ILSI Europe Activities in (Bio)Markers

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Biomarker:
“a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to an intervention”

Biomarkers

• are measurements that reflect biological processes
• can be physiological measurements; analyses of tissues, blood or other body fluids; metabolic data; genetic data; or measurements from bio-images
• new technologies have enabled the simultaneous measurement of genetic sequences, messenger RNAs, peptides, proteins, or metabolites resulting in patterns (or “signatures”) as biomarkers
• have relevance to medical practitioners and other healthcare professionals, researchers, the general public, patient sub-groups, industry, healthcare funders, regulators, and policy makers
The terminology can be confusing

Biomarker vs risk factor vs endpoint

Biomarkers are biological characteristics that are measured and evaluated.

Risk factors are variables that are related to an increased probability of developing a disease or injury; they may include biomarkers but also social and environmental factors.

Endpoints are clinical outcomes or events.
Biomarkers, risk factors and endpoints are all very relevant to nutrition research and are widely used.

However, nutrition researchers are often interested in a broader range of exposures and outcomes. These may include food, nutrient and non-nutrient intake from the diet; behaviour in the context of food or nutrient exposure; psychological as well as physiological outcomes; and well-being.

This goes beyond biomarkers -> markers
The conduct of high quality nutrition research requires the selection of appropriate markers, for example as indicators of food or nutrient intake, nutritional status, physiological or psychological function, health status or disease risk.

The selection of suitable markers will allow the research question to be robustly addressed, but such selection requires detailed knowledge of the markers, and consideration of the factors that may influence the measurement of these markers, other than the effects of nutritional change.
A framework to guide selection of markers within nutrition research studies would be a valuable tool for researchers in the field.

In this context, a key conclusion of the European Commission-funded project PASSCLAIM was that there is a lack of adequate markers in nutrition sciences and that there is a high need for such markers.

ILSI Europe therefore launched an activity, “Marker Initiative on Nutrition Research”, with the aim of identifying and reviewing criteria for validation of markers.
Step 1 - 2011

Approach A
Review of existing criteria for selecting marker
Done by a selected expert group through literature search covering various fields of nutrition research

Approach B
Collaboration of 9 ILSI Europe task forces
Identification of criteria for selecting markers on broadly used markers in various fields of nutrition research

- Probiotics
- Dietary Carbohydrates
- Nutrient Requirements
- Eating Behaviour & Energy Balance
- Functional Foods
- Nutrition & Mental Performance
- Metabolic Imprinting
- Nutrition & Immunity
- Addition of Nutrients to Food

Step 2 - 2012

Obtaining consensus on the criteria for evaluating markers in nutrition research
by discussing the results of step 1 in a workshop in 27-29 June 2012, Lisbon Portugal


Challenge and refine the criteria
Done by a selected expert group by the testing the criteria on markers in various fields of nutrition research

Application of criteria
Application of criteria on broadly used markