

PROFESSORS PAUL SCHEEPERS AND THOMAS GÖEN DISCUSS THE DIAGNOSIS, MONITORING AND PREVENTION OF EXPOSURE-RELATED NON-COMMUNICABLE DISEASES (DiMoPEX - A COST NETWORK)

Searching for signatures

Non-communicable diseases (NCDs) are associated with environmental exposures. The WHO predicted that global mortality due to NCDs will increase to 55 million by the year 2030. The analysis of body fluids is used to study the linkage between chemical exposure and NCDs by use of biomarkers. If causality is supported by research, steps can be taken to reduce these exposures and prevent the occurrence of NCDs. The COST Action DiMoPEX was begun to explore research opportunities in a European network.

Environmental factors

In addition to genetic factors, environmental factors are driving the morbidity and mortality of some NCDs. An important component of these environmental factors is represented by chemical compounds which have been developed, produced, used and emitted into the environment since the beginning of the Industrial Revolution. They are called 'xenobiotics' because they are external and 'strange' to our body, as well as a potential threat to our homeostasis, and exposure to them at either high levels or prolonged durations can be harmful. Xenobiotics may also take the form of toxic residues in food and consumer products, while people may also become exposed due to an unhealthy lifestyle, including smoking, alcohol consumption and recreational drugs.

Together with our genetic constitution, xenobiotics in part determine the risk of acquiring health complaints and disease. As long as pathogens are not involved, the disease outcome is often referred to as an NCD, and this includes cancer, diabetes, cardiovascular, neurological, respiratory and many other diseases. Most NCDs can be treated and have, in part, been converted into chronic diseases, leading to an increased contribution of NCDs to overall disease burden.



Fig. 1 Gas chromatograph mass spectrometer (GC-MS) in the Research Lab Molecular Epidemiology

The European Cooperation in Science and Technology (COST) is a funding agency for research and innovation networks. COST Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.



Because of the long latency of most NCDs, it is difficult to identify factors that cause or modify the risk of their formation. The COST Action Diagnosis, Monitoring and Prevention of Exposure-Related Non-communicable Diseases (DiMoPEX) tries to improve research methods to study environmental exposures that lead to NCDs (<http://dimopex.eu/>). These insights could contribute to the prevention of these adverse health outcomes.

Unique chemical signatures

If exposures to xenobiotics leave a chemical trace, it is possible to detect this in chemical analysis, especially if this trace carries the chemical signature of the xenobiotic (Fig.1). For some persistent synthetic chemicals it is obvious that the exposures are derived from environmental pollution and, depending on their properties, they may accumulate over a long period of time.

In 2003 Margot Wallström, at that time a European commissioner, agreed to have her blood tested by the WWF for 77 of these persistent chemicals. This resulted in the finding of environmental pollutants such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Some of these chemicals are known to have endocrine disruptive properties that may impede development during childhood and puberty.

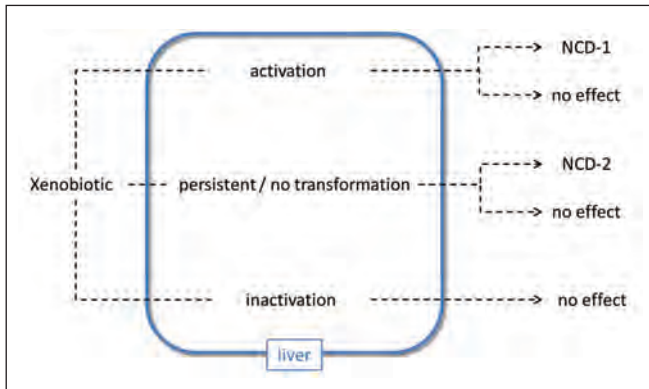


Fig. 2 The liver may change the chemistry and toxicity of the xenobiotics

Other xenobiotic substances are biotransformed soon after they reach the liver (Fig. 2). Examples are modern pesticide residues in the diet or plasticisers in food packaging. Most of these substances are detoxified by the liver and readily excreted.

