Identifying suitable biomarkers of biological effects (BOBEs) for evaluating reduced risk products

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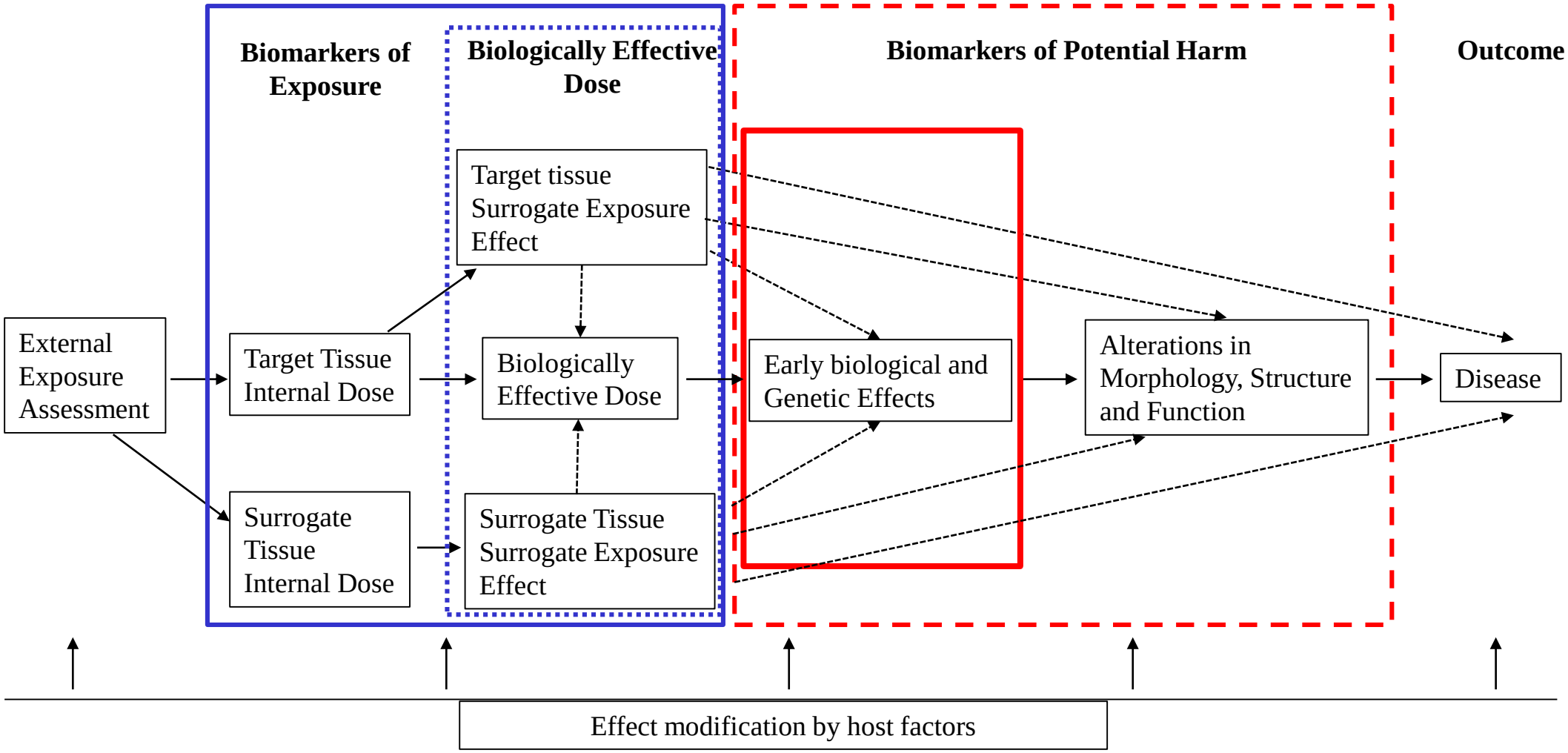
Outline

