APPENDICES *with paper:* From ideas to studies: how to get ideas and sharpen them into research questions

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APPENDIX A: Examples

Example A1: Clinical observations lead to a genomic 'breakthrough': mitochondrial or maternal diabetes

A 1992 breakthrough in understanding some forms of diabetes, which was reported as a triumph of genomics, was actually based on careful clinical observation. A diabetologist was consulted by a young woman with diabetes who wished to start a family, but who was worried because she and all her eight siblings had developed diabetes and several had hearing problems. The diabetologist made a rudimentary family tree and showed it to a colleague – the colleague offered, quite tentatively, the opinion that there were rare forms of cytoplasmatic (mitochondrial) inheritance wherein all children of a mother would develop a disease (as the mitochondria in human cytoplasm are inherited almost solely from mothers). Intrigued, the diabetologist went on to make a full family tree and the cytoplasmatic (i.e., maternal, mitochondrial) inheritance became clear. This led to genetic investigations on the mitochondrial DNA in the family, and to the discovery of a 'new' cause of diabetes (which might be as old as the human genome in its present form). Subsequent to this publication[1] and others, there was an 'epidemic' of newly discovered patients with mitochondrial diabetes in the literature – even if the mutations that are the culprits are probably tens of thousands of years old.

Example A2 : *Observation of adverse effects in patients leads to study the effect of usual practices: anticoagulation with chronic hemofiltration.*

A resident in internal medicine wanted to do a research project, but had no predefined idea. He was asked by one of us [JPV] whether he recalled any patients whose disease course had been unusual, and whether he would like to do to research on what had happened. He had just spent a rotation on the intensive care unit, and had witnessed several patients who bled severely during continuous haemofiltration – a procedure used for acute kidney failure; the procedure requires continuous anticoagulation by heparin.

The resident wondered whether anything went wrong with the heparinisation procedure in these patients and whether he could study this. We asked whether it would be possible to identify all patients on haemofiltration, and also those who had bled severely. This was possible by using the nursing logs at the intensive care unit. An epidemiologic staff member with experience in intensive care proposed a design based on person-time calculations. The activated Partial Prothromblastine Time (aPTT) which monitors the degree of anticoagulation was measured every six hours during the procedure, possibly followed by an adaptation of the administered heparin dose. Calculating the person-hours that patients spend on different aPTT levels would permit calculation of the incidence rate of severe bleeding per number of patient-hours at different aPTT levels. Also, the flip-side could be investigated: if the patients were not sufficiently anticoagulated, the haemofiltration filters might clot. A U-shaped curve emerged:

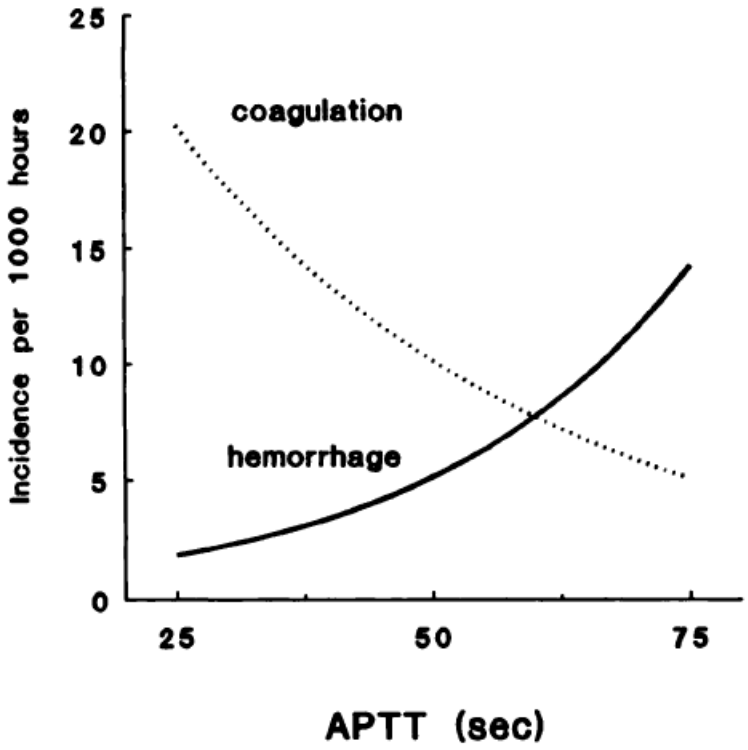

Figure 3. Incidence rates of patient hemorrhage and filter survival time in patients treated with CAVH/DF. Incidence rates were estimated using a Cox's proportional hazards model adjusted for the type of filter, mean arterial blood pressure, decreases in blood pressure, and platelet count.

FIGURE from reference [2]

It showed on the one side an increased incidence of bleeding, and on the other an increase in clotting of the hemofiltration filters, with an optimum level of anticoagulation in between.

Example A3: Challenge received wisdom: NSAIDs, ABO blood group and bleeding peptic ulcers

Ever since the discovery of Helicobacter pylori (*H. pylori*), it has become fashionable to state that this discovery has wiped out all old myths about peptic ulcer: that gastro-duodenal ulcers are due to stress, alcohol use, personality type etc. Peptic ulcer is now seen as an infectious disease that can be eradicated once and for all by proper antibiotic treatment. Apparently, *H. pylori* is a necessary cause for peptic ulcers. However, it cannot be a sufficient cause: at least one third or more of the adult population, also in industrialised countries, is infected with Helicobacter - yet, not all these persons develop peptic ulcers! Clearly, some additional causes are necessary. This idea led to the question whether there might be some truth in the 'old myths'. For example, in the 1970s it was noted that ABO blood group was linked with peptic ulcer disease. People with blood group 0 would develop bleeding peptic ulcers more often (perhaps because persons with blood group 0 have lower levels of coagulation factor VIII). Small but consistent relative risks were found.

One resident in internal medicine set out to study whether this still would be true, and whether having blood group 0 would be an additional risk factor in the causation of bleeding peptic ulcer among people carrying *H. pylori* and/or using NSAIDs. The project involved listing a case series of all patients admitted with bleeding peptic ulcers during one time period at the Leiden University Hospital (excluding those with obvious other causes like anticoagulation, etc.). The frequency of the various blood groups, NSAIDs use, and *H. pylori* among those patients was compared with the known frequencies in the general population in the Netherlands (assuming that the general population was the source population of the patients with acute gastro-intestinal bleeding, which is a reasonable assumption because it is an emergency leading to admission to the nearest hospital). In this way, combinations of causes that were present in the patients could be examined, because the expected combinations of these causes in the

general population can be calculated under the (likely) assumption that they are independent.

