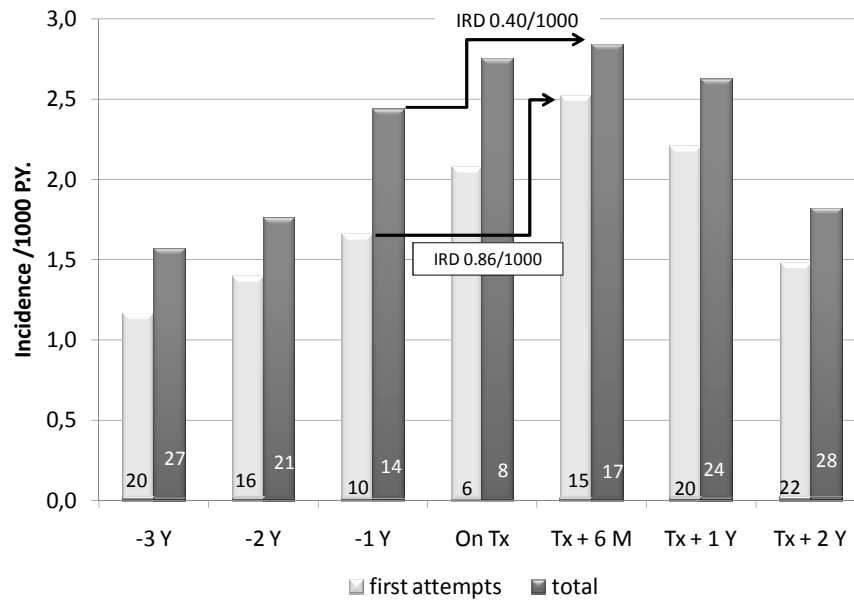
The observation that the incidence rates and SIRs for suicide attempts were rising already before start of treatment with isotretinoin is an indication that the underlying severe acne was an important risk-factor for attempting suicide. The results, however, do not exclude the possibility that some individuals, with an assumed vulnerability to isotretinoin, may have initiated a suicidal behavior due to the treatment.

Methodological aspects

Confounding by indication

With the possible exception of studies on vaccinations in healthy subjects and use of hormonal contraception in healthy women, all observational pharmacoepidemiological studies should consider even the remotest possibility of confounding by indication. All medical treatment is, or at least should be given for a reason – the indication – and that indication may be a risk-factor for the adverse reaction under study. The distinction should be made between "confounding by indication" and "reversed causality" (also called "protopathic bias"). Reversed causality is at hand when the exposure occurs only *after* the debut of the disease or outcome being studied. An example of this is presented in Paper I and is further discussed in another section of the thesis. Confounding by indication, on the other hand, is at hand when the underlying condition for taking the drug is also a risk factor for the reaction that is being studied, but the use of the medication precedes the adverse drug reaction.

It is normally very difficult, some would say impossible in a non-experimental setting, to distinguish the association between the underlying disease and the outcome on the one hand, and the treatment for the disease and the supposed adverse reaction on the other hand.

In paper I this is an issue of importance in the interpretation of the increased relative risk to develop acute pancreatitis in users of acid-suppressing drugs (proton-pump inhibitors and H2-antagonists). Among the indications for use of these drugs are gastritis and GERD, and an increased risk for acute pancreatitis was indeed observed among those suffering from these conditions. It will be stated in a later section of the thesis that a dose-response relationship can be considered a strong indicator of a causal relationship. However, the observed increased risk for the outcome associated with higher doses of acid-suppressing drugs may reflect a more severe gastritis/GERD, and thus the dose-response relationship may reflect a situation of confounding by indication. It was also observed, however, that the relative risk was higher in patients treated with these drugs for less than six months, compared with those who had used it for longer periods. Should the association between treatment and outcome be entirely explained by confounding by indication, one must draw the conclusion that a recent condition of gastritis/GERD would entail a higher risk for developing acute pancreatitis than such a condition over a longer period of time. Intuitively, it may be more reasonable to assume that the relationship resides in the association between new start of a treatment, than in a recent diagnosis, but the results do not allow for a definite conclusion.

In paper II, three drugs – albeit one not with statistically significant ORs - with different pharmacodynamics and spectrums of ADRs were observed to be positively associated with the development of VTE. Even if it's not impossible that these drugs are in fact causally related to VTE, these observations also raise the suspicion of the presence of confounding by indication. An argument in favour of this hypothesis is that a diagnosis of anemia (or an Hb < 11.5 g/L), a possible indication of a more pronounced menorrhagia, was also associated with the development of VTE. Given the complexity of the delicate coagulation cascade, it should therefore not be inconceivable that menorrhagia, in some individuals, is part of one of the many pathways leading to VTE. The association between menorrhagia and laboratory abnormalities in hemostasis has for example been described (Kouides 2007).

In paper III, an association between antiretroviral therapy and hernia of the abdominal wall in HIV-infected patients was observed. It is easily suspected that confounding by indication could explain this observation since HIV-infection is a serious condition, especially if AIDS-events occur.

However, there are at least three arguments indicating that ART entails an increased risk to develop hernias. First, the incidence of hernias in ART-naïve patients was quite near the incidence in the Hernia Quality register, even if only three such cases were observed in the study cohort. (Paper III, Table 2) Secondly, variables indicative of disease severity, *i.e.* duration of HIV-infection, HIV-RNA- and CD4-counts did not confound the association between the exposure and the outcome, be it if the HIV-RNA- and CD4-counts were considered as time-dependent variables, or if highest and lowest, respectively for viral and CD4-counts, were used as indicators of disease severity. Thirdly, there was a relationship between duration of PI-containing ART and the observed HRs for both outcomes.

In paper IV, presence of confounding by indication is not only demonstrated as present, but the extent and magnitude of this confounding could also be assessed. In this paper, the association between treatment with isotretinoin for severe acne and suicide attempts was analyzed. Severe acne is in itself a condition with increased risk for psychiatric disorders, including depression, suicide attempts and –ideation. (Purvis 2006; Magin 2008)

Selecting untreated controls to patients with severe acne was judged difficult given the presumably strong association between the underlying condition and the suspected outcome under study. The solution to this was to consider a self-controlled "exposed only" method: information on the complete history of hospitalizations were available for the treated patients not only during and after treatment, but also before treatment started. The permission to start treatment with isotretinoin was conditional on having attempted con-

ventional treatment with tetracyclines for at least six months without improvement. It follows that for at least six months before being starting treatment with isotretinoin, the patients were in fact suffering from severe acne, but without being exposed to the drug. By establishing the incidence rates of suicide attempts during this time-window, it was possible to estimate the risk for suicide attempts in the presence of severe acne, without isotretinoin exposure. This was then compared with the incidence rates during and after isotretinoin treatment, giving an indication of the magnitude of the possible increase in risk that treatment supposedly entailed.

