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Title: Cancer subtypes in aetiological research.

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Abstract

Researchers often attempt to categorize tumors into more homogeneous subtypes to better predict prognosis or understand pathogenic mechanisms. In clinical research, typically the focus is on prognosis: the tumor subtypes are intended to be associated with specific responses to treatment and/or different clinical outcomes. In aetiological research, the focus is on identifying distinct pathogenic mechanisms, which may involve different risk factors. We used directed acyclic graphs to present a framework for considering potential biases arising in aetiological research of tumor subtypes, when there is incomplete correspondence between the identified subtypes and the underlying pathogenic mechanisms. We identified two main scenarios: (i) *weak effect*, when the tumor subtypes are identified through combinations of characteristics and some of these characteristics are affected by factors that are unrelated with the underlying pathogenic mechanisms; and (ii) *lack of causality*, when the set of characteristics corresponds with a mechanism that is actually not a cause of the tumor of interest. Examples of the magnitude of bias that can be introduced in these situations are provided. Although categorization of tumors into homogenous subtypes may have important implications for aetiological research and identification of risk factors, the characteristics used to classify tumors into subtypes should be as close as possible to the actual pathogenic mechanisms to avoid interpretative biases. Whenever our knowledge of these mechanisms is limited, research into risk factors for tumor subtypes should first aim to causally link the characteristics to the pathogenic mechanisms.

Keywords: cancer subtypes, molecular characteristics, bias, disease classification, aetiological research

Introduction

Clinical and aetiological research often aims to categorize disease entities into more homogeneous subtypes to better predict prognosis, or to improve the understanding of pathogenic mechanisms.

Typically, a disease is classified into subtypes that share specific characteristics. In particular, any characteristic associated with prognosis and/or response to treatment may identify subtypes that differ regarding their clinical outcome; however, the same characteristics may not correspond to specific aetiological mechanisms, and vice versa.

Research into the identification of disease subtypes has recently increased due to advances in high-throughput techniques to explore molecular patterns, the availability of large biological databases, and the implementation of collaborative initiatives to obtain and analyze large numbers of tissue samples in a standardized fashion. This increased research activity is tightly linked with the concept of precision medicine which involves the identification of disease subtypes to better target the treatment, estimate prognosis and/or manage clinical follow-up. This concept is having a particular impact on clinical oncology and cancer research [1], but is being adopted also in non-malignant diseases, including, for example, asthma [2], cardiovascular disease [3] and diabetes [4].

A related development is to use disease subtypes in aetiological studies. Through unsupervised cluster analysis, for example, breast cancer researchers have identified subtypes of breast cancer, based on the expression of ESR1 (estrogen receptor), PGR (progesterone receptor), and ERBB2 (HER2) [5]. While this classification is usually adopted for prognosis and to inform therapeutic choices [6], it has been recently proposed to use it for etiological purposes. Epidemiological studies have shown that triple negative breast cancer may have specific risk factors [7].

Traditionally, tumor classification has been primarily based on the anatomical site of origin of the tumor [8]. Then, within each tumor site, stratification is typically carried out on the basis of the cell type of origin and, sometimes, other specific characteristics. It is now becoming increasingly

possible to further subdivide the tumors on the basis of their molecular features [9]. Thus the process of identification of tumor subtypes currently goes from the identification of an organ-specific tumor (e.g. “breast cancer”), to the classification of that tumor into specific subtypes (e.g. “triple-negative breast cancer” or even finer subtypes [10, 11]).

It is debatable whether such tumor subtypes represent subtypes of a single disease, or should be treated as different diseases. We will approach this issue pragmatically assuming that an organ-specific tumor type has been identified, and the issue is whether and how to divide this into subtypes. This approach mimics the current clinical approach to tumor heterogeneity: the characterization of tumor subtypes – often based on molecular analyses of the tumor tissue - logically follows the diagnosis of the tumor as an organ-specific entity. For example, a patient is first diagnosed with a breast cancer and, then, after having the results of immunohistochemistry assays on the tumor tissue, that tumor is further subclassified on the basis of the receptor status.