- Background
- Suitability criteria for biomarkers of biological effects (BOBEs)
  - Association with disease
  - Established differences between smokers and non-smokers
  - Dose-response relationship
  - Reversibility
  - Kinetics after smoking cessation
- Smoking-related diseases and biological effects
  - Possible BOBEs
  - Established BOBEs / Evaluation
  - Future BOBEs
- Summary and conclusions

Background


Biomarkers of Exposure
Biologically Effective Dose
Biomarkers of Potential Harm
Outcome
Target tissue Surrogate Exposure Effect
External Exposure Assessment
Target Tissue Internal Dose
Biologically Effective Dose
Early biological and Genetic Effects
Alterations in Morphology, Structure and Function
Disease
Surrogate Tissue Internal Dose
Surrogate Tissue Surrogate Exposure Effect
Effect modification by host factors

Suitability criteria for BOBEs

- Association with smoking-related diseases
  - Cancer, cardiovascular diseases (CVD), respiratory diseases
  - Adverse effects: Oxidative stress, lipid peroxidation, inflammation
- Differences in BOBE levels between smokers and non-smokers
  - Consistency over many studies
  - Effect size
- Dose-response relationship (DRR)
  - Smoking dose: CPD, pack-years, biomarkers of exposure (BOE)
  - Consistency over many studies
- Reversibility
  - Levels in ex-smokers: similar to those of never smokers
- Kinetics after smoking cessation
  - Short-term (< 1 week), medium-term (weeks to months), long-term (> 12 months)
  - Consistency over many studies

Respiratory diseases (1): *General considerations*

- Most important for smoking:
  - Chronic obstructive pulmonary diseases (COPD)
  - Bronchitis
  - Emphysema
- Pathological mechanisms involved:
  - Inflammation
  - Protease/anti-protease imbalance
  - Oxidative stress
  - Epithelial injury
- Potential biological matrices:
  - Plasma/serum, urine, saliva
  - Exhaled breath (volatile markers)
  - Exhaled breath condensate (EBC, semi-volatile, non-volatile markers)
  - Bronchoalveolar lavage fluid (BALF, invasive sample collection)

Respiratory diseases (2): *Potential BOBEs*

- Lung function:
  - Forced expiratory volume in 1 s (FEV_1)
  - Forced expiratory flow between 25 and 75 % (FEF_{25-75})
  - Forced volume capacity (FVC)
  - Lung diffusion capacity for CO (D_{LCO})
- NOS activity/inflammation in the respiratory tract
  - Fractional exhaled NO (FeNO)
- Clara cell (CC) proteins and surfactant associated proteins (SPs) :
  - CC16, CC10 in serum
  - SP-A, SP-B, SP-D
  - Exhaled breath condensate (EBC, semi-volatile, non-volatile markers)
  - Bronchoalveolar lavage fluid (BALF, invasive sample collection)
- BOBEs in EBC (partly also in sputum and BALF)
  - Carbonyls (MDA, 4-HNE, n-hexanal, n-heptanal)
  - LTB4, IL6 (inflamation)
  - 8-epi-PGF2a, H_2O_2 (oxidative stress)
  - Untargeted (omics) approaches

Evaluation of BOBEs

- Effect size (d): $d = \frac{x_s - xns}{SD}$ (x_s, x_{ns}: mean BOBE levels of smokers and non-smokers; SD: pooled standard deviation)

  $d \leq 0.2$: small

  $d \leq 0.5$: medium

  $d \geq 0.8$: large (Cohen, 1988)

- Four (residual) suitability criteria (semi-quantitative evaluation, scale: 0 - 1):
  - Association with

    Disease / DRR / Smoking cessation (CE) / Kinetics:

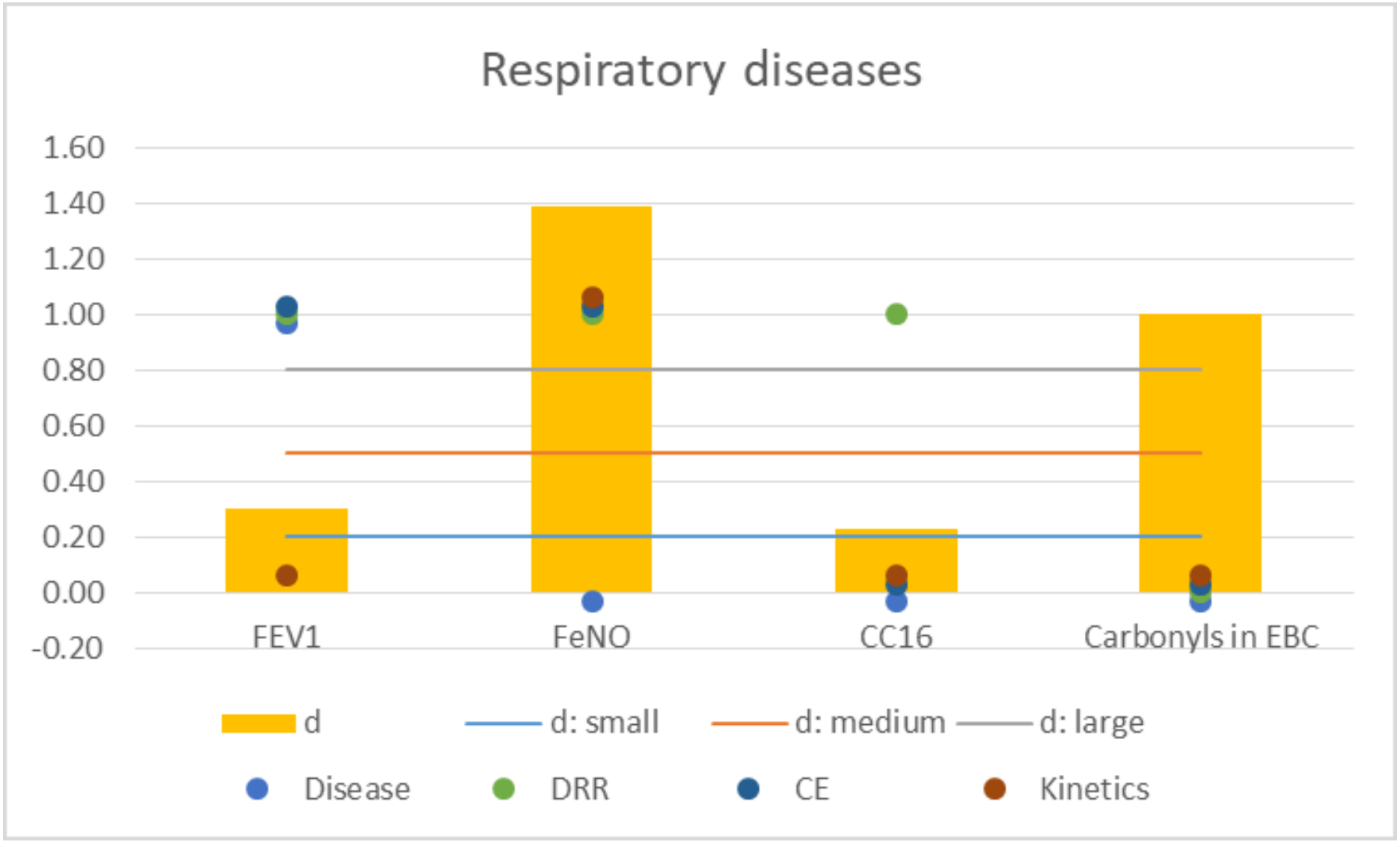
    0.0: low/weak/no data

    0.5: medium

    1.0: high/strong

- Limitations:
  - Only those BOBEs were evaluated for which data from (preferably) 3 – 5 larger studies were available