In order to take into consideration the temporal trends in the incidence rates of suicide attempts in the general population, an external comparison was nevertheless made. By use of age-, sex- and calendar-year specific incidence rates of suicide attempts in the national patient register, the age-, sex- and calendar-year specific expected number of suicide attempts could be calculated, allowing for SIRs to be estimated.

This "cohort crossover" approach can thus be considered a way of identifying and estimating the magnitude of confounding by indication: the SIR estimated immediately before treatment gives an idea of the relative increase in risk in patients who will subsequently cross over from "untreated" to "treated" with the drug under study. By comparing the incidence rates internally in the cohort, before and during/after treatment, the incidence rate difference and incidence rate ratio can be calculated, in principle an estimation of the absolute and relative increase in risk entailed by the addition of the drug to the already present risk due to the underlying disease.

Figures 3 and 4 give schematic illustrations to the cohort crossover approach presenting the internal comparison (figure 3), and the external comparison (figure 4).

Figure 3 Schematic illustration of cohort crossover design, internal comparison. Tx: Treatment

The Cohort Crossover approach: _internally_

Longitudinal data: medications and morbidity

| Underlying disease present | Start - duration of Tx | End of Tx – follow-up |

| Person-time Events → *Pre-Tx Incidence* | Person-time Events → *Tx Incidence* | Person-time Events → *Post-Tx Incidence* |

Incidence rate difference
Incidence rate ratio

The Cohort Crossover approach: *externally*
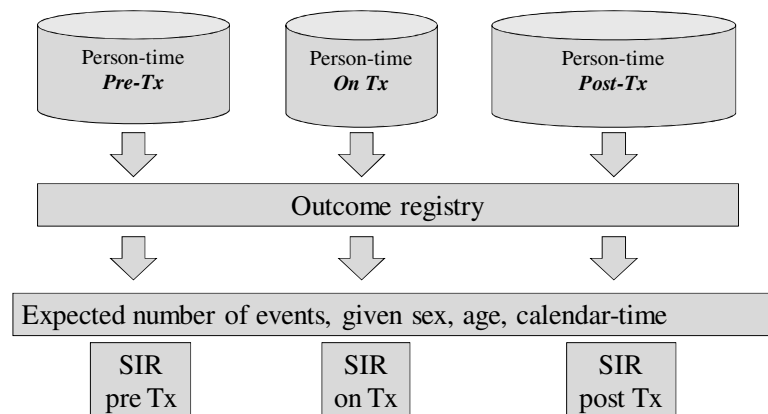
This approach to use "self-controls" - as means of eliminating control-selection bias in observational pharmacoepidemiology - has been widely applied in case-crossover studies (a "case-only" design, as opposed to the "exposed-only" design of the cohort crossover method). It was originally designed to analyse transient exposures such as physical exertion and their associations with acute events, such as myocardial infarctions. (Maclure 1991, Mittleman 1993) Subsequent pharmacoepidemiological studies have used this design, one of which was the first observational study demonstrating a relationship between use of isotretinoin and psychiatric side-effects. (Azoulay 2006) More refined variants of this design have been proposed by *e.g.* Suissa (1995): The "Case-time-control design".

In the basic case-crossover design, the case-event is used as a point of reference regarding exposure preceding the event, and this exposure status is contrasted with one or more time-control windows - *i.e.* periods without presence of the condition under study - are selected for the same individual, and the exposure status in that time-window is assessed. Using a *matched* case-control analysis, an OR can then be calculated.

A brief note on the properties of individually matched analysis in case-control studies: in such analyses, only the discordant pairs contribute to the OR. However, the confidence interval of the OR is also only based on the discordant pairs, in fact, in a crude 4x4 calculation for a matched case-control study, the number of concordant pairs has no effect on the precision expressed as the 95% confidence interval, as illustrated by figures 5 and 6 (Screen dumps from Stata 11, Stata Corporation, Collage Station, TX, USA).

Figure 5. Matched OR: 5 + 5 concordant pairs

Figure 6. Matched OR: 5000 + 5000 concordant pairs

Using register-based data that are easily available, this fact may be less of an issue, but in the case of data-collection by means of *e.g.* interviews or questionnaires, this may lead to some disheartening feelings.

However, the risk estimate obtained in the case crossover design is thus a relative one, and even if the strength of the association is of interest, it is also informative to have an idea of the risk in absolute numbers, *e.g.* with an incidence rate difference and a "Number Needed to Harm" (NNTH). Even if the NNTH is possible to calculate using case-control data (Bjerre 2000), it should be an advantage to use, if available from *e.g.* a large database, the entire experience of all exposed patients identified in a cohort, instead of just those experiencing the adverse event under study.

Further, in a case-crossover analysis, as is the case in "traditional" control-selection, there is a concern on how to select a relevant control-time window; using the entire experience of an identified exposed cohort, this possible source of bias is eliminated. (Maclure 2000)

A crossover design (of cohort type) is relatively often used in controlled trials: according to clinicaltrials.gov (accessed 12 April 2010), there were 25,148 open, interventional phase I-IV studies registered, of which 1,671 (6.6%) had the word "crossover" in the title or description of the trial. However, the cohort crossover design does not seem to have been used very often in observational pharmacoepidemiological studies, even if it is mentioned by Maclure (2000), who notes the possibility of calculating the incidence rate difference as an advantage of this approach. One example of this method has been published by Marcil and Stern, who report a nested cohort crossover study of 28 patients suffering from psoriasis, analysing the frequency of squamous-cell carcinoma before and after first use of ciclosporin. (Marcil 2001)

Another design of the type "exposed-only" was introduced by Hallas: The prescription symmetry sequence analysis. (Hallas 1996) In this design, the sequence of prescription of two drugs or group of drugs is analyzed; if one drug "A" is prescribed more often before drug "B" than the other way around, there may be reason to assume that drug "A" has caused an adverse drug reaction, subsequently identified by the treatment with drug "B". Should there be no association at all between "A" and "B", no difference in prescription sequence should be at hand. In a recent paper, the symmetry analysis has expanded to include the sequence of prescriptions and hospital care events. (Tsiropoulos 2009)

In recent years, the use of propensity score models has been increasing as a method of overcoming the problem of confounding by indication. (Rosenbaum 1983, Arbogast 2009) A detailed presentation of that method is however deemed beyond the scope of this short text.