We support the need to reach a clearer definition of tumors in terms of pathogenic mechanisms. However, in this paper we argue that aetiological research into tumor subtypes may, under some circumstances, be problematic. A better understanding of these limitations is required for appropriate planning and interpretation of aetiological studies based on tumor subtypes. In this paper, we first present a conceptual framework for considering the correspondence between pathogenic mechanisms and the identification of the disease subtypes. We then assess two scenarios in which there is not a direct correspondence between the characteristics and distinct pathogenic mechanisms: (i) *weak effect*, when the tumor subtypes are identified through combinations of characteristics and some of these characteristics are affected by factors that are unrelated with the underlying pathogenic mechanisms; (ii) *lack of causality*, when the set of characteristics corresponds with a mechanism that is actually not a cause of the tumor of interest. Finally we

provide some suggestions on how to conduct research to establish the links between the characteristics and the pathogenic mechanisms.

Tumor subtypes and pathogenic mechanisms

In the context of aetiological research, tumor subtypes have aetiological value if they identify distinct pathogenic mechanisms. In Fig. 1 we use directed acyclic graph (DAG) to depict the supposed causal relationships between the diagnosis of a tumor, the identification of the tumor subtypes, and the associated pathogenic mechanisms: E denotes an exposure, A denotes a pathogenic mechanism causing an organ-specific tumor T and C is a characteristic that is used alone or in combination with other characteristics to define the tumor subtypes S once the tumor T has been diagnosed. Both of the pathogenic mechanisms (A_1 , A_2) lead to the development the tumor T. However tumors caused by the two mechanisms will show different characteristics (C_1 and C_2), which are then used to define the disease subtypes S. It is also possible that the characteristics used to define the tumor subtypes contribute to the initial diagnosis of the tumor, in which case there would be arrows from C_1 and C_2 to T. However, in the current example, we assume that the characteristics that are used to define the subtypes are not used to reach the first diagnosis, but are evaluated after the diagnosis of the tumor.

For simplicity, in Fig. 1 and throughout the article we assume that all variables are binary. It is thus assumed that a tumor can have two subtypes: the first is due to A_1 (and identified through C_1), the second is due to A_2 (and identified through C_2). If it is biologically possible that A_1 and A_2 coexist (as, for example, in the case of intratumor heterogeneity), then the two subtypes can also coexist. A simple example of the scenario described in Fig. 1 is the sub-classification of tumors on the basis of the cell type of origin. For example germ-line testicular cancer can be divided into seminomas and

nonseminomas: these are biological and, potentially, clinical different subtypes, and it is not uncommon that a seminoma and a nonseminoma coexist in the same patient.

In the model proposed in Fig. 1, the subtypes directly correspond to pathogenic mechanisms. The exposures may act specifically on a pathogenic mechanism (E_1 or E_3) or may be shared by the different pathogenic mechanisms (E_2). It should be noted that C_1 and C_2 may be a set of characteristics (rather than single characteristics), but should still be specific for a single pathogenic mechanism.

Problems of causal interpretation

It is possible that the assumption of direct correspondence between the characteristics used to define the subtypes and the pathogenic mechanism is not valid. This may affect the interpretability of aetiological studies of tumor subtypes. We discuss two scenarios, which are shown, respectively, in Fig. 2 (hereafter labeled as “weak effect”) and Fig. 3 (hereafter labeled as “lack of causality”)

Scenario 1: Weak effect

In the scenario depicted in Fig. 2a, the set of characteristics C_2 is caused by both pathogenic mechanisms A_1 and A_2 , thus a subtype is defined by the presence of C_1 and C_2 , while the other subtype has only C_2 features. This scenario may apply whenever the tumor subtypes are defined by combinations of characteristics (the definition of breast cancer subtypes, for example, requires the combination of ESR1 and PGR expression with ERBB2 expression). The situation depicted in Fig. 2a would lead to correct causal interpretations. However there may be problems of causal interpretation if, as shown in Fig. 2b, some of the characteristics used to define the tumor subtypes are affected by factors that are unrelated with the pathogenic mechanisms. In Fig. 2b, the exposure

E_4 affects the characteristic C_1 without acting on the pathogenic mechanism A_1 . Since C_1 is explained also by E_4 , the pathogenic mechanism A_1 is not a necessary and sufficient cause of C_1 and thus its effect on C_1 is weakened (hence the label *weak effect*).