NSAIDs use had the strongest association with bleeding peptic ulcers. NSAID use and H. pylori infection seemed to be separate risk factors and did not really potentiate each other's effect. Moreover, blood group O did not potentiate the strong effect of NSAIDs. It was concluded that H. pylori infection may add only a little to the important risk of NSAID use in the development of bleeding peptic ulcers.[3]

Example A4: How much should you read? The role of literature. Pregnancy as a risk factor for aplastic anaemia

At the Leiden University Medical Center, where one of us worked [JPV], residents in internal medicine were allowed to spend one training period of 4 months in the Department of Clinical Epidemiology. Usually, they did a small research project of their own. This always greatly taxed our ability to develop a good research project. One resident who wanted to continue training in haematology was told by her future clinical supervisors that she might “do something” about aplastic anaemia — as there was a list of patients with that disease at the Leiden University Hospital (something like a box of cards with names on them). She was told that perhaps she might study something about prognosis or therapy. She plunged into the literature, and found thousands of references on “aplastic anaemia, prognosis, therapy” and felt discouraged: how could she ever add anything to the literature?

We focussed on a recent review paper, which we read in detail to see whether there might be unsolved issues that might be researched by a patient series. Pregnancy was listed as a possible cause, but with a question mark. Upon reading more about this ‘pregnancy association’, we learned that ever since the original description of aplastic anaemia by Ehrlich in 1888 in a pregnant woman, pregnancy was always listed as one possible cause of aplastic anaemia - but over the years doubts had been growing because the combination of aplastic anaemia and pregnancy was not seen very frequently.

From the patient series present at Leiden, all women of reproductive age were identified, and it was checked whether they were pregnant at the time (or in the months before) the diagnosis of aplastic anaemia. By way of comparison, it could be calculated easily (from birth rate and length of pregnancy) how many ‘prevalently’ pregnant women one might expect in a ‘random’ group of women in the Netherlands. Although the numbers in the case series were small, some 35

women of reproductive age, the observed number of pregnancies (1 woman developed the disease during pregnancy, 1 other immediately after pregnancy) was well within range of the expected of roughly 4.4%. When we went back to the literature, we found that this was only the second patient series ever to have looked at the problem systematically (the other series had identical results and was of similar magnitude). Thus, the vague overall idea of “doing something” with the patient series, was narrowed down to a solvable question about a problem that had been lingering in the literature for more than a century—in a matter of a few weeks of work.[4]

Example A5: A discovery in the data: Factor V Leiden and Oral Contraceptives;

The discovery that young women who carry the factor V Leiden (FVL) mutation and at the same time use oral contraceptives, are at a much higher risk of venous thrombosis than women with either risk factor alone, was not at all pre-planned. It was made by examining the data of an ongoing case-control study on hereditary and other risk factors for venous thrombosis.

The background of this study is recounted as example 7. As described in example 7, the FVL mutation was discovered in the midst of an ongoing case control study. The FVL mutation leads to a coagulation abnormality (APC resistance) and increases the risk of venous thrombosis.

Once factor V Leiden was accepted as a cause of APC resistance that leads frequently to venous thrombosis, it was determined in all patients in the case control study. While heterozygosity for the factor V Leiden mutation abounded amongst the patients with thrombosis (see example 7), also a handful of patients with thrombosis was found who were *homozygous* carriers of the mutation. Quite surprisingly, almost all of the homozygotes were young women – even if the case-control study contained patients of both sexes and all ages. There was no reason why a collection of consecutive patients with venous thrombosis, in whom the presence of a hitherto unknown mutation on an autosomal chromosome was determined, would show a predominance of homozygotes among the younger women. This struck one of us [JPV] because, ever since the 1970s several epidemiologic studies had shown quite a strong association between the use of oral contraceptives and the risk of venous thrombosis – but doubt remained because there was no clearcut mechanism; older studies on coagulation effects of oral contraceptives had only shown minor changes. It was found that most of these homozygous women used oral contraceptives. Because of this new observation in a very small part of the data, this was taken as the starting point for an analysis about the interaction between oral contraceptives and FVL in the development of venous thrombosis – combining homozygotes and heterozygotes (there were many more

homozygotes).

It was found that oral contraceptives increased the risk of venous thrombosis about 4-fold, factor V Leiden about 7-fold, but that the joint effect was a more than 30-fold increase.[5] The following table was adapted from a rearrangement of the tables of the original paper, which was proposed as a teaching example by Botto and Khoury [6].

Factor V†	OC†	Cases	Controls		OR†
+	+	25	2	ORge†	34.7
+	–	10	4	ORg†	6.9
–	+	84	63	ORe†	3.7
–	–	36	100	Reference	Reference
Total		155	169		

In retrospect it seems quite likely that the life-long preoccupation of one of the lead investigators [JPV] with adverse effects of drugs, and his knowledge about the adverse effects of oral contraceptives led him to ‘see’ the possibility that the strange clustering of homozygosity amongst young women might be due to oral contraceptives. Personal interests of scientists colour what they ‘see’ and subsequently discover.

Example A6: Variation over time and place: Asthma in Poland

An analysis of time trends in asthma prevalence in Poland, following its accession to the European Union, has led to striking new findings which call into question the established theories of asthma causation. Farming environments may protect against atopy, asthma and hay fever. In 2003 Sozanska et al.[7] measured a striking, age-related difference in the prevalence of atopy between village and small-town populations in south west Poland. Nine years later, the same group undertook a second cross-sectional survey of the same area to assess whether rapid changes in farming practices, driven by accession to the European Union in 2004, would be reflected in an increase in atopy, asthma and hay fever in these villages. Between 2003 and 2012, the introduction of the Common Agricultural Policy (CAP) resulted directly in a major shift in village farming practices, so that the keeping of large animals, in particular cows, became uneconomical and virtually disappeared. The study found a three-fold increase in the prevalence of atopy in small Polish villages in this nine-year period. These changes were, in part, expected because of priori knowledge about the possible protective effects of farming exposures on asthma and atopy. What was not entirely expected was that these changes happened across all age-groups, in contradiction to the established dogma that the first few years of life are crucial for the causation of asthma and allergy. Thus, these findings are important not only in terms of the specific exposures, but also for challenging the dogma that the first few years of life are crucial in terms of asthma risk.[8] It appears that, instead, the first few years of a new exposure are probably crucial, and that this could involve the first few years of life, the first few years in a new environment (e.g. a new country, a new job), or the first few years following a change in the environment (as happened in Poland). See also related Example 8.