The cohort crossover design used in the present analysis could possibly, for certain indications, treatments and adverse outcomes, be of further use.

As is the case in Sweden (and most of our Nordic neighbouring countries), the availability of nationwide registers on filled prescriptions, in- and out-patient hospital care, cause of death, cancer *etc.* allows for the analysis of certain events in populations before a given treatment is prescribed.(Wettermark 2007, Furu 2010) If the treated population is large enough, the period immediately preceding the new treatment can give insights into the incidence of diseases and conditions that occur as part of the natural history of the underlying indication for treatment. This natural incidence - the underlying basis for confounding by indication - can then be compared with the incidence following treatment, and possible differences can be assumed to be caused by the addition of treatment more safely than is the case when "healthy" non-exposed controls are used. The setting in paper I is probably a near-ideal one: presence of the severe disease (severe acne) before treatment is certain, and the event studied is an acute one (suicide attempt) that can also be repeated. However, it should be possible to consider an outcome that is of a more chronic character. *E.g.* diabetes is a relatively common condition that can occur in large enough populations before introducing a new treatment making possible an estimation of the "natural" occurrence of diabetes in the patient population to be treated. Use of this method for treatments of indications of a more acute nature may be more problematic since no underlying condition can then be assumed in the period before the new treatment. On the other hand, the characteristics of the population developing this acute condition leading to treatment should be more similar within itself, than compared with untreated or "healthy" controls.

A matter of time I: Dose-response/Duration-response

Generally, a dose-response relationship between exposure and outcome can be considered indicative of a causal relationship. However, absence of such relationship needs not contradict a causal relationship. For example, in the case of allergic reactions, the threshold dose at which such a reaction will occur in a susceptible patient may be so low that it is not possible to establish or even measure. However, when possible, an analysis comparing risk estimates for different doses should be made. In paper I, such difference in dose-response relationship was observed. At daily doses of proton-pump inhibitors and H2-antagagonists less than one DDD, the ORs were considerably lower than for daily doses at one or more DDDs. The distribution of number of daily doses was however narrow and it was only possible to divide the doses in those two categories.

A more substantial example of dose-response relationship was demonstrated by Eland (2006) in an article on antihypertensive medications and acute pancreatitis, from the European Case-Control Study on Drug-Induced Acute Pancreatitis (EDIP). The Swedish study was part of EDIP, and the same interview program was used. The EDIP-protocol was very similar to the Swedish, with some exceptions: patients with previous history of gallstones were included as were those with a previous episode of acute pancreatitis, the controls were individually matched to cases by sex and age, and the lower age-limit was at 40 years of age. In that article, the overall OR for any use of ACE-inhibitors in the week before the index-date was 1.5 (95% CI 1.1-2.2). When the daily dose was divided in three categories, a statistically significant trend of increasing OR was observed with increasing daily doses (OR, 95% CI): < 1 DDD: 1.2 (0.6-2.3); 1-2 DDDs: 1.6 (1.1-2.5); > 2 DDDs: 3.3 (0.8-14.3).

Analysing different doses can be of considerable importance: if the true underlying risk of an outcome only occurs starting at a minimal specific dose, an analysis that lumps together all exposures regardless of dose may lead a dilution of the association, with an underestimation of the risk (and/or possibly a lack of precision with statistically "insignificant" results) as a consequence, and thus a missed or disregarded association. Conversely, a statistically significant increased risk for an exposure that has not been divided in doses may hide a much stronger association for higher doses, and a non-association for lower ones.

The question on duration-response relationships is related to the daily doses, *i.e.* differences in the duration of treatment is a factor to be considered in the same way as daily doses. In fact, in some cases the effect may well be a product of both dose and duration: the duration of treatment is in other words also measure of the total accumulated dose. Depending on the type of reaction and exposure being analysed, the daily dose may actually be of less or no importance, compared with the total longitudinal dose-load.

The question of duration of treatment is possibly a more important factor than the (daily) dose-response relationship as an indicator of a causal relationship, since a higher dose may indicate a more severe underlying condition when confounding by indication is suspected. One can in fact wonder if it is at all possible that the risk for an adverse drug reaction can be the same for a patient treated for several years with a drug, as for one who started treatment just weeks before the ADR. Either, a patient who starts treatment with a new drug has a genetic or other type of predisposition for the ADR, and such patients will never be treated for long periods of time; patients who are treated for long durations simply tolerates the drug – this pattern of difference in risk has been described as "depletion of susceptibles" (Moride 1994), of which the results in paper I and III (decreased risk in

those treated for long durations) are examples. A thorough presentation of various patterns of time-risk relationships is found in Einarson (1997).

Assuming a causal relationship when subjects who are exposed for very varying periods of time are lumped together can probably not always be done. One possible scenario, however, is treatment with warfarin: a patient can be treated with warfarin for a long period of time without problems, but the addition of another drug or the occurrence of an additional disease may disturb the levels of warfarin with large effects on the INR, and hence provoke a bleeding or an ischemic attack. In most cases, however, an association between a treatment and an adverse reaction should definitely be analysed taking duration of treatment into consideration. When there is indeed a pattern of varying risks depending on treatment duration, this would be indicative of a possible causal relationship, also in the case when the risk actually decreases the longer the treatment lasts.

In paper I, the duration of treatment with acid-suppressing drugs was analysed. It was observed that when taking any exposure into consideration, *i.e.* lumping together those who started treatment during the past six months with those who had used proton-pump inhibitors or H2-antagonists for longer periods of time, the ORs were lower, than when distinction was made between new starters and long-time users. (Paper I, Tables 2 and 4) This difference in risk depending on duration of use was interpreted as one indication of a causal relationship between treatment with proton-pump inhibitors and H2-antagonists and the development of acute pancreatitis.

In paper II on the association between treatments for menorrhagia and the development of VTEs, no analyses were possible regarding duration-response patterns for the different treatments. Such analyses could maybe have shed some light on the association between the use of mefenamic acid and the outcome.

In paper III the importance of duration of treatment was also illustrated. When the exposure to protease inhibitors (PI) containing regimens was defined as a dichotomous variable, not taking duration of treatment into consideration, the adjusted HR for this drug group was 7.3 (95% CI 0.96-55.47) and 3.07 (95% CI 0.89 – 10.57) for midline and inguinal hernias respectively. When the treatment duration was divided in groups, a rather clear duration-response pattern can be seen, indicating a stronger and statistically significant association in the second year of treatment, for both outcomes.