The scenario described in Fig. 2b, in which the characteristic C_1 is caused by both the pathogenic mechanism A_1 and the exposure E_4 , may imply that: (i) some individuals have the set of characteristics C_1 for mechanisms that are unrelated with the pathogenic mechanism A_1 ; or that (ii) the pathogenic mechanism A_1 does not always produce the associated characteristics C_1 . These two mechanisms are not mutually exclusive. Their consequence is that individuals who experience the pathogenic mechanism A_2 can have both C_1 and C_2 and be spuriously attributed to the pathogenic mechanism A_1 , and, vice versa, individuals who experienced the pathogenic mechanism A_1 can have only C_2 and be spuriously attributed to the pathogenic mechanism A_2 . These effects can be seen in Fig. 2b when considering the effect of conditioning on C_1 on its two causes A_1 and E_4 . If all variables are binary (0=absent, 1=present): (i) $C_1=1$ does not imply necessarily that $A_1=1$, as presence of C_1 could be due also to $E_4=1$, and (ii) $C_1=0$ does not imply necessarily that $A_1=0$ if there is an interaction between E_4 and A_1 that produces $C_1=0$ when, for example, $A_1=1$ and $E_4=1$. It follows that presence of C_1 may occur in absence of A_1 and absence of C_1 may occur in presence of A_1 .

It should be noted that the problems described here relate to the underlying biological mechanisms leading to the tumor subtypes, and are therefore different from problems of measurement error and misclassification of the characteristics C_s . They occur even if the characteristics C_s are perfectly measured.

Scenario 2: Lack of causality

A different problem may occur if one of the supposed pathogenic mechanisms leading to the characteristics used to define the subtypes is not a cause of the tumor. The DAG shown in Fig. 3 depicts such a scenario in which A_2 is not a cause of the disease, and thus there is not an arrow from A_2 to T . The corresponding characteristic C_2 could still have a prognostic role, but it would not be a marker of a pathogenic mechanism. For example, methylation in the promoter of the O6-methylguanine methyltransferase (MGMT) gene in glioblastoma affects response to treatment with telozolemid (and it is thus relevant clinically), but this molecular alteration might not be a driver in the pathogenesis of this cancer type. Under this scenario, any risk factor for A_2 (and thus C_2) would be associated with the both disease subtypes defined on the basis of C_2 (presence or absence of C_2) even if this risk factor does not have any aetiological role for the tumor of interest.

Numerical examples

In Table 1, we use the scenario of Fig. 2b and consider a tumor caused by two possible pathogenic mechanisms: A_1 which always causes the characteristics C_1 and C_2 , and A_2 which always causes only the characteristic C_2 . We also assume that an exposure E_3 doubles the risk of A_2 . We assume that the population risk of A_1 is 1 per 1000 and that the population risk of A_2 is 1 per 1000 when $E_3=0$ and 2 per 1000 when $E_3=1$. We assume complete case ascertainment and diagnosis, irrespective of the mechanism involved, and that all variables are measures with no error. We also assume a 10% risk of developing the characteristic C_1 for reasons that are unrelated with the disease of interest (compared to 100% of subjects who are affected by A_1). Finally, we assume that the two mechanisms A_1 and A_2 can coexist, even if in this particular example, this assumption has negligible effects because the risks of A_1 and A_2 are low.

Using these assumptions, Table 1 gives an example of the *weak effect* scenario caused when C_1 can occur also in individuals without the disease of interest. Although the exposure E_3 affects only the pathogenic mechanism A_2 (which corresponds to tumor subtype 2 – S_2 –), analyses based on tumor subtypes would suggest that E_3 also affects S_1 (which corresponds to the unaffected pathogenic mechanism A_1).

In Table 2, we also assume that only 50% of the subjects having the pathogenic mechanism A_1 will in fact have the characteristic C_1 . This, in combination with the occurrence of C_1 in subjects without the disease, further contributes to the interpretative problems due the *weak effect* scenario: the risk ratio for S_2 (corresponding to the pathogenic mechanism A_2) underestimates the effect of E_3 on A_2 (RR of 1.67 vs. a true RR of 2.00), while the risk ratio for S_1 further overestimates the effect of E_3 on A_1 (RR of 1.15 vs. a true RR of 1.00).