Example A7. Imagination Roaming: Hereditary causes of venous thrombosis.

A major research undertaking in one of our departments (JPV) was motivated by being scientifically challenged - and being angry about the challenge. Rare hereditary deficiencies in protein C, S and antithrombin that lead to familial venous thrombosis were an important subject of the Leiden Haemostasis and Thrombosis research unit at the University Hospital of Leiden, The Netherlands. Patients with the presumption of a familial venous thrombosis syndrome (venous thromboembolism occurring at young ages, without provoking general medical conditions, and in several family members) were referred from far and wide in the Netherlands to the Leiden clinic. In 1987, the importance of the genetic abnormalities was challenged when a paper was published in the NEJM by a leading group of US haematologists, who expressed doubt about the importance of several of these deficiencies. [9] In a large group of blood donors, the US authors had found a rather large number of people with these coagulation deficiencies who had never experienced venous thrombosis. This publication seemed to challenge the importance of the work of the Leiden people (and others) who had devoted their lives to work on these mutations. A first reaction was that something in the challenge must be wrong, but what? And how could we prove this? The Leiden haematology researchers wondered whether they should do a similar survey among blood donors or in the general population.

When JPV was consulted (who had at that time just taken up the chair of clinical epidemiology at Leiden)[10] he responded to his Leiden colleagues that they should stop collecting ‘rare postal stamps’. Rather than conducting research with patients referred from far and wide, JPV proposed to set up a case-control study with successive patients as they presented in daily practice, with an accompanying control group from the population – and to determine the presence of the coagulation abnormalities in which the researchers were interested in *both* groups. In itself, using this design was a major innovation within this branch of coagulation research at the time it was proposed. The

research was set up in intensive cooperation with clinicians and basic scientists.

The reason for pursuing the matter with this design was that the NEJM authors had a point: what is the importance of these abnormalities outside of rare families? To know that for sure, you need to study consecutive patients with venous thrombosis, as they enter the hospital, one after the other, irrespective of their family history, and compare with general population controls. The difference in coagulation abnormalities between the consecutive cases and the population will tell the impact of these abnormalities in the causation of venous thrombosis.

Thinking along these lines also made it immediately obvious that blood donor surveys will not help. Blood donors are selected for being healthy, and might thus include precisely those people who did not develop venous thrombosis despite their haemostatic abnormality. Indeed, one does not expect a one-to-one correspondence between having the mutation and developing thrombosis (that the correspondence was not one-to-one was already known from the family studies). In a certain sense, blood donors might be better suited as a control group in a case-control study (to estimate how frequent the mutation is in the population). Likewise, a survey in the general population will not tell you what the relation is with venous thrombosis, as you will have to rely on the memory of people of previous venous thromboses.

Thus, a population based case-control study was started to determine the impact of protein C, S and antithrombin deficiencies on venous thrombosis. Half-way the study, however, a new hereditary cause of venous thrombosis was detected by Björn Dahlbäck in Sweden, called “Activated Protein C resistance”.[11] The Leiden researchers were the only ones who could immediately study this new abnormality in consecutive cases in an epidemiologic study. A few months after the Swedish discovery, they published that this new hereditary cause was not simply an oddity in rare families, but that no less than one in five of all patients with venous thrombosis carried this abnormality.[12] Afterwards, the Leiden group determined the genetic basis of the mutation which came to be known as

“Factor V Leiden”.[13, 14] Roughly 5 per cent of Caucasians carry the mutation, and it increases the risk of venous thrombosis five to twentyfold – depending on the presence of other risk factors. (See also Example 5 about the presence of one other risk factor, oral contraceptive use). In the aftermath of these exciting discoveries, the original aims of the project were almost forgotten, and the publications about protein C, S and antithrombin came much later.

Example A8: Imagination roaming: Protective effects of farming on asthma

Bacterial endotoxin exposure in childhood is associated with a reduced risk for allergy and asthma. However, although commonly assumed, it is not clear whether these effects only occur in *early life* (see related example 6). In fact, some recent epidemiological studies suggest that “immune deviation” may take place throughout life. If endotoxin exposure *later in life* also has the potential to shift the Th_1/Th_2 balance (favouring Th_1 white blood cells that lead to delayed immunity, and inhibiting atopic Th_2 immunity that leads to immediate allergy) then the protective effect would not be limited to children. In principle, these exposures may then even *reverse* atopy and any related atopic diseases such as allergic asthma, hay fever, and eczema. This is also relevant to studies which show that growing up on a farm may protect against the development of asthma and atopy, and that adult farming exposures may even reverse atopic status.[15] Farmers are a difficult group to study because they have such a wide range of exposures. Endotoxin is of particular interest, but it is ‘mixed in’ with many other exposures in farmers. Thus, Douwes et al decided to study whether endotoxin exposure *later in life* is associated with a reduction in atopy.[16] They did this in a prospective cohort of previously unexposed allergic adults who are starting a work career in chicken production facilities. These involve high endotoxin exposure, but avoid some of the other exposures involved in farming. The hypothesis is that endotoxin exposure could not only protect against atopy in new non-atopic chicken workers, but could also reverse atopic status in new workers who are already atopic.

Example A9. Innovative solutions: getting deeply immersed in the problem.

To think of innovative solutions to solve a problem demands to be acquainted with a problem in depth: to understand how it occurs, what the background biochemistry and physiology is (as far as known), how it is seen in the general population, in different communities, how it is seen in health care – as well as a thorough understanding of epidemiologic principles of bias and confounding.