Respiratory diseases (3): *Evaluation*



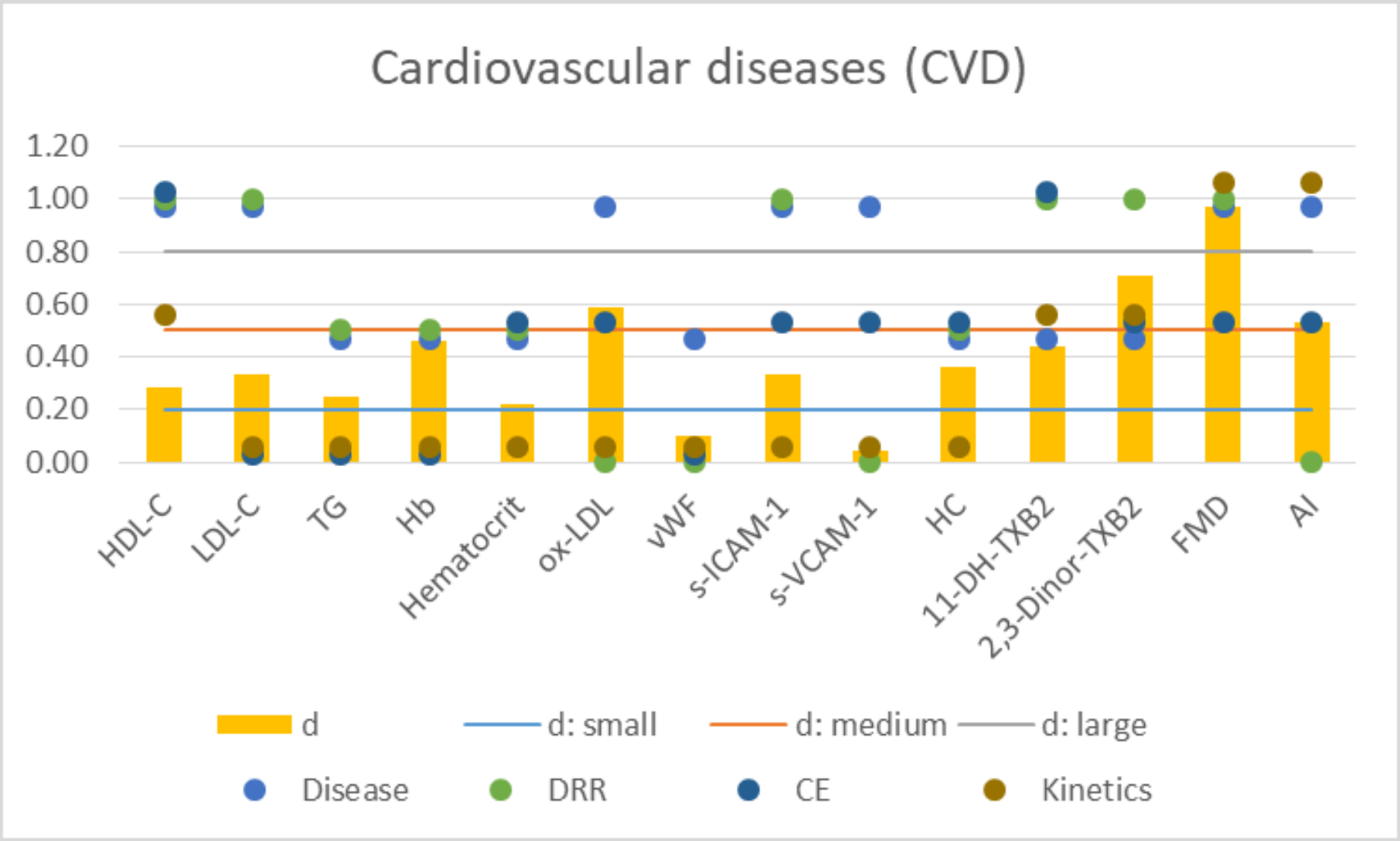
Cardiovascular diseases (1): *General considerations*