If only a dichotomous exposure variable for PI-ART had been used in the analysis, the non-statistically significant HRs may well have led to a decision not to publish, and maybe even not to submit for publication.

In paper IV, neither daily dose, nor duration of single treatment episodes *per se* were identified as important factors. An analysis of the daily prescribed dose is however difficult to make in the case of isotretinoin: the dosage is based on body weight, a variable that was not recorded. Individuals treated with high doses according to body-weight could therefore not be identified. However, the duration of treatment counted as the number of treatment episodes was statistically significantly higher for females that made more than one suicide attempt. Receiving more than one treatment episode is an indication of more severe or at least more difficultly treated acne, and this may be interpreted as a result of confounding by indication, and not a result of a higher accumulated total dose: first, no such difference was noted in male patients, and secondly, there is evidence that the psychiatric effects of acne are more pronounced in females than in males. (Aktan 2000)

A matter of time II: Reversed causality

Reversed causality is sometimes called "protopathic bias", *i.e.* a systematic error in close temporal connection with the disease under study. It can be argued that reversed causality is actually a case of misclassification of exposure, since the alleged exposed case was, in the case of "protopathic bias", exposed only *after* the debut of the disease. The problem of reversed causality can present when the time of the presumed adverse reaction and the exposure of the drug is very close in time, *i.e.* very early symptoms of the disease leads to use of drugs to treat these very symptoms, in which case the outcome precedes the exposure, a true violation of causality for which a quite definitive prerequisite is that the exposure must precede the outcome.

In the case of acute pancreatitis, as presented in paper I, it is shown that reversed causality is probably at hand. In fact, use of locally acting antacids were seen to be associated with developing acute pancreatitis when the index-date – defined by the expert-group as the first date of symptoms of the study subjects suffering from acute pancreatitis – was used as a cut-off when defining the etiologically relevant exposure time-window. Such association was deemed questionable, reason for which the index-date, for both cases and controls, was moved up to three days *before* the defined index-date. When defining the etiologically relevant time-window for exposure to start at the third day before the index-date and the five preceding days, and not at the index-date established by the expert-group and five days before, the association between locally acting antacids and the outcome disappeared. (Table 1, *supra*) The interpretation of this was that reversed causality was at hand: on average, already three days before the assumed first day of symptoms, the cases did actually experience early signs of the pancreatitis to come, and took antacids for that reason. The ORs for the acid-suppressing drug-groups proton-pump inhibitors and H2-

antagonists also decreased when adjusting the index-date, even if they remained statistically significantly associated with the outcome even after that.

At the time of conducting this study, antacids were available without prescription, whilst all proton-pump inhibitors and H2-antagonists were sold on prescription only. This may be an explanation as to why the reversed causality was most clear regarding these OTC-drugs. Presently, in 2010, both proton-pump inhibitors and H2-antagonists are available without prescription, and it may be assumed that a similar study in present days would demonstrate as large effects of reversed causality for these latter drugs as for antacids. In this context, a study subject in the English part of the EDIP study who developed acute pancreatitis should be mentioned here. She stated that her physician had prescribed an H2-antagonist just days before developing the pancreatitis, since "the doctor thought I had an ulcer".

In paper II, an unexpected association between use of mefenamic acid and development of VTE was observed. There is a slight possibility that early signs of a VTE (*e.g.* pain in legs) have led to the GP prescribing mefenamic acid, and a case of reversed causality would then be at hand. This can never be established with certainty in a data source such as the GPRD, since the correlation between date of prescription and date of ingestion of the drug can be unclear.

To sum up: in studies where the natural course of the disease or adverse reaction is very acute and short, the correct timing of use of drugs is of high importance when early symptoms may lead to use of drugs that are actually under study. When this is the case, use of register-data on *e.g.* prescriptions of drugs - as in the GPRD - or dates of dispensing prescriptions - as in the Swedish prescribed drug register (Wettermark 2007) - information on exact timing of taking the drugs cannot be assumed; it is only with difficulty that one can envisage study designs other than personal interviews, and possibly questionnaires, able to ascertain timing of drug-use with an exactitude satisfactory enough to identifying and avoiding reversed causality.

A matter of time III: Recall bias

The problem of recall bias, as identified in paper I, concerns the possibility that cases interviewed *after* a disease episode are more likely to remember use of medications than "healthy" controls, thus introducing a differential misclassification of exposure. Normally this is not an issue in so called retrospective studies using registry-based data, since the information on exposure and subsequent adverse reactions has been collected independently of each other. In fact, true retrospective studies are those where information on ex-

posure was collected *post factum*, as in case-control studies using the study subjects as sources of information. On the contrary, retrospective register-studies, be they of cohort or case-control design, most often use exposure information that has been collected independently of the outcome.

When the information of drug use originates from the study subjects, there is however a possibility that cases, those who have suffered a disease or possible adverse drug reaction, recall use of *e.g.* drugs better than control-subjects that have not suffered such disease. Such recall bias may therefore lead to an over-estimation of risks associated with the exposure.

In paper I, where personal interviews were the main source of information, this was an issue. The possibility of recall bias as a source of error was assessed by estimating the association between use of Vitamin C and loratadine as independent variables in the analyses. Use of these drugs during the six months preceding the index-date yielded ORs very close to one.

Further, the possible effects of recall bias was sought to be diminished by excluding potential cases staying at hospital for more than 30 days; it was assumed that staying in hospital longer than 30 days would make the correctness of drug use prior to admission less accurate. Forty-two of 2,453 (1.7%) potential cases of acute pancreatitis were excluded due to this reason. (Blomgren 2002;1).

Finally, in the publication of Eland *et al* (2006), it was stated that study subjects from the Netherlands were validated regarding use of ACE-inhbitors, by comparing data from the interview with data on dispensing from local pharmacies, and no indications of recall bias was identified.

Recall bias may thus be a source of error when the information originates from the actual study-subjects, and when the information is collected after the event under study. However, by use of "control-exposures", allegedly non associated drug-use, or history of diseases unrelated to the outcome, can be used to assess presence of recall bias. Yet another way to overcome such problems may be to use a control-population that has suffered from other diseases than the one under study. For example, in studies of specific birth defects, controls may be selected among those who have given birth to children with different types of malformations (Lieff 1999). The propensity for correct recall should than be similar in both cases and controls.