One way of elucidating understanding is by *'triangulation'* of research findings.[17] In a dispute about the effects of breast feeding, and the possibility that any effect in affluent societies was linked to the education of the mother (mothers with higher education do breastfeed more often in affluent societies), a 'triangulation' comparison was made by looking at the same research question in a less affluent society where the richer women paid themselves the luxury of buying easy to prepare infant feeding so as to avoid breast feeding that was still very prevalent among the poor.[18]

Another example of triangulation is the use of 'negative controls' Two main types of negative controls are exposure controls and outcome controls.[19] An example was the finding of an association of smoking habits with pregnancy outcomes. It might be argued that this might be due to other characteristics of smoking pregnant women. However, smoking by fathers had very little relation to the same pregnancy outcomes, which strongly points to an effect of maternal smoking and not of putative other characteristics.[20] As yet another example, the causality of 'arms sports' in causing venous thrombosis in the arm of persons who were competitively involved in 'arm sports', was greatly enhanced by the finding that in 'one-arm' sports the thrombosis was much more often seen in right arms than in left arms. (Tennis is a one-arm sport; swimming a two-arm sport; running a no-arm sport.) Actually, these highly physically active persons had a deficit of thrombosis overall, but an increase in the arm that was put under strain.[21] Finding such comparisons presupposes a deep understanding the problem, and knowledge of how it plays out in different circumstances.

Example A10: Inventing a new design: Mortality in families with hereditary predisposition to venous thrombosis.

We were sitting around a table with a number of clinical, epidemiologic and basic scientist, all interested in hereditary venous thrombosis. One of the great problems in clinical management of hereditary thrombosis is: what to advise if you find an hereditary predisposition (say, protein C, S or antithrombin deficiency) in a family wherein several members experienced venous thrombosis at a young age. Often the family stories were dramatic: venous thrombosis had occurred at young ages with severe consequences (which is why they came to attention). In families that came to attention and in whom these deficiencies were found, almost half of the family members who had experienced venous thrombosis, had developed it before age 50.

Question: should you screen the other family, i.e., members who never had a venous thrombosis for the presence of these mutations? And if they have the same genetic defect, should you propose anticoagulation to these otherwise healthy people, during high risk situations or perhaps for life? The ideal study, of course, would be a randomised controlled trial. However, for each of these rather rare genetic defects, there were only a few families known in the Netherlands, and if you started with people in their twenties, you might have to wait for 30 years to obtain an answer. The alternative of only randomising anticoagulation during known risk periods (pregnancy, surgery etc.) was equally unattractive since the number of risk episodes is not that large and would necessitate again a life-long follow-up.

We continued the discussion to pinpoint what the therapeutic dilemma really was. Finally we articulated it as follows: it is known for sure that by chronic anticoagulation you will kill or inflict a lifelong severe handicap (say, a brain haemorrhage or another severe bleeding requiring hospitalization) to close to 5 patients per thousand per year of treatment, *and we do not know whether this is a worthwhile risk to take.* When realising that this was our problem, we immediately saw that we already had the answer to this question, at least in principle. The question now became: would the death rate in those families be higher than in the general population, and most importantly, would the

difference be more than, say, 5 deaths or very handicapping events per 1000 per year – the maximally imaginable penalty of anticoagulation? When posing the question that way, we immediately realized that we could know the answer by simply looking at the past survival in the families and compare it to the general population. If mortality was clearly higher than in the general population (by a difference of at least 5 per 1000 per year), it would be worthwhile to give anticoagulation to carriers; if not, treatment would do more harm than good.

Next we realised that we should not study the whole family, but only the family members carrying the mutation. But how could we determine mutations in people who had already died? We proposed that if we have a family, e.g., with two nephews with venous thrombosis who both have the defect, we will know at once (1) that their parents (who are sibs) also carried the defect, (2) that the sibs of their parents would have a 50% probability of carring the effect, and we know (3) that their grandparents on that side of the family would also have 50% probability of carrying the defect. If the family was larger, and if we had information about cousins once removed, etc., we might even know for certain which grandparent carried the defect. The principle is shown in Figure 1.

The task was simple (at least in principle): construct the family trees, identify obligatory carriers (transmitters of the gene) and likely carriers (i.e. those with 50% certainty of carriership because they are parents, brothers or sisters of carriers); the calculate their mortality, and compare this with the mortality of their contemporaries of the same age and sex and calendar years in the general population by a well-known epidemiologic procedure called the Standardized Mortality Ratio. In the real life example in the figure this is slightly more complicated, because some living family members not having thrombosis were already tested for heterozygosity (so, their status was known and did not need to be calculated). Survival of persons in the family tree was only calculated from age 20 onwards, because persons with the possibility of transmitting the gene must have lived at least until the age of procreation. While conceptually simple, the task involved looking up family trees in genealogy registers which often was complicated and time consuming.

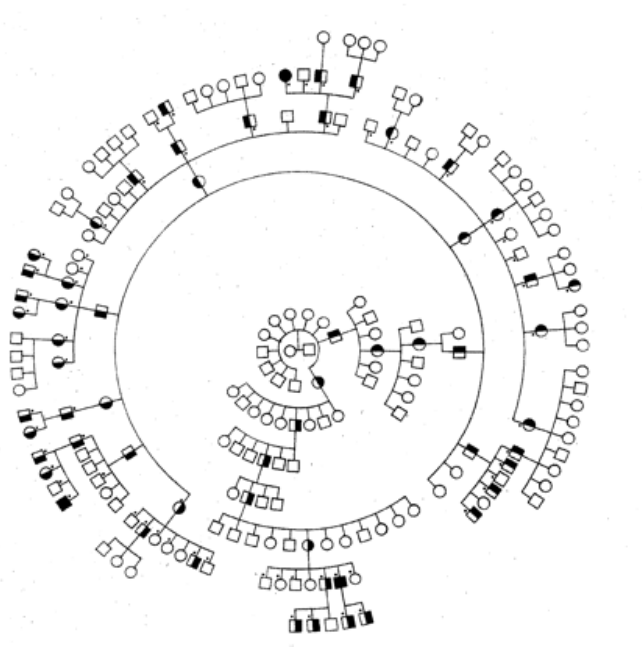

FIGURE 1 Legend: Pedigree of family 7, the largest family, derived from families of three probands. Squares represent men, circles women. Tiny black dots indicate later testing for heterozygosity. Black symbols indicate original three probands; black and white symbols indicate certain heterozygous individuals (either proved by testing or "obligatory" because of their place in pedigree); white symbols indicate possible heterozygous individuals (50% genetic probability, including individuals who later had normal test result).[22]

The intriguing possibility of this design was that we could measure "the effect" of these genes back in time, even in the 19th and early 20th century, when neither this type of familial disease was known (certainly existed, but was not described), certainly the gene was not known, and no treatment existed (as there was no anticoagulation before the second World War).[22] The results are shown in figure 2, in which it is clear that there never was any marked overall increase in mortality in these families (see the data for the whole period 1820-1993 on the right).