- CVD include a number of various diseases:
  - Coronary heart disease (CHD)
  - Cerebrovascular disease
  - Atherosclerosis
  - Others
- Pathological mechanisms involved:
  - Inflammation
  - Damage of arterial endothelial function
  - Endothelium-dependent vasodilation
  - Oxidative stress
  - Platelet aggregability (thromboxane)
  - Blood viscosity and coagulability
  - Insulin and lipid metabolism
- Potential biological matrices:
  - Whole blood
  - Urine, saliva

Cardiovascular diseases (2): *Potential BOBEs*

- Blood cells:
  - Hemoglobin (Hb)
  - Hematocrit
- Blood lipids:
  - HDL-C
  - LDL-C
  - ox-LDL
  - Triglycerides (TGs)
- Adhesion, coagulation, thrombogenesis:
  - Soluble intercellular adhesion melocule-1 (s-ICAM-1) in serum
  - Soluble vascular adhesion molecule-1 (s-VCAM-1) in serum
  - 11-Dehydro-thromboxane-B2 (11-DH-TXB2) in urine
  - 2,3-Dinor-thromboxane-B2 (2,3-dinor-TXB2) in urine
  - D-Dimer in serum
  - Von Willebrand Factor (vWF)
  - NO
  - Asymmetric dimethylarginine (ADMA) in serum
  - Homocysteine (HC) in plasma
- Cardiovascular functional parameters
  - Heart rate, blood pressure
  - Flow-mediated dilation (FMD)
  - Augmentation index (AI)

Cardivascular diseases (3): *Evaluation*



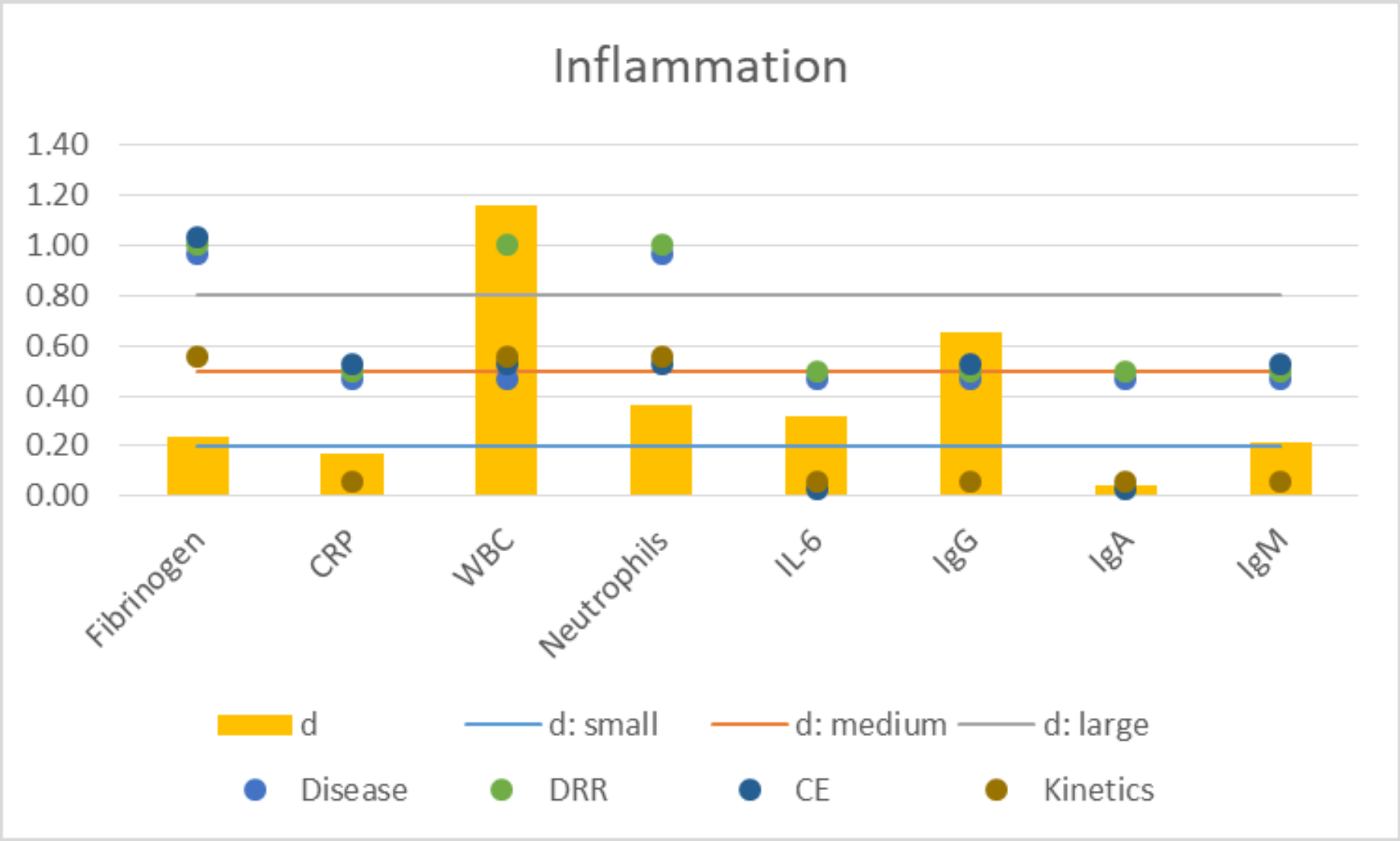
Inflammation (1): *General considerations*