Effects of under-reporting and missing data

Life-style factors such as high BMI, use of tobacco and alcohol are often important con-comitant risk-factors or confounders for many diseases. In the nation-wide health regis-tries in Sweden, such data are normally not present. They may however be part of quality registries on specific diseases and conditions; a presentation of the Swedish Quality Reg-isters has been published by the Swedish Association of Local Authorities and Regions (Anonymous 2007). As regards electronic medical charts in primary care, information on these factors may be recorded, but most often as free-text, and hence not easily extracted for analysis. Further, the degree with which primary care physicians in Sweden enter these data in the charts is mostly unknown. When data on life-style factors are indeed recorded, there is still a problem in the timing of recording these data. Habits on smoking and alcohol use change, as well as weight. If these data are only recorded at one, or very few occasions, it may well have changed at the time of establishing associations with an event. Considerable misclassification of exposure may then be at hand, and should be considered during analysis.

In the GPRD, some structured information on smoking, alcohol use and BMI can be found. However, the proportion of patients without such information recorded is high. In paper II, the association between treatment for menorrhagia and subsequent development of venous thromboembolism was investigated using the GPRD. BMI was missing for approximately 20% of both cases and controls; smoking status was unknown for 18% of the cases and 14% of the controls; alcohol use was missing for 25% of the cases and 21% of the controls. (Paper II, Table 1) In a GPRD-based study by Seaman et al (2001) on the association between liver damage and use of minocycline the proportion of study subjects with missing data on alcohol use was: 34.4% of the cases and 36.3% of the controls.

However, even when *e.g.* alcohol use is recorded in the medical records, they are at risk of being underestimations of the actual quantities consumed. This issue of underreporting is of special concern in studies where information is collected from the study subjects, as in interviews or questionnaires.

In paper I, the association between use of acid-suppressing drugs and the development of acute pancreatitis was investigated using personal telephone interviews with cases and controls. In the interview, use of alcohol was assessed separately for beer, wine and liq-uor. The normal frequency of use was requested, and the normal/average number of drinks taken per day at those occasions. This information was transposed to number of grams of alcohol ingested per week. In the analyses, it was only a high weekly consump-tion, ≥ 520 grams per week (≈ 2.3 bottles of 70 centilitres at 40% alcohol by volume) that yielded an adjusted OR with statistical significance: 8.7 (95% CI 2.2-36), and for quanti-

ties ≥720 grams/week (≈3.2 bottles of liquor) the OR increased to 9.1 (95% CI 2.3-1.4). Information on alcohol use was not obtained from 7.6% of the cases and 7.7% of the controls. The quantities of alcohol that was reported by the controls were compared with official data-sources on alcohol use in Sweden, and a good coherence was observed (Blomgren 2002:1)

The possibility of under-reporting on use of alcohol was recognized during the analyses, and instead of making a simple statement on this source of error, with more or less advanced speculations on its effect on the risk estimates, we chose to perform simulations in the study material: what actual level of under-reporting of alcohol use would be needed in order to observe a null association (OR = 1) between use of proton-pump inhibitors and the development of acute pancreatitis? (The simulations were made for this drug group and not for the H2-antagonists, since the number of exposed cases and controls was higher for the former.)

It was only when a 90% under-reporting was assumed (78% users of proton-pump inhibitors were defined as ingesting ≥520 grams of alcohol per week, instead of 7% as obtained from data in the interview), *solely* in cases reporting use of proton-pump inhibitors, and for *no other cases*, that the OR for this drug group was decreased to 1. Such a high and selective degree of under-reporting was judged highly unlikely. However, should it be the case that only 3 out of 16 users of proton-pump inhibitors reported their true alcohol consumption, it follows that the OR for the interaction-term "proton-pump inhibitors and alcohol use ≥520 G/w" would be extremely high. The observed and simulated data are presented in table 6.

Table 6. Observed and simulated data on high consumption of alcohol and use of proton-pump inhibitors (PPI)

Exposure		Number of Cases	Number of Controls	OR$_{unadjusted}$
Alcohol ≥520 G/w, **not** PPI		21	5	17.3
Alcohol ≥520 G/w **and** PPI	Observed	1	1	4.1
	Simulated	*11*	*1*	*45.4*
PPI, Alcohol <520 G/w	Observed	13	13	4.1
	Simulated	*3*	*13*	*1.0*
Alcohol <520 G/w, no PPI		427	1762	reference

What is possibly forgotten when statements such as "the association between drug x and reaction y is most probably due to under-reporting of confounder z", is that exposure to the drug still remains, even if the confounder has been incorrectly measured. As seen in table 1, the cases exposed to proton-pump inhibitors don't disappear, they just move from the category "PPI without alcohol" to the category "PPI with alcohol". Hence the interaction-term high alcohol use together with use of proton-pump inhibitors yields an extremely high OR of 45. Even if the true risk of developing acute pancreatitis is not so extreme for this group, it should in general be of clinical interest if a risk for an adverse reaction can be identified as more relevant in specific groups of patients, such as smokers, obese or users of excessive quantities of alcohol.

Therefore simulations such as the one presented here could be of interest in other studies where missing or under-reported exposures are believed to have effects on observed risk estimates.

Data Sources

In the papers included in this thesis, there were four different types of data sources used. Some remarks on the advantages and disadvantages of these respective methods will be briefly presented.

The Study Subjects – interview or questionnaire

Collecting data from the study subjects by means of interviews or questionnaires may be more or less challenging: it can be a time-consuming project, and hence the cost considerable. The problem of non-participation is also an important issue, since the characteristics of non-participants may differ significantly, in a medical sense, from those ultimately participating.

In the case of personal interviews performed by different study-monitors, the importance of consistency – both between interviewers and over time in specific interviewers – must be emphasized. (Blomgren 2006:3) Compared with questionnaires distributed by mail or filled in on-line, the personal interview nevertheless has the advantage of giving the interview subject the chance to inquire about *e.g.* unclear questions. The credibility of the interview subject can also be – albeit roughly – assessed by the interviewer.

In paper I, analysing the association between use of medicines and the acute pancreatitis, a condition with a rapid course, the use of personal interviews was instrumental in identifying the reversed causality between locally acting antacids and the outcome. (Paper I, Table 5). Using a database with prescriptions (such as the GPRD) or with filled prescriptions (such as the Swedish prescribed drug register) cannot provide data with enough temporal granularity to distinguish actual exposure from one day to another. Other outcomes that thus would need to collect data from the study subjects would be *e.g.* allergic reactions and anaphylactic chocks.