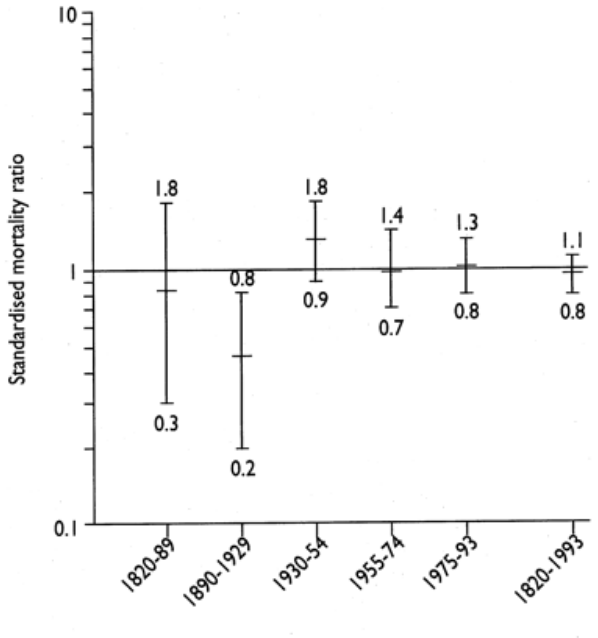

FIGURE 2: Standardized mortality ratio in families over calendar time (the complete 1820 – 1993 period is at the right)[22]

Later, the same principle was applied to study what mortality was associated with several other diseases, such as familial hypercholesterolemia in the past,[23] renal syndromes,[24] skin cancer mutations,[25] mutations that increase the risk of cardiac arrhythmias,[26] etc. And also for mortality in Huntington's disease in the past which was used as a positive control since we expected that there would be a clear signal.[27]

Example A11. Role of emotions: Radiology vs. Gastroscopy:

One of the nicest answers about the reasons for doing the research came from a radiologist who said (more or less): "I am angry with gastroenterologists who think that by doing a gastroscopy they can see much more lesions and much better than we can by radiology with contrast media. I want to prove them wrong". Emotions like these are very useful starting points for research. Of course, whether the gastroenterologists will be proven wrong is another story. However, it is immediately clear what the radiologist wants: to prove that he can visualise as much lesions as a gastroenterologist. Or, on further probing, whether he can visualise all the clinically important lesions as well as a gastroenterologist for lesser cost or lesser patient discomfort.

Example A12: Advice to be self-critical about potential severe validity problems: The diagnosis of venous thrombosis.

Early in the design of the case-control study on venous thrombosis and its genetic and biochemical risk factors that was used in examples 5 and 7, one of us [JPV] discussed the study with Alvan Feinstein. Our initial idea was to accept the diagnosis of 'venous thrombosis' as given by doctors, even if we knew that in the absence of any imaging (e.g., Doppler, which was in those years not yet mandatorily used), there would be substantial misclassification. We reasoned that 'at most' this might result in 'random misclassification' and would only attenuate the results. Feinstein told us that it would be much better to only include patients in whom there had been an objective diagnosis, because this was a scientific undertaking and we should not put ourselves into a position of needing to shield behind fairly general excuses after the study would be completed. Indeed, we did not know how large the effects would be, and how much they would be attenuated by the misclassification (and we had to assume that any misclassification was non-differential). The advice by Feinstein proved to be excellent – even if it took a lot of extra work to secure sufficient patients - as we could continue to write in all publications variants of the sentence that 'only patients with an objective diagnosis' entered the study.

Example A13. Two examples of settling for a 'lesser question'.

A13.1: The usefulness of haemodialysis in the elderly

There is widespread concern about the usefulness of haemodialysis as renal replacement therapy in the elderly. One might, of course, think about doing an RCT of dialysis vs. supporting (palliative) care. However, besides the costs, it is foreseeable that for a host of reasons (ethical, patient and doctor's choices) such an RCT might be very difficult to carry out. A published alternative was to look at the survival of elderly and seriously ill persons who had started haemodialysis. It was found that after onset of dialysis survival was on average very short – in seriously ill patients much shorter than patients and doctors generally expected that it would be.[28] While it is of course possible that without haemodialysis, the survival would have been even shorter, which cannot be known from these data (as they are not a randomized comparison) these data give an important background to discuss the usefulness of the procedure among health care providers and with patients in this condition.

A13.2 The usefulness of the Intensive Care Unit….

A young doctor who is confronted with all the chaos, the suffering and the human drama on an intensive care unit may well wonder what the ultimate benefit is for patients. She might wonder whether she might do a study about "An inventory of the use and the evaluation of the usefulness of intensive care units". Such a question is much too broad, and unfeasible. Firstly, doing 'an inventory' is a phrase that does not really cover a research question, because it runs the danger of not clearly formulating any aim about a problem that needs to be solved *[See Note 1 in Appendix B below].* Secondly, the idea of 'usefulness' is much too broad *[Note 2 in Appendix B below].* The budding researcher might be attempted to make lists of all newly entered patients at an intensive care unit and follow them up. This will lead to hopelessly muddled data sets that will not answer any research question. She would do better to limit herself to one or more specific

diseases, for instance to patients with ascending neuropathy (the Guillain Barré syndrome). In some of these patients the respiratory muscles become temporarily affected and they will be transferred to an intensive care unit to wait until the neuropathy clears by itself. As the short term benefit is rather apparent (the paralysis is often transient and the patient survives thanks to the artificial respiration in the intensive care unit), a remaining question might be the long-term outcome of patients who have received artificial ventilation in an intensive care unit – e.g., their physical and emotional functioning after a year or longer. That might be a feasible project that might fill a gap in our knowledge about the long-term prognosis of patients with severe Guillain-Barré syndrome. Of course, this seems far removed from the original thoughts that led to this piece of research, but it is useful prognostic data and might lead either to some sobering reflections or to a rather enthusiastic account. [Note: since formulating this example, more than two decades ago in the course of teaching about study design by JPV, several studies have been done on the prognosis of Guillain-Barré].