- Inflammatory processes are involved in many smoking-related diseases:
  - COPD
  - Artherosclerosis
  - Cancer
- Pathological mechanisms involved:
  - Not entirely clear
  - Ox-LDL and lipid peroxidation may play roll
  - Aldehydes possibly involved
- Potential biological matrices:
  - Whole blood
  - Plasma/serum
  - Urine, saliva
  - EBC
  - Sputum, BALF

Inflammation (2): *Potential BOBEs*

- Blood proteins:
  - Fibrinogen in plasma
  - C-reactive Protein (CRP)
  - ox-LDL
  - Triglycerides (TGs)
- Blood cells:
  - White blood cell count (WBC)
  - Neutrophils
- Mediator molecules:
  - Interleukins: IL-6, IL-8, IL-12
  - Leukotrienes: LTB4, LTE4
  - Monocyte chmotractant protein-1 (MCP-1)
  - Tumor necrose-factor-alpha (TNFα)
- Immunoglobulins:
  - IgG
  - IgA
  - IgM

Inflammation (3): *Evaluation*



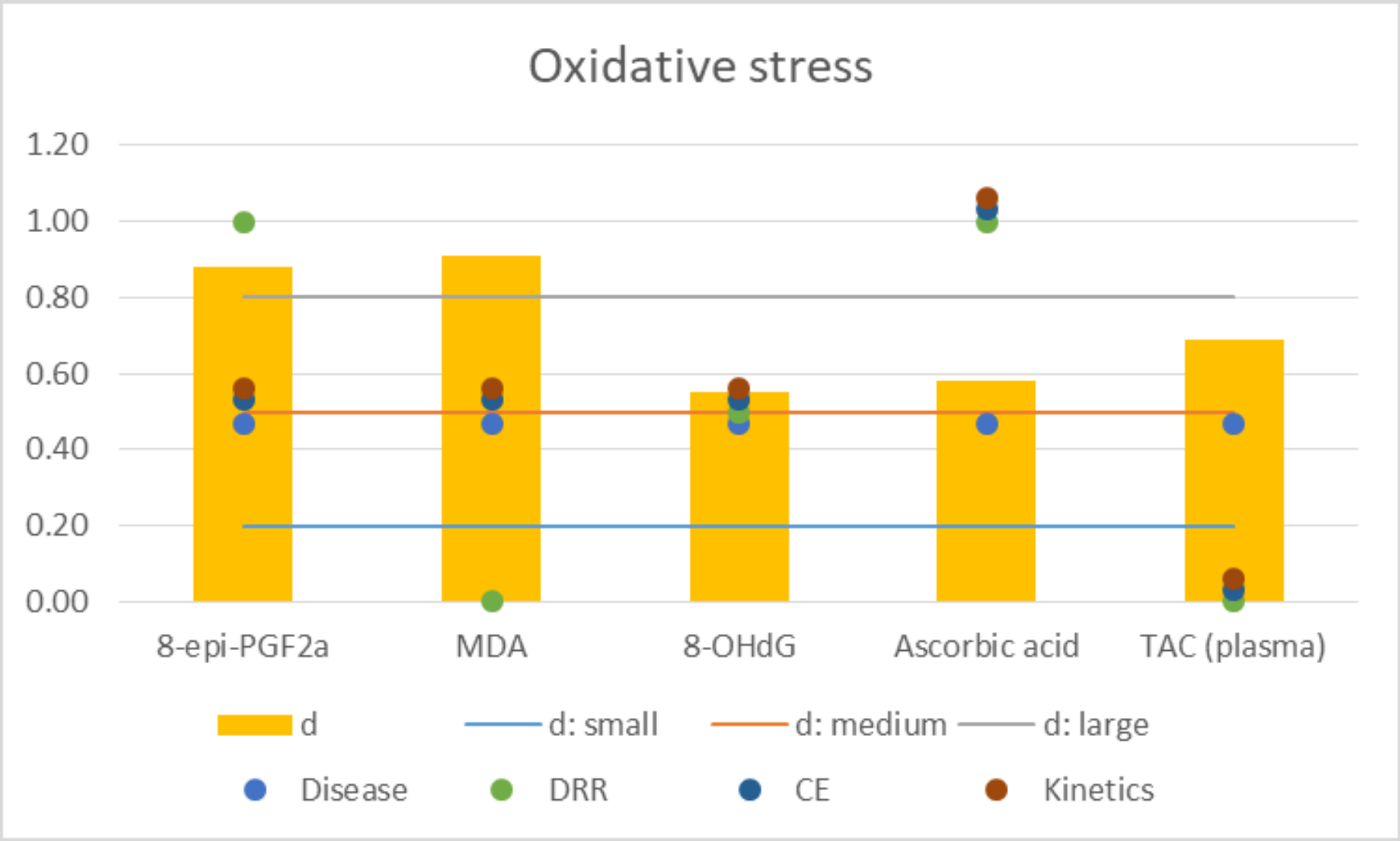
Oxidative stress (1): *General considerations*

- Oxidative stress (including lipid peroxidation and anti-oxidant capacity) is involved in many smoking-related diseases:
  - COPD
  - CVD
  - Cancer
- Pathological mechanisms involved:
  - Physiological redox equilibrium is disturbed
  - Radicals in tobacco smoke play an important role
  - Endogenous generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species) is induced by smoking
  - Enzymes (SOD, catalyse, MPO) and antioxidants (GSH, folic acid, etc.) can play important roles and are influenced by smoking
  - Micronutrients (vitamines C, E, tocopherols, carotenes, lycopene) are important as well
- Potential biological matrices:
  - Whole blood
  - Plasma/serum
  - Urine, saliva
  - EBC
  - Sputum, BALF