There is a third group of chemicals that represents a risk due to bio-activation in the liver. Some of these xenobiotics are formed during food preparation such as polycyclic aromatic hydrocarbons and amines in fried, broiled, smoked and barbecued meat and acrylamide in fried potato and toasted wheat products. 14 years after Wallström had her blood tested, the European Commission launched a European-wide biomonitoring programme (HBM4EU) to systematically search for xenobiotics in body fluids in the European population (<http://www.eea.europa.eu/themes/human/human-biomonitoring>).

Biomarkers bridge a gap in knowledge

In the onset of an NCD, many known (and often also unknown) factors may be involved. Population research often provides the first evidence of an association between the environmental factors and the disease outcome. To understand the biological plausibility and causality, most pieces of the puzzle come from animal testing and, increasingly, from bioassays that do not require experimental animal models. For most xenobiotic factors, this chain of events is difficult to unravel and there are many knowledge gaps in the chain from cause to disease outcome.

The finding of biomarkers in body fluids can help to bridge these gaps in knowledge (Fig. 3). Is a chemical activated or deactivated by metabolism? How does a chemical or product of biotransformation interact with DNA, RNA, or

proteins? What are the target tissues? Do changes to tissues accumulate or do they disappear due to repair mechanisms and, when it comes to the changes that persist, are they considered benign or adverse?

Chemical analysis of body fluids can help to find answers to these questions. This is called 'human biological monitoring' (HBM), and the field where these biomarkers are applied in population-based research is indicated as molecular epidemiology.

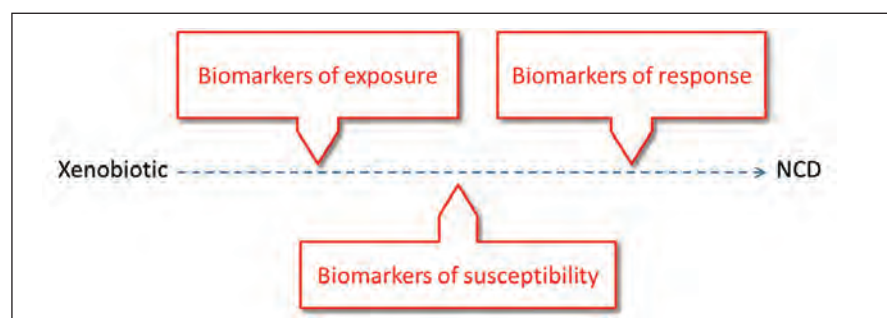
Biomarkers of exposure

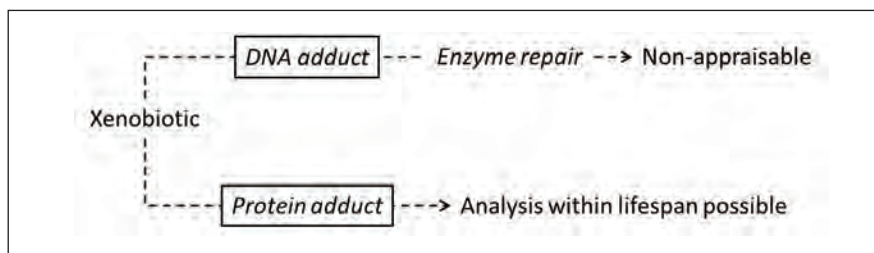
Different types of biomarkers will tell different parts of the story 'from cause to effect'. Measurement of the parent substance in blood indicates to what extent a xenobiotic was taken up over time and distributed over the entire body. It is important to know if a substance is still free in solution or immobilised in a protein complex – only the fraction that is free in solution can interact with a receptor or become a substrate in enzymatic conversions that may change its activity. In the latter case, a product of biotransformation (metabolite) is often used as biomarker. Especially for metals, the chemical form (oxidation state and organic or inorganic form) and its 'free' plasma concentration are important to predict toxicity. All of these chemical species are called 'biomarkers of exposure' and demonstrate how much of a chemical substance is circulating and in what chemical form: active or inactive.