APPENDIX B: Further background for sharpening research questions (Verschuren & Umberto Eco)

B.1: The idea that a research question should have an aim 'to change or to accomplish' was strongly emphasized by Verschuren whom we cited for his wrist-watch metaphor in the main paper. He wrote about do's and don'ts in formulating a research question.[29] [Translation from Dutch by JPV].

"Do not: say that you will be occupying yourself with some subject
Do: make up your mind to change something"

"Do not: say that you will try to formulate a good subject
Do: tell what you want to accomplish with the subject"

B.2: A warning that young researchers will tend to formulate too ambitious questions was made explicit by Umberto Eco in his book *"Come si fa una tesi di laurea"* [*How to write a thesis.*].[30] destined to help Italian students with writing a Master's thesis. He devotes a whole chapter to the choice of subject and writes with his usual humour: "*If the student is interested in literature, he will start by wanting to write a thesis entitled "the literature now". When forced to limit the subject, he might try to settle for "The Italian literature from the second world war until the 1960s*". [Translation from Italian by JPV]. Eco explains that for 20-year-old students, these are impossible subjects in which they will always fail: the simple perusal of the table of contents by a supervisor will immediately spot grave omissions and defects. Eco continues and describes what is more appropriate: *"Therefore it is desirable that the student… chooses a more modest title. I tell you immediately what would be ideal: not "the novels of Fenoglio", but the different editions of "Il partigiano Johnny". Boring? Maybe, but much more interesting as a challenge.*" [Translation from Italian by JPV] Thus, Umberto Eco advises the young scientists to make a study of a single book by the author and to focus on the differences in the successive editions. .

APPENDIX C: ‘Serendipity vs. ‘Chance favours the prepared mind’.

“*Le hasard ne favorise que les esprits préparés*” (Pasteur 1854)[*]

Of course, simply looking for regularities or anomalies is not enough. Thousands of researchers can see the same phenomena without noticing a regularity or anomaly, or without recognising its significance. You need to have at least some idea of what you are looking for.

This can be illustrated by the famous discovery of penicillin by Alexander Fleming. Usually the story is told that Fleming got the idea when looking at a petri dish that had accidentally been forgotten on a window sill, and saw after the week-end that a mould had started growing – and lo and behold(!) no bacteria were seen anymore around the spots where the mould grew. The mould was later shown to produce penicillin. Whether or not this actually (could have) happened is still a matter of speculation. Whatever happened, the most important part of the story is left out: Fleming as a researcher was constantly on the look-out for substances that killed bacteria![31] He tried all kinds of things; he made his laboratory technicians weep by putting a drop of lemon in their eyes and had their tears fall on bacterial cultures to see whether these body substances also inhibited bacterial growth (they did, later the responsible agents became known as lysozymes). So, whatever really happened at the time of discovery of these moulds, Fleming knew quite well how ‘inhibited’ bacterial cultures looked like, and he had an acute interest in them.

This is rather different from the usual notion of “serendipity” -- i.e., discovering things that you were not looking for. Research always has aims and reasons; in fact all exploratory actions have aims and reasons – one does not start looking aimlessly

[*] The original French expression was “Dans les champs de l’observation, le hazard ne favorise que les esprits preparés”

around to find something new (in this respect, we cited Rothman in the main text of the paper[32]). The Dutch social scientist Verschuren described that "Columbus did not sail around the world aimlessly, he sailed in western direction based on a likely theory".[29] Indeed, Columbus chose a particular latitude (in his days longitude could only be determined very approximately while sailing) and kept to it in Western direction. He had expectations of landing in India. Although these were over-optimistic and involved ‘cooked’ figures to favour a much too small earth to win the argument that his expedition was possible.[33] Still, because off his expectation, it soon became clear that he had discovered something new. More generally: it is only when you have a very clear expectation that you can see an anomaly and understand its importance.

APPENDIX D: COMMON PROBLEMS AND SOLUTIONS ABOUT RESEARCH QUESTIONS AND STUDY PLANS [Hulley et al.[34]

Table 2.2.
The research question and study plan: Problems and solutions

Potential problem	Solutions
1. Vague or inappropriate	• Write the research question at an early stage • Get specific in the 1-2 page study plan about - how the subjects will be sampled - how the variables will be measured • Think about ways to make - the subjects more representative of the population - the measurements more representative of the phenomena of interest
2. Not feasible	
Too broad	• Specify a smaller set of variables • Narrow the question
Not enough subjects available	• Expand the inclusion criteria • Eliminate exclusion criteria • Add other sources of subjects • Lengthen the time frame for entry into study • Use more efficient variables or designs
Methods inadequate or beyond the skills of the investigator	• Consult experts and review the literature for alternative methods • Learn the skills • Collaborate with colleagues who have the skills
Too expensive	• Consider less costly study designs and measurement methods • Seek additional financial support
3. Not relevant or novel	• Modify the research question
4. Uncertain ethical suitability	• Consult with institutional review board • Modify the research question to avoid potentially unethical elements

APPENDIX E: PRINCIPLES OF A PILOT STUDY

The following questions need to be answered in a pilot study.

1. Are the data available? Can they be collected and abstracted? How much time will that take?

Even for the simplest forms of research, e.g. research that is completely based on existing clinical patient records, or on administrative records, or on 'available computer data', you should do a pilot.

For clinical records: is the piece of information that you need really present in the patient records? How much time will you need to make a list of patients with a particular diagnoses? Is making such a list possible at all in your own clinical setting – which may differ if you need patients from other settings? Would you need computerised discharge records, or alternatively, financial reimbursement records about certain procedures to identify the patients? Or records from lab files? Or evidence that certain procedures were carried out (e.g. rooms used for procedures, billing records)? Are the data that you need really in the patient records, or do you need to go back to the clinical or pathology lab or to other sources?

Next, if you try to abstract the information, how much time do you need per record? Thus, if you think you will need a few hundred patient records, and that you can obtain them very easily in your institution, then impose yourself this restraint: start out with only the first dozen and do everything on them as if it was your final study. Thereafter pause and ponder what you have learned about the feasibility of your project.