Oxidative stress (2): *Potential BOBEs*

- Isoprostanes:
  - 8-iso-$PGF_{2\alpha}$ in plasma and urine (also in EBC)
  - Other F_2-isoprostane species in plasma and urine
- Lipid peroxidation products:
  - Malondialdehyde (MDA)
  - 4-Hydroxy-2-nonenal (4-HNE)
  - Thiobarbituric acid reactive substances (TBARS)
  - Carbonylated proteins
- Oxidative DNA damage:
  - 8-Hydroxy-2‘-deoxyguanosine (8-OHdG)
  - Thymidine glycol
  - 5-Hydroxymethyluracil in urine
- Antioxidants:
  - Ascorbic acid
  - GSH
  - Antioxidative capacity (AC) in plasma

Oxidative stress (3): *Evaluation*



Oral cavity and buccal mucosa changes

- Of particular interest, since …
  - … first contact tissue and cells for both cigarettes and new generation products (NGPs)
  - … non-invasively available biological samples of target tissue: exfoliated buccal cells, saliva
  - … association with important tobacco-related diseases: oral cancer, periodontitis, etc.
- BOBEs for DNA damage in buccal cells:
  - Micronuclei
  - COMET assay
  - Chromosomal aberrations
  - Gene mutations
- DNA adducts (derived from endogenous genotoxic compounds):
  - 8-Hydroxy-2‘-deoxyguanosine (8-OHdG)
  - Etheno adducts (1,N^2-ethenoguanosine, ethenocytosine)
  - 1,N^2-propanodeoxyguanosine (derived from acrolein, crotonaldehyde)

Future methodologies for BOBEs

- Metabolomics
  - Can be performed in the ‘usual’ bodyfluids (blood, urine, saliva, EBC)
  - Clinical studies require strict control of other factors (particularly diet)
  - Can lead to the identification of new BOBEs (which then can be applied in targeted approaches)
  - We recently showed that in smokers who quit the metabolome changes in the direction of that of non-smokers

- Adductomics (comprises the reaction products of exogenous and endogenous electrophiles with physiological nucleophiles)
  - DNA adductomics (e.g. buccal cell DNA)
  - Protein adductomics (e.g. N-terminal valine of Hb, Cys 34 in human albumin)
  - GSH adductomics (e.g. pattern of urinary mercapturic acids)

- Micro-RNA (miRNA) patterns
  - miRNA are small (~ 22 nucleotides), non-coding RNA pieces which regulate the DNA transcription
  - miRNA can be analysed in the ‘usual’ bodyfluids (blood, urine, saliva, EBC)
  - miRNA patterns differ between smokers and non-smokers. Differences can be related to various diseases.

Summary and conclusions

- Switching from conventional cigarettes to new reduced risk products (RRPs) is an effective approach for tobacco harm reduction, provided that the new products are indeed less harmful when used over long time periods
- Application of suitable biomarkers of biological effect (BOBEs) in clinical studies would represent a practical way of proving reduced risk if favorable changes could be shown in acceptable time periods after switching (days to months).
- We propose 5 criteria for the evaluation of BOBEs, including (1) association to disease, (2) difference between smokers and non-smokers, (3) dose-response relationship (DRR), (4) reversibility, (5) kinetics upon smoking cessation.
- The second criterion can be best quantified by the parameter effect size (d), which also allow the estimation of group sizes for clinical studies. The other criteria were semi-quantitatively rated (0, 0.5, 1.0).
- Application of this evaluation system was possible for 41 established BOBEs, for which sufficient published data were available. Ten BOBEs (FeNO, 11-dh-TXB2, 2,3-dinor-TXB2, FMD, AI, WBC, IgG, 8-epi-PGF2α, MDA, 8-OHdG, ascorbic acid) were identified to be of practical value for clinical studies (~ 60 subjects/group, days to weeks duration).
- Other BOBEs could be also suitable, however would require more subjects, longer study durations or (simply) more data for evaluation.

More details, including references …

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Suitability of biomarkers of biological effects (BOBEs) for assessing the likelihood of reducing the tobacco related disease risk by new and innovative tobacco products: A literature review

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