Finally, Table 3 gives an example of the *lack of causality* scenario. There are only two possible subtypes (based on the presence or absence of C_2) as A_2 is not causal for the disease, and thus the pathogenic mechanism A_1 (and $C_1=1$) is a necessary cause. In Table 3 we assume that the population risk of A_1 is 1/1000 irrespectively of the level of the exposure E_3 , while E_3 doubles the risk (from 10% to 20%) of another mechanism A_2 unrelated with the tumor of interest. We also assume that A_1 always causes C_1 , A_2 always causes C_2 , no case is left undiagnosed and all variables are measured correctly. Since the characteristic C_2 is unrelated with any pathogenic mechanism for the disease, when the analyses are stratified on C_2 , the overall group of cases is divided into two groups which are in fact homogeneous in terms of aetiology. An exposure E_3 affecting C_2 would thus increase the number of patients labeled as having a “ C_2 tumor subtype”, and thus decrease the

number of patients with a “non-C₂ like tumor”. It follows that E₃ would be incorrectly considered to be a risk factor for the C₂ tumor subtype and a protective factor for the non-C₂ tumor subtype..

Discussion

Categorization of tumors into homogenous subtypes can help to identify specific pathogenic mechanisms and thereby specific risk factors [12]. There has recently been increasing interest in the definition and detection of tumor subtypes. This has been given impetus by recent advances in various omics technologies and the resulting increase in the amount of clinically relevant information that is available. Intertumor heterogeneity [13] and molecular pathological epidemiology [14, 15] are fast developing and influential concepts in cancer research. A similar increasing attention to the identification of disease subtypes has been occurring for non-cancer diseases. For example, already in 1995, an influential paper identified three asthma phenotypes based on combinations of age at onset and persistency of wheezing [16]. This classification, as well as subsequent developments including additional clinical characteristics and inflammation markers [17] have been used in a large number of aetiological studies on the assumption that “considering these more homogeneous phenotypes in future studies may lead to a better identification of risk factors for asthma” [18].

As discussed in this paper, the markers used to sub-classify tumors into tumor subtypes should be as close as possible to the actual pathogenic mechanisms. Although this correspondence is crucial to avoid interpretative biases, our knowledge of the pathogenic mechanisms is often too limited to assess whether this important criterion has been achieved. The classification of breast cancer subtypes, for example, based on the expression of the estrogen and progesterone receptors and ERBB2, has been suggested through a cluster analysis; even if its clinical value has been proven,

distinct pathogenic mechanisms for the different subtypes have not yet been demonstrated. In fact, a recent commentary on breast cancer suggests the existence of only two aetiological components (which would correlate with the expression of the ESR1); it argues that, even if the model may seem too simplistic clinically, it is not too simple for aetiological purposes, considering that many molecular alterations may be more linked with tumor progression than with its development [19].

We suggest that aetiological research into tumor subtypes should first aim to connect the pathogenic mechanisms to the relevant characteristics, and then use these characteristics to assess whether the disease subtypes have different risk factors. Biological knowledge is a key factor. For example, when the subtypes are identified on the basis of the cell type of origin, it can be reasonably assumed, solely on a biological basis, that different cell types are involved in different pathogenic mechanisms. They may share risk factors, as, for example, small cell lung carcinoma and lung adenocarcinoma are both affected by smoking [20], but the pathogenic mechanisms remain different as they involve different cell types.

Often, however, biological knowledge is not sufficient to link a characteristic to a pathogenic mechanism and research should be conducted with the primary aim of establishing such a link. There are several options. First, characteristics that are evident at an early tumor stage are more likely to be causally linked to its aetiology than late characteristics. Tumor cells evolve during the tumor lifespan, acquiring new and complex molecular features. If we are however interested in primary prevention and early development of the tumor, molecular characteristics acquired at a later stage are less relevant and may easily be affected by mechanisms that are not related with the risk factors of interest. Thus, studies that have access to pre-diagnostic tissues are highly informative to define tumor subtypes for aetiological studies [21]. Once the subtypes are defined, they can be identified also on the diagnostic tissue, but an initial step involving pre-diagnostic tissue and early molecular characteristics would greatly enhance the potential to validly interpret subsequent studies.