If you need to collect new data from patients, it is even more mandatory to pilot. Suppose you want to mail a questionnaire to a few hundred patients, to obtain baseline information or follow-up information. You should first make the questionnaire and

discuss it in depth with others, and read the literature about similar questionnaires. Then, you give it to three or four patients and say: "*This is a test run, tell me what you think of the questionnaire and whether you are able to complete it*". Next try mailing 15 or 20 patients, to see how quickly the questionnaires return, how they are completed, and phone the patients afterwards to ask their comments. This is already a two stage pilot.

Multistage pilots of data collection are necessary in studies where you collect new data prospectively, like in randomised trials. The final pilot for a randomized trial is to execute it 'pocket size' – inclusive or randomisation. However, you might first try out the data collection on existing records of a few patients, to see what forms you will need. Second, try the forms and try having the extra collection of data (blood, lab determinations) on a few new patients to see whether this data collection works and what unexpected hurdles you meet (unwilling labs, uncooperative secretaries, sudden irritation in another clinical department who consider the patients "their own", etc.). Thereafter, try out the randomization procedures, in particular if you were never involved in a randomized trial, to see if you can manage the randomisation, blinding etc (it has happened that people discovered after a trial that the randomisation schedule was wrong). This then leads to the final pilot: the whole study on a limited number of patients.

Collecting patient data can get extremely complicated, due to appointment schedules, collaboration with labs and other people that are involved, etc. In contrast to usual clinical practice, it is *you*, the researcher who should be flexible and beg and cajole everyone else to comply with your wishes. Clinical and epidemiologic research needs the art of continuous negotiation: at each level, from patients to hospital management and data-base holders, you will have to explain anew what you want and why. Better try this out beforehand to avoid nasty surprises.

When data are available 'administratively', there is the tendency to think that you will be able to use them. Not so! It has quite often happened that administrative data are available in formats that almost prohibit making the lists you want.

> *Example E1:* information about blood transfusions is in most hospitals ‘readily available on computer’. That information is excellent for clinical use in a single patient; however if you want to have, for example, a complete list of patients who developed antibodies after a first transfusion, and you want a list over may years, that demand can be quite taxing on the system and might need a lot of negotation.

Even if the data are completely available from a previous scientific study, because you want to do a secondary analysis, you should do a pilot to see how the data look, and check a few things, e.g. whether you can also obtain the original results,and whether you can get back to original records for verification of what was coded and how it was coded.

2. Can your new data be entered on computer?

Can you enter the newly abstracted or collected data on computer forms, e.g., in database programs? Often, you will need some help to accomplish this. Even if in the final project you will have a research assistant to help you, you should to the first few dozen yourself: otherwise, how will you ever supervise your assistant? This is the same principle as in lab research: young researchers should first gain the lab skills themselves before they are given lab assistants.

3. *Are you able to use the data for statistical analysis?*

Can you display the data in some statistical package? Can you produce the single two-by-two table, or the single figure that you dreamt of when you were discussing the research question? Of course, the numbers in the pilot study will be too small to be meaningful. However, when you are able to produce the tables that you dreamt of, based on your pilot data, you know that you are on the right track.

4. Now that we see this analysis, is this really what we wanted?

A final moment of reflection: we can obtain the *type of* data that we dreamt of, but will this convince others when they see it?

> *Example E2. Go virtual.* A lot of skills can be learned and tried out "virtually". In one project, a resident had to wait a long time before results on some genetic polymorphism came in. All clinical and epidemiologic data were already available. So, we decided to randomly assign "mutations" to some of the people in the database. Thereafter, the resident did all the analyses as if they were for real, and then wrote the paper. When the final data came in, after several months, plugging them in and doing the analysis was a matter of hours. The paper also needed little adjustment – as it gave a 'null' result.

Problems of pilots with ethics committees, particularly with randomized trials

The need for a pilot can necessitate some negotiation with ethics committees: some actions that you need to pilot may demand informed consent, and/or agreement of ethics committees first. This is a difficult area, in which wisdom should prevail, but the general principle is: never be shy to ask help, explaining why and how you do a pilot. It might also help to split the pilot phase into several stages, as described for randomized trials above.

Proposing separate pilots on different parts of the study might also be a way to discuss the permission to do pilots with and ethics committee or review board.

A good general recent reference about pilot studies is by Thabane et al.[35]

APPENDIX F: Some notes on writing, and the role of reporting guidelines

About writing

Writing involves two types of difficulties: style and ‘doing it’. Many books are written about writing; here we will present ideas that we found useful ourselves, and in addition we will point at the most useful guidelines to report research for different types of research designs (such as randomized trials, observational studies, systematic reviews….).

The theme of writing and rewriting was taken up masterly by Strunk in his booklet "*The elements of style*": “*Vigorous writing is concise. A sentence should contain no unnecessary words, a paragraph no unnecessary sentences, for the same reason that a drawing should have no unnecessary lines and a machine no unnecessary parts. This requires not that a writer makes all his sentences short, or that he avoid all detail and treat his subject only in outline, but that every word tell.*” [36] [see also below for online pdf in “useful books”]. To write briefly and retain all meaning is the most exacting task for any writer. Rumour has it that Hemingway’s “*Old man and the sea*” originally was over a thousand pages, and that it took him a decade or longer to trim it to slim novel that may have clinched his Nobel Prize in literature.

The favourite format for a young researcher - in particular when you are writing a paper ‘in advance’ (before the results are in – see main paper) should therefore be the most difficult format: the "short report”. Major general medical journals have the possibility of publishing short reports: often just 500-800 words, sometimes a maximum of 1200 words, one or two tables, five or six references. Once you can write a 600-1000 word short report with all the essential information about your research project in it, you are ready to write longer papers – the inverse is not necessarily true.

To help you write a first draft of a short paper, you might think of it as an ‘extended abstract’ (if applicable, modelled on a PICO – Patient, Intervention, Control group, Outcome): approximately 500 words plus a table and figure. The main advantage is that writing a short paper might take less time than a full paper - but still a very tough task to have everything that is important in it! Moreover, co-authors and busy physicians will be more willing to read one page and offer feedback than take the time to read a lengthy full manuscript. [See for inspiration some BMJ guidelines for short writing].[37]

Writing a short paper also helps to think clearly about the paragraph structure of a text. The very first paragraph of a text is usually a summary – in a short report that can be one sentence. Thereafter, each following paragraph should express a single idea, and the first sentence of each paragraph should announce it. A well written text can be understood by reading the first sentences of all paragraphs. (You can verify this if you take a quality daily newspaper and read a front page article or an editorial).