When the interview is performed after a disease-episode, recall bias is a concern; the case is more prone to remember details on things occurring before the disease-episode than "healthy" controls. In the interviews made for the pancreatitis study, in the section on drug-use, the subjects were asked to bring their medical containers and boxes to improve recollection. In the analysis, "control-exposures" such as vitamin C were analysed, and found not to be associated with the outcome.

Database - medical records

The General Practice Research Database, the GPRD, is a widely used database for pharmacoepidemiological research. It holds the medical records from a representative sample of general practitioners in the UK, and currently ≈7% of the population is covered in the database, or 4 million "current" patients, and 9 millions historically. (www.gprd.com, accessed 13 April 2010).

When data was extracted for the study presented in Paper II, the electronic medical charts were the only source of information. Presently, the possibility to link identities in the GPRD to various NHS-registers has been added, such as centrally held death data, full hospitalisation records, disease registers, socio-economic class and other census data to small area level. This means that some, but not all previous disadvantages of analysing GPRD-data have disappeared. If diseases requiring in-hospital care are now identifiable via record-linkage, it is no longer necessary to rely on the treating GPs to record such information in the local medical records, something that was previously the case. Also, identifying deaths in the GPRD was not a straightforward task, something that record-linkage to death data makes easier.

However, the problem still exists, that patients changing GP, because of change of residence or any other reason, are in fact lost to follow-up regarding the regular primary care, especially regarding the prescriptions of drugs. Other similar databases with either primary care data (in the EU) or claims databases in the US supposedly suffer from the same problem of mobility of the source population.

Also, since only prescriptions made by the GPs end up in the medical record, no treatments handled by specialists are likely to be present in the records. For example, the association between use of isotretinoin for severe acne and development of psychiatric side-effects was analyzed using the GPRD. (Jick 2000). In that study, only 346 users of isotretinoin, out of a source population of ≈4 million people during an unknown study period, were identified, reflecting the fact that specialists and not GPs handle such treatments. The ideal association to study using a database such as the GPRD, would thus be one where the drugs are prescribed by the GP; that the induction time is not too long; and preferably that the adverse event is actually diagnosed by the GP him- or herself.

Medical records from specialist care

Manual extraction of information from medical records may, just as interviews and questionnaires, be a costly and logistically challenging task. The necessity of initial training and follow-up of the extractors is as relevant as for personal interviewers. Lack of consistency in extracting and coding data, both between extractors and individually, may result in more or less significant misclassifications. One advantage, however, is that the source information is not interpreted and thus simplified into "one dimensional" codes, which is actually the case in *e.g.* the patient register that only contains discharge codes. Also, the copies of the relevant records may be kept as a source document enabling subsequent validations of extracted data.

In paper III, specially trained specialists and research nurses extracted information on ART, disease activity, metabolic and other lab-values and suspected adverse events. As regards information on exposure to ART, solely data on prescribed treatment was recorded, and not actual use. However, one to two times per year, the patients' treating physician completed a form in which information on *e.g.* "number of missed doses past week" was recorded.

Nation-wide health-care registers

Sweden and the Nordic countries share the advantage - regarding research purposes - of a publicly managed health care system, with individualized, continually updated data on prescribed drugs, in- and out-hospital care, diagnoses of cancer, and causes of death. (Bergman 1992, Wettermark 2007, Furu 2010) Several other register exist on *e.g.* defined chronic and acute conditions, procedures and treatments, so called Quality Registers. (Anonymous 2007) The unique personal identification number used throughout the health-care system allows for linking between these registers. Even if the compliance of the patients in taking their drugs is unknown, data on drug use is more reliable in the Swedish setting in which actual purchases at the pharmacy are registered, and not only the prescriptions as in for example in the GPRD.

The lack of information on life-style factors is however problematic, since many adverse events can be associated with *e.g.* high BMI, smoking habits and use of alcohol. Such data should however be recorded in primary care, but to what extent that is done is not well established. By extracting data from electronic medical charts in primary care centres in well-defined geographical regions, at least some information on these factors can be obtained, and if permission is given, linking such data to other registers is possible.

There are of course challenges in using the nation-wide registers, despite their coverage and richness in data: the tradition in and quality of coding of diagnoses may vary over time and geographically. The quality of care varies in terms of which drugs and treatments are preferred in different regions and in patients with different social and ethnic background. A recent publication from the National Board of Health and Welfare and the Swedish Association of Local Authorities and Regions presents detailed information on regional differences in health-care in Sweden. (Anonymous 2009)

However, one of the most obvious advantages of the Swedish system is the possibility of using an entire country as a cohort, in which practically no "loss to follow-up" can occur, with the exception of those who emigrate. In the future, true long-term - even life-long - follow-up studies of drug use and its positive and negative effects will thus be possible.

Conclusions

In Paper I, we identified statistically significant odds ratios associating the use of proton-pump inhibitors or H2-antagonists with the development of acute pancreatitis. There is a possibility that confounding by indication was present since a diagnosis of gastritis/gastro-esophageal reflux disease (GERD) was also associated with the outcome. However, because of the observation that only short term use (< 6 months) of the drugs was associated with the outcome, and not long term use, it was concluded that a causal association is probable.

The study presented in paper II, on the association between use of tranexamic acid for the treatment of menorrhagia and the risk to develop venous thromboembolism, was inconclusive because of the very few cases and controls exposed to that drug, even if the point estimate (OR 3.2) indicated a possibility of an increased risk. Use of other drugs for menorrhagia, mefenamic acid and norethisterone, were however statistically significantly associated with the development of VTE, as was a diagnosis of anaemia. Because of the latter observation, and because all drugs studied were associated with the outcome, it was concluded that menorrhagia in itself may possibly constitute a risk factor for VTE.

In paper III, increased risks to develop inguinal and midline hernias were observed in patients treated with protease-containing antiretroviral treatment, but this risk was restricted to the second and third year of treatment. By comparing the observed number of male patients developing inguinal hernia with the expected number, given the sex- and age-specific incidence in the general population, it was shown that inguinal hernia was twice as common in the study population, regardless of treatment status or -duration.

Finally, the association between use of isotretinoin for severe acne and the risk for suicide attempts was analysed in paper IV. It was concluded that the underlying condition, severe acne, was associated with an increased risk for suicide attempts since the SIR, standardized by age- sex- and calendar-year, was observed to be rising already during two years prior to isotretinoin treatment. The SIR was highest within six months after treatment, and within three years after treatment, the SIR was close to one. On the population level, the results thus suggested that isotretinoin does probably not entail a significant risk for suicide attempts in addition to the risk attributed to the underlying disease. On the individual level, however, it cannot be excluded that certain individuals, supposedly sensitive to isotretinoin, reacted negatively to the treatment in a way leading to a suicide attempt.