Second, the risk of interpretative bias may be reduced by defining tumor subtypes on the basis of subtype-specific sets of characteristics (i.e. each subtype has different identifying characteristics) instead of combinations of characteristics partially shared by different pathogenic mechanisms. This should be taken into account, for example, when the tumor subtypes are defined on the basis of an unsupervised cluster analysis, and then a set of markers is chosen to characterize each specific cluster. For aetiological research, it is perhaps safer if the characterizing sets of markers do not overlap among clusters. Third, in some instances it is possible to directly test whether a characteristic is causally involved in tumor development. For example, to understand whether gene-specific methylation is causally involved in tumor development, it is possible to study the association between germ-line variants in the DNA methylation machinery genes and cancer incidence [22]. If an association is found, methylation markers are more likely to be causally involved instead of being just epiphenomena. This approach, which is based on the concept of Mendelian randomization, can be carried out almost systematically, at least for characteristics that are known to be affected by germ-line variants.

In conclusion, categorization of tumors into homogenous subtypes may have important implications for aetiological research and identification of risk factors. However, it is essential that the characteristics used to classify tumors into subtypes should be as close as possible to the actual pathogenic mechanisms to avoid interpretative biases. Whenever our knowledge of these mechanisms is limited, research into risk factors for tumor subtypes should first aim to causally link the characteristics to the pathogenic mechanisms.

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Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793-5.
2. Muraro A, Lemanske RF Jr, Hellings PW, Akdis CA, Bieber T, Casale TB et al. Precision medicine in patients with allergic diseases: Airway diseases and atopic dermatitis- PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol*. 2016;137(5):1347-58.
3. Pitt GS. Cardiovascular precision medicine: hope or hype? *Eur Heart J*. 2015;36(29):1842-3.
4. Pearson ER. Personalized medicine in diabetes: the role of 'omics' and biomarkers. *Diabet Med*. 2016;33(6):712-7.
5. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Med*. 2010;7(5):e1000279.
6. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol*. 2013;24(9):2206-23.
7. Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL et al. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Inst*. 2011;103(3):250-63.

8. WHO/IARC Classification of Tumours, <http://publications.iarc.fr/Book-And-Report-Series/Who-Iarc-Classification-Of-Tumours>. Accessed on 28 July 2016.
9. Song Q, Merajver SD, Li JZ. Cancer classification in the genomic era: five contemporary problems. *Hum Genomics*. 2015;9:27.
10. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Med*. 2010;7(5):e1000279.
11. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70.
12. Morton LM, Slager SL, Cerhan JR, Wang SS, Vajdic CM, Skibola CF et al. Etiologic heterogeneity among non-Hodgkin lymphoma subtypes: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. *J Natl Cancer Inst Monogr*. 2014;2014(48):130-44.
13. Zellmer VR, Zhang S. Evolving concepts of tumor heterogeneity. *Cell Biosci*. 2014;4:69.
14. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. *J Natl Cancer Inst*. 2010;102(6):365-7.
15. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol*. 2013;26(4):465-84.
16. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*. 1995;332(3):133-8.
17. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;185:716-25.
18. Siroux V, Basagaña X, Boudier A, Pin I, Garcia-Aymerich J, Vesin A et al. Identifying adult asthma phenotypes using a clustering approach. *Eur Respir J*. 2011;38(2):310-7.
19. Anderson WF, Rosenberg PS, Prat A, Perou CM, Sherman ME. How many etiological subtypes of breast cancer: two, three, four, or more? *J Natl Cancer Inst* 2014;106(8):dju165.
20. Pesch B, Kendzia B, Gustavsson P, Jöckel KH, Johnen G, Pohlmann H et al. Cigarette smoking and lung cancer--relative risk estimates for the major histological types from a pooled analysis of case-control studies. *Int J Cancer*. 2012;131(5):1210-9.

21. Kadara H, Scheet P, Wistuba II, Spira AE. Early Events in the Molecular Pathogenesis of Lung Cancer. *Cancer Prev Res (Phila)*. 2016;9(7):518-27.
22. Sung H, Yang HH, Zhang H, Yang Q, Hu N, Tang ZZ et al. Common genetic variants in epigenetic machinery genes and risk of upper gastrointestinal cancers. *Int J Epidemiol*. 2015;44(4):1341-52.

Fig. 1. Simplified diagram showing the relationship between exposures (E), pathogenic mechanisms (A), the organ-specific tumor (T), characteristics (C) and subtypes (S).

Fig. 2. Diagram depicting a situation in which the set of characteristics (C) used to define the disease subtypes (S) are not always specifically linked to a given pathogenic mechanism (A). **2a)** E denotes an exposure potentially causing at least one of pathogenic mechanisms and T denotes the organ-specific tumor diagnosis. **2b)** A further exposure (E₄) causes one of the characteristics used to define the subtypes but it is not a cause of any of the pathogenic mechanisms of interest.