If you feel terribly at loss about how to take up the task of writing, in particular of a larger paper, why not start with the part that you feel secure about, whatever it is: e.g. results or introduction. Have a folder with several headings or submaps labelled “Summary, Introduction, Materials & Methods etc…”. Start writing anywhere. Next time, begin somewhere else, or if you have an additional idea about some of the sections, while doing something totally different, write that up also and throw it in. Keep going until you have a rough draft of the whole paper – it doesn’t have to be perfect. Once you have done a draft, editing is often much easier.

A good rule in (learning to) write is to enlist the help of others to read your drafts: sympathetic colleagues who do not know your project intimately but who know the general area of research: they will tell you whether they understand what you wrote. Once you become very skilled at it, you will learn to see your own writings with the eyes of others – which is really what you need in order to write well. In a sense this aspect of writing is the same as what good visual artists do: the secret of drawing is to be able to see your own drawing with the eyes of others.

Maria Gardiner and Hugh Kearns advised in Nature website that you should:[38]

- Write before you feel ready — because you might never feel ready. It's amazing how people magically feel ready when there is an imminent deadline.
- Don't wait to have a clear picture of the paper. As you start putting down your ideas, you may actually clarify them.
- Snack write — work in short, frequent bursts instead of waiting to sit down for big blocks of time. Those blocks hardly ever come, and when they do, they don't usually get used very productively.
- Set specific times in your schedule for writing — don't leave it to chance, because chances are it won't happen.
- Writing means putting new words on the page or substantially rewriting old words. It does not mean editing, reading, referencing or formatting — and it definitely does not mean composing e-mails.
- If you refrain from writing because you worry that what you write won't be good enough, try noting the adage that to write well, you first have to write.
- To really increase the quality and quantity of your writing, get feedback from mentors and colleagues — it can be painful, but it works.

See: (http://www.nature.com/naturejobs/2011/110707/full/nj7354-129a.html)

Reporting guidelines

Over the past few years, several reporting guidelines have been published. Several of them have been made ‘obligatory’ for authors who want to submit in the larger and more interesting journals. While there remains some uncertainty about their value for journal editors and reviewers, they are for sure a great help for junior (and not so junior) researchers.[39] They help researchers because they specify what should be covered in each section of a paper, from the title onwards, up to the conclusion and the acknowledgements.

A rather complete list of reporting guidelines can be found on the EQUATOR Network (Enhancing the QUAlity and Transparency Of health Research) website http://www.equator-network.org

Two types of guidelines exist: one about particular designs, and one geared towards subject matter or specific research problems.

- Examples of well-known reporting guidelines for specific study designs: CONSORT (Consolidated Standards of Reporting Trials) for randomized trials, STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) for observational studies
- Examples of well-known reporting guidelines for specific research problems: PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) for systematic reviews and meta-analyses of RCTs, STARD (STandards for the Reporting of Diagnostic Accuracy Studies) for diagnostic studies.

While they offer a very useful template, a commentary warned that slavishly following these reporting guidelines might lead to sterile, standard reporting that gives no feel for the reasoning behind the piece of research; it was advocated that additional 'narrative' might be useful about the why, the how and the assumptions that are guiding the research – if necessary in Appendices.[40]

Useful books on writing:

- One book that went to many editions and reprints and contains a wealth of humorous advice about writing papers for biomedical journals: Day RA, Gastel B. How to Write and Publish a Scientific Paper (7th Edition). Greenwood, 2011
- The advices of a former editor of the Annals of Internal Medicine: Huth EJ, Writing and publishing in medicine, 3rd Ed. Williams and Wilkins, 1999. have a clarity and logic that is very appealing.
- A successor to Huth's book: Lang TA. How to Write, Publish, & Present in the Health Sciences. American College of Physicians; 2010. Its forword has the lines that

summarize all of it: *"….. the secret to good medical writing is to: have something to say, say it, stop.*" The book itself is not short, but full of detailed very good advice.

- The 1918 US classic that continues to be reprinted - about writing succinctly, and which we cited above and was judged in 2011 by Times Magazine as one of the 100 best and most influential books written since 1923: Strunk W, White EB. The elements of style. Several pdf files of this book are available on the internet. http://faculty.washington.edu/heagerty/Courses/b572/public/StrunkWhite.pdf

- From a different field, very good general writing advice by a former editor of the UK newspapers *Sunday Times* and *Times*; often reprinted up to the 1990s: Evans H. Newsman's English: how to write clearly, concisely, vividly. Heinemann Professional Publishing, 1972

APPENDIX G: Further reading

- An impressive chapter about thinking about the research question and the design of your research is: Feinstein AR. Chapter 14: Preliminary appraisal of the outline. IN: Clinical Epidemiology: The Architecture of Clinical Research, Philadephia, Saunders 1985.
- A general and very useful chapter about finding as well as refining your research question: Hulley SB, Cummings SR. Eds. Chapter 2: “Conceiving the research question”. IN: Designing Clinical Research, an Epidemiological Approach, Baltimore: Williams & Wilkins, 1988. We refer to the 1st edition, since we prefer the wording of this chapter in that edition.
- A view about what can be achieved and what not by clinical and public health research: Crombie IK, Davies HTO. Chapter 2: The Foundations of Research. IN: Research in health care; design, conduct and interpretation of health services research. Chichester, Wiley 1996. In the first paper we advocated Chapter 3 where you can also find good advice about pruning.
- A general paper about how to be a (clinical) biomedical researcher: Kahn CR. Kahn CR. Picking a research problem. The critical decision. N Engl J Med. 1994;330:1530. Worthwhile advice that complements the advice by Medawar.

For those who like to probe deeper:

- Medawar PB. “Advice to a young scientist”. New York. Harper and Row, 1979. A slim volume with gems of insight still worth reading for younger and older researchers.
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- Vandenbroucke JP. On the new clinical fashion in epidemiology. Epidemiol Infect 1989; 102:191-6. (subheading: The daily practice of clinical epidemiology). Another view on clinical epidemiology with emphasis on observational pathophysiologic research and helping the clinician to sharpen research questions.

- Vandenbroucke JP. Alvan Feinstein and the art of consulting: how to define a research question. J Clin Epidemiol 2002;55:1176-1177. Description of some elements of the reasoning processes that help to define a restricted subject for a piece of research that can be set up with limited time and funds.

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