An activated chemical could interact with critical tissues or biomolecules such as DNA, RNA or protein. A much-used biomarker is an addition product or adduct; this is the product of a chemical reaction of the activated form of the xenobiotic with a biomolecule. The benefit of using an adduct as a biomarker is that the structure contains the unique chemical signature which allows the positive identification of the causing chemical species. This is the ultimate proof of a xenobiotic interacting on a molecular level with the body, without knowing if the outcome will be adverse or not.

A sophisticated system of enzymatic DNA repair removes the DNA adduct, usually in a matter of a few days (Fig. 4). For proteins, however, there is no known repair system, which means that protein adducts will prevail, usually for as long as the lifespan of the native protein. This makes the protein adduct the ideal dosimeter because the groups where the xenobiotic binds to a protein are of a similar chemical nature as in DNA. Haemoglobin adducts have a lifespan of four months and are very useful to integrate exposure over this period of time. This means that for a considerable time after exposure it is still possible to tell if an individual was exposed and to what specific xenobiotic [see proof of principle on page 133].

Fig. 3 In the chain from cause to disease, different types of biomarkers can be used for different purposes





Biomarkers of response

Another group involves the 'biomarkers of response'. This is a difficult category because it contains very different types of responses that can be protective or harmful. Only in a few cases do these response biomarkers reflect outcomes that can be interpreted as 'adverse effects' or 'health effects'. A merit of the biomarkers of response is the 'early warning' that they can provide at a stage when the impact on health can still be reversed without medical intervention, i.e. by reducing the exposure. Many of these biomarkers indicate harm at the molecular level that cannot be traced back to a specific chemical exposure. An example is oxidative damage to DNA and proteins caused directly or indirectly by a wide range of toxic substances. Observing this type of molecular damage indicates that normal defence mechanisms were bypassed or overloaded, which could suggest an increased risk for an adverse outcome. On the other hand, most of the limited/putative oxidative damage is also quickly repaired and may not have an effect that is noticeable.

Some cytogenetic response biomarkers are associated with cancer risk. The type of molecular damage that can be related to such adverse events is at the chromosome or cellular level and includes micronuclei, sister chromatic exchanges, and chromosome aberrations. Currently, other new types of structural changes in DNA or the protein part of the cell nucleus are being studied to find out about their value as early indicators of adverse events and their possible link to the onset of disease, e.g. methylation of DNA, DNA-protein cross links, post-translational changes to proteins in the chromatin, etc. The limitation of these biomarkers is that they do not carry a unique chemical signature that allows direct translation to the causing agent.

Vulnerable groups

In a third group, biomarkers do not have a direct relationship with exposure but instead characterise an individual and whether or not he/she is above average with regard to susceptibility or resistance to a certain level/duration of exposure, or if he/she can recover from reversible changes that may be caused. These biomarkers are called 'biomarkers of susceptibility'. In short, they represent properties that explain why one individual falls ill while the health of another, with a similar pattern of exposure, is not affected.

Susceptibility biomarkers often reflect genetic polymorphisms in enzymes that activate or deactivate xenobiotics. On a population level, such inter-individual differences in enzyme activity have been shown to have an influence on NCD prevalence and morbidity. These biomarkers can be used in molecular epidemiology to study gene-environment interactions and contribute to the understanding of risk factors on a population level.

By comparing disease patterns, we now understand the impact of genetic factors on cancer occurrence. In populations with a high intake of amines

from fish consumption, the bladder cancer risk was observed to be lower in individuals with a fast N-acetylase genotype (NAT2). This particular enzyme changes the chemistry of the amines, resulting in a lower urinary concentration of the reactive intermediate that is implicated as a risk factor for bladder tumour induction.