As regards methodological issues, confounding by indication was a potential source of error in all four papers; in two of them it could be challenged (paper I and III); in one, the suspicion of its presence indicated menorrhagia as a new potential risk-factor for the de-

velopment of venous thromboembolism (paper II), and in one case (paper IV), the observed increase in risk for suicide attempts was interpreted as mainly caused by the underlying disease.

The importance of analysing dose-response and duration-response patterns was illustrated in paper I and III, in which the presence of these patterns were judged as indicators in support of an interpretation of causal relationships between exposure and the outcome.

The presence of reversed causality was clearly illustrated in paper I, and a method that probably eliminated this source of error was presented.

Recall bias, the risk for differential misclassification of exposure due to better recollection of ingestion of drugs by cases as compared to controls in a retrospective interview setting, was identified as a possible source of error in paper I, but its presence was successfully challenged.

In order to estimate the importance and consequences of a presumed underestimation of reported alcohol use in paper I, a simulation was used that showed what extent of differential underreporting of alcohol use was actually needed for the association between exposure and outcome to disappear.

Finally, the different data-sources used in the four studies were discussed; the importance of selecting sources of information that contain adequate data for the assessment of the associations to be studied were demonstrated and emphasized.

Acknowledgements

References

Aktan, S., Ozmen, E. and Sanli, B. (2000). "Anxiety, depression, and nature of acne vulgaris in adolescents." Int J Dermatol **39**(5): 354-357.

Anonymous (2007). National Healthcare Quality Registries in Sweden. Stockholm, Swedish Association of Local Authorities and Regions.

Anonymous (2009). Öppna jämförelser av hälso- och sjukvårdens kvalitet och effektivitet. Stockholm, Sveriges Kommuner och Landsting & Socialstyrelsen.

Appelros, S. and Borgström, A. (1999). "Incidence, aetiology and mortality rate of acute pancreatitis over 10 years in a defined urban population in Sweden." Br J Surg **86**(4): 465-470.

Arbogast, P. G. and Ray, W. A. (2009). "Use of disease risk scores in pharmacoepidemiologic studies." Stat Methods Med Res **18**(1): 67-80.

Azoulay, L., Oraichi, D. and Berard, A. (2006). "Patterns and utilization of isotretinoin for acne from 1984 to 2003: is there need for concern?" Eur J Clin Pharmacol **62**(8): 667-674.

Becquemont, L. (2009). "Pharmacogenomics of adverse drug reactions: practical applications and perspectives." Pharmacogenomics **10**(6): 961-969.

Bergman, U. (1992). "Pharmacoepidemiological perspectives." Journal of Clinical Epidemiology **45**(3): 313-317.

Berntorp, E., Follrud, C. and Lethagen, S. (2001). "No increased risk of venous thrombosis in women taking tranexamic acid." Thromb Haemost **86**(2): 714-715.

Bjerre, L. M. and LeLorier, J. (2000). "Expressing the magnitude of adverse effects in case-control studies: "the number of patients needed to be treated for one additional patient to be harmed"." BMJ **320**(7233): 503-506.

Blomgren, K. J., Sundström, A., Steineck, G., Genell, S., Sjöstedt, S. and Wiholm, B. E. (2002). "A Swedish case-control network for studies of drug-induced morbidity--acute pancreatitis." Eur J Clin Pharmacol **58**(4): 275-283.

Blomgren, K. J., Sundström, A., Steineck, G. and Wiholm, B. E. (2002). "Obesity and treatment of diabetes with glyburide may both be risk factors for acute pancreatitis." Diabetes Care **25**(2): 298-302.

Blomgren, K. J., Sundström, A., Steineck, G. and Wiholm, B. E. (2006). "Interviewer variability - quality aspects in a case-control study." Eur J Epidemiol **21**(4): 267-277.

Bourke, J. B., McIllmurray, M. B., Mead, G. M. and Langman, M. J. (1978). "Drug-associated primary acute pancreatitis." Lancet **1**(8066): 706-708.

Brelsford, M. and Beute, T. C. (2008). "Preventing and managing the side effects of iso-tretinoin." Semin Cutan Med Surg **27**(3): 197-206.

Corfield, A. P., Cooper, M. J. and Williamson, R. C. (1985). "Acute pancreatitis: a lethal disease of increasing incidence." Gut **26**(7): 724-729.

Dai, W. S., LaBraico, J. M. and Stern, R. S. (1992). "Epidemiology of isotretinoin expo-sure during pregnancy." J Am Acad Dermatol **26**(4): 599-606.

Einarson, T., Bergman, U. and Wiholm, B. (1997). Principles and practice of pharmacoe-pidemiology. In: Drug Treatment. Speight, T. and Holford, N (Eds). Auckland, Adis International Ltd**:** 371--392.

Eland, I. A., Alvarez, C. H., Stricker, B. H. and Rodriguez, L. A. (2000). "The risk of acute pancreatitis associated with acid-suppressing drugs." Br J Clin Pharmacol **49**(5): 473-478.

Eland, I. A., Sundström, A., Velo, G. P., Andersen, M., Sturkenboom, M. C., Langman, M. J., Stricker, B. H., and Wiholm, B.E. (2006). "Antihypertensive medication and the risk of acute pancreatitis: the European case-control study on drug-induced acute pancreatitis (EDIP)." Scand J Gastroenterol **41**(12): 1484-1490.

Furu, K., Wettermark, B., Andersen, M., Martikainen, J. E., Almarsdottir, A. B. and Sørensen, H. T. (2010). "The Nordic countries as a cohort for pharmacoepidemio-logical research." Basic Clin Pharmacol Toxicol **106**(2): 86-94.

Hallas, J. (1996). "Evidence of depression provoked by cardiovascular medication: a pre-scription sequence symmetry analysis." Epidemiology **7**(5): 478-484.

Hazen, P. G., Carney, J. F., Walker, A. E. and Stewart, J. J. (1983). "Depression--a side effect of 13-cis-retinoic acid therapy." J Am Acad Dermatol **9**(2): 278-279.

Jaakkola, M. and Nordback, I. (1993). "Pancreatitis in Finland between 1970 and 1989." Gut **34**(9): 1255-1260.