Fig. 3. Diagram depicting a situation of lack of causality. The set of characteristics (C) used to define the disease subtypes (S) are linked with pathogenic mechanisms (A) which are (A1) or are not (A2) causes of the disease T of interest. E denotes an exposure potentially causing at least one of pathogenic mechanisms.

Table 1. Example of a *weak effect* scenario. Probabilities of different combinations of presence of an exposure E_3 , pathogenic mechanisms A_1 and A_2 , characteristics C_1 and C_2 and subtypes S_1 ($C_1=1$ and $C_2=1$) and S_2 ($C_1=0$ and $C_2=1$), when the characteristic C_1 may occur also among subjects without the disease. Relationships among the variables are described in Fig. 2b.

Exposure	Pathogenic mechanism ^a		Subtype ^b	
	A_1	A_2	S_1 ($C_1=1, C_2=1$)	S_2 ($C_1=0, C_2=1$)
$E_3=1$	0.001	0.002	0.0012	0.0018
$E_3=0$	0.001	0.001	0.0011	0.0009
Risk ratio ^c	1.00	2.00	1.09	2.00

^a These probabilities are assumed by design (see text for details)

^b These probabilities are obtained on the basis of A_1 and A_2 , assuming that A_1 always generates C_1 and A_2 always generates C_2 and a 10% risk of developing the characteristic C_1 for reasons that are unrelated with the disease of interest (see text)

^c Risk ratios are calculated by dividing the disease probabilities in $E_3=1$ for disease probabilities in $E_3=0$. Other approaches are possible, which, however, would give the same results as we assumed a rare disease

Table 2. Example of a *weak effect* scenario. Probabilities of different combinations of presence of an exposure E_3 , pathogenic mechanisms A_1 and A_2 , characteristics C_1 and C_2 and subtypes S_1 ($C_1=1$ and $C_2=1$) and S_2 ($C_1=1$ and $C_2=1$), when the characteristic C_1 may occur also among subjects without the disease and the pathogenic mechanism A_1 does not always produce C_1 . Relationships among the variables are described in Fig. 2b.

Exposure	Pathogenic mechanism ^a		Subtype ^b	
	A_1	A_2	S_1 ($C_1=1, C_2=1$)	S_2 ($C_1=0, C_2=1$)
$E_3=1$	0.001	0.002	0.00075	0.00225
$E_3=0$	0.001	0.001	0.00065	0.00135
Risk ratio ^c	1.00	2.00	1.15	1.67

^a These probabilities are assumed by design (see text for details)

^b These probabilities are obtained on the basis of A_1 and A_2 and assuming that A_1 always causes C_2 and causes C_1 in 50% of the affected subjects, while A_2 always causes C_2 . We also assume a 10% probability of C_1 in subjects not having A_1 (see text)

^c Risk ratios are calculated by dividing the disease probabilities in $E_3=1$ for disease probabilities in $E_3=0$. Other approaches are possible, which, however, would give the same results as we assumed a rare disease

Table 3. Example of a *lack of causality* scenario. Probabilities of different combinations of presence of an exposure E_3 , pathogenic mechanisms A_1 and A_2 (the latter is not a cause of the disease of interest), characteristics C_1 and C_2 , and C_2 -subtype ($C_1=1$ and $C_2=1$) and non C_2 -subtype ($C_1=1$ and $C_2=0$). The relationships among the variables are described in Fig. 3

Exposure	Pathogenic mechanism ^a		Subtype ^b	
	A_1 (causing the disease)	A_2 (not causing the disease)	non C_2 -subtype	C_2 -subtype
$E_3=1$	0.001	0.2	0.0008	0.0002
$E_3=0$	0.001	0.1	0.0009	0.0001
Risk ratio ^c	1.00	2.00	0.89	2.00

^a These probabilities are assumed by design (see text for details)

^b These probabilities are obtained on the basis of A_1 and A_2 and assuming that only A_1 causes the disease of interest

^c Risk ratios are calculated by dividing the disease probabilities in $E_3=1$ for disease probabilities in $E_3=0$. Other approaches are possible, which, however, would give the same results as we assumed a rare disease