Fig. 4 Some adducts are repaired; others can be used as biomarkers

Analytical challenges

For the detection of biomarkers relevant to NCDs, analytical instruments are required that allow the full chemical characterisation of a substance, even when only a trace of a xenobiotic is available. Many such ultrasensitive detection systems currently rely on mass spectrometry (MS) – and due to the use of biomarkers in clinical research and practice, the capabilities of MS-based analytical equipment have significantly improved over the past three decades. As a result, biomarkers can be analysed from more complex matrices and in smaller volumes of biological sample due to miniaturisation (Fig. 5).

Similarly, improvements in both hardware and software allow a more reliable identification of the structure of the biomarker and improve the possibility of quantification, even when detecting only the smallest trace. This means that it will be possible to detect a background exposure to xenobiotics in human populations. Furthermore, this adds the 'reference level' of exposure to molecular epidemiology studies and holds a promise for the future, namely the ability to identify subpopulations with lower or higher exposures and/or lower or higher health risks. These datasets will help to further explore factors that attenuate the relationship between chemical exposure and NCD prevalence and morbidity. This will lead to the identification of susceptible subgroups and the identification of environmental factors that can be assigned 'protective' or a 'risk factor' for a particular NCD.

Fig. 5 UPLC system with tandem triple quadrupole mass spectrometer used for ultrasensitive detection of unique chemical signatures of xenobiotics in body fluids





Proof of principle

On 4 May 2013 a train derailed close to the small village of Wetteren in Belgium. Some tank wagons carried the chemical substance acrylonitrile, which was dispersed in the environment during the fire that followed. 33 people were injured and there was one fatality.

Many first responders and inhabitants reported to hospital emergency rooms with health complaints. In the second and third week after the incident, several thousand blood samples were collected and analysed for N-(2-cyanoethyl) valine, an adduct of acrylonitrile with blood haemoglobin.

The concentration of this adduct was used to identify exposed and non-exposed. For non-smokers it was possible to make an estimate of the exposure at the time of the accident; this was not done for smokers, because smoking also leads to an increased blood level of this adduct.

Picking up chemical signatures

The chemical signatures may consist of a mass spectrum of complete xenobiotics molecules or their products of biotransformation, including the products of covalent binding of a reactive xenobiotic or an activated metabolite to a macromolecule (adduct). If the biomarker has unique chemical properties, this helps the biomarker of interest to stand out from the complex biochemical pool that a sample may represent. Examples of such chemicals are organic fluorides, chlorides and bromides that have characteristics that allow more easy identification and detection than chemical entities that are more abundant, such as organic amines.

Sometimes it is possible to add a chemical label to improve detection capabilities (derivatisation). For some xenobiotics the chemistry does not allow sensitive detection or the chemical signatures are not unique and instead drown in a multitude of endogenous substances.

Another problem may be that the biomarker allows sensitive detection but is itself not unique to xenobiotics exposure. A classic example is hippuric acid, which is a metabolite of the industrial chemical toluene. However, it can also be formed as a metabolite of benzoic acid, which is commonly used as a food additive and preservative (E210).

Emerging risks

New analytical methods allow a more explorative approach in what is called a 'non-targeted'

analysis. In the near future, it is expected that more chemical signatures will be picked up from biological samples that will turn out to be meaningful in terms of real-life exposures. This adds another perspective to the process: instead of a deterministic approach of looking for specific 'knowns', it will be possible to start looking for the 'unknowns' in a more empirical way. The question is how these emerging chemical signatures can be related to exposure patterns in the population and also how they relate to trends in the prevalence and morbidity of NCDs. Such exposures are more likely to involve complex mixtures than single factors.

Several EU projects like DiMoPEX have been started (including HBM4EU, EXPOsOMICS and HEALS), using advanced scientific methods to study the role of environmental exposures across different critical life stages. Of particular interest is the impact of environmental factors in early life. How do perinatal exposures determine the health after birth? How does the exposure of the parents affect the susceptibility in their children and grandchildren?

In these studies, existing and new scientific hypotheses on the influence of epigenetic factors will be studied. These will include exposures to single factors but also to mixtures – an area where much more knowledge is necessary.

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