Jick, S. S., Kremers, H. M. and Vasilakis-Scaramozza, C. (2000). "Isotretinoin use and risk of depression, psychotic symptoms, suicide, and attempted suicide." Arch Dermatol **136**(10): 1231-1236.

Källén, B. (1999). "Restriction of the use of drugs with teratogenic properties: Swedish experiences with isotretinoin." Teratology **60**(2): 53.

Kimmel, S., Leufkens, H. and Rebbeck, T. (2005). Molecular Pharmacoepidemiology. In: Pharmacoepidemiology. Strom, B. L. (ed). Chichester, John Wiley & Sons, Ltd**:** 571-586.

Kouides, P. A. and Kadir, R. A. (2007). "Menorrhagia associated with laboratory abnor-malities of hemostasis: epidemiological, diagnostic and therapeutic aspects." J Thromb Haemost **5 Suppl 1**: 175-182.

Lammer, E. J., Chen, D. T., Hoar, R. M., Agnish, N. D., Benke, P. J., Braun, J. T., Curry, C. J., Fernhoff, P. M., Grix, A. W., Jr., Lott, I. T. and et al. (1985). "Retinoic acid embryopathy." N Engl J Med **313**(14): 837-841.

Lieff, S., Olshan, A. F., Werler, M., Savitz, D. A. and Mitchell, A. A. (1999). "Selection bias and the use of controls with malformations in case-control studies of birth defects." Epidemiology **10**(3): 238-241.

Maclure, M. (1991). "The case-crossover design: a method for studying transient effects on the risk of acute events." Am J Epidemiol **133**(2): 144-153.

Maclure, M. and Mittleman, M. A. (2000). "Should we use a case-crossover design?" Annu Rev Public Health **21**: 193-221.

Magin, P. J., Pond, C. D., Smith, W. T., Watson, A. B. and Goode, S. M. (2008). "A cross-sectional study of psychological morbidity in patients with acne, psoriasis and atopic dermatitis in specialist dermatology and general practices." J Eur Acad Dermatol Venereol **22**(12): 1435-1444.

Marcil, I. and Stern, R. S. (2001). "Squamous-cell cancer of the skin in patients given PUVA and ciclosporin: nested cohort crossover study." Lancet **358**(9287): 1042-1045.

McBride, W. G. (1961). "THALIDOMIDE AND CONGENITAL ABNORMALITIES." The Lancet **278**(7216): 1358-1358.

McGuire, S. (1996). "Saquinavir gets accelerated approval." Posit Aware **7**(1): 8.

McKay, C. J., Evans, S., Sinclair, M., Carter, C. R. and Imrie, C. W. (1999). "High early mortality rate from acute pancreatitis in Scotland, 1984-1995." Br J Surg **86**(10): 1302-1305.

Mittleman, M. A., Maclure, M., Tofler, G. H., Sherwood, J. B., Goldberg, R. J. and Muller, J. E. (1993). "Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators." N Engl J Med **329**(23): 1677-1683.

Moride, Y. and Abenhaim, L. (1994). "Evidence of the depletion of susceptibles effect in non-experimental pharmacoepidemiologic research." J Clin Epidemiol **47**(7): 731-737.

Nelson, R. (2006). "AIDS treatment enters its 25th year." The Lancet Infectious Diseases **6**(8): 466-477.

Nitsche, C. J., Jamieson, N., Lerch, M. M. and Mayerle, J. V. (2010). "Drug induced pancreatitis." Best Pract Res Clin Gastroenterol **24**(2): 143-155.

Nolan, D., Reiss, P. and Mallal, S. (2005). "Adverse effects of antiretroviral therapy for HIV infection: a review of selected topics." Expert Opin Drug Saf **4**(2): 201-218.

46

Purvis, D., Robinson, E., Merry, S. and Watson, P. (2006). "Acne, anxiety, depression and suicide in teenagers: a cross-sectional survey of New Zealand secondary school students." J Paediatr Child Health **42**(12): 793-796.

Reisler, R. B., Han, C., Burman, W. J., Tedaldi, E. M. and Neaton, J. D. (2003). "Grade 4 events are as important as AIDS events in the era of HAART." J Acquir Immune Defic Syndr **34**(4): 379-386.

Rosenbaum, P. R. and Rubin, D. B. (1983). "The central role of the propensity score in observational studies for causal effects." Biometrika **70**(1): 41-55.

Seaman, H. E., Lawrenson, R. A., Williams, T. J., MacRae, K. D. and Farmer, R. D. (2001). "The risk of liver damage associated with minocycline: a comparative study." J Clin Pharmacol **41**(8): 852-860.

Spirito, A. and Esposito-Smythers, C. (2006). "Attempted and completed suicide in adolescence." Annu Rev Clin Psychol **2**: 237-266.

Spitzer, W. O., Lewis, M. A., Heinemann, L. A., Thorogood, M. and MacRae, K. D. (1996). "Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. Transnational Research Group on Oral Contraceptives and the Health of Young Women." BMJ **312**(7023): 83-88.

Suissa, S. (1995). "The case-time-control design." Epidemiology **6**(3): 248-253.

Taparia, M., Cordingley, F. T. and Leahy, M. F. (2002). "Pulmonary embolism associated with tranexamic acid in severe acquired haemophilia." Eur J Haematol **68**(5): 307-309.

Tran, D. and van Schilfgarde, R. (1994). "Prevalence and mortality from acute pancreatitis in the Netherlands during 1971 - 1990." Digestion **55**(5): 342-343.

Tsiropoulos, I., Andersen, M. and Hallas, J. (2009). "Adverse events with use of antiepileptic drugs: a prescription and event symmetry analysis." Pharmacoepidemiol Drug Saf **18**(6): 483-491.

Wettermark, B., Hammar, N., Fored, C. M., Leimanis, A., Otterblad Olausson, P., Bergman, U., Persson, I., Sundström, A., Westerholm, B. and Rosén, M. (2007). "The new Swedish Prescribed Drug Register--opportunities for pharmacoepidemiological research and experience from the first six months." Pharmacoepidemiol Drug Saf **16**(7): 726-735.

Woo, K. S., Tse, L. K., Woo, J. L. and Vallance-Owen, J. (1989). "Massive pulmonary thromboembolism after tranexamic acid antifibrinolytic therapy." Br J Clin Pract **43**(12): 465-466.

Wysowski, D. K., Pitts, M. and Beitz, J. (2001). "An analysis of reports of depression and suicide in patients treated with isotretinoin." Journal of the American Academy of Dermatology **45**(4): 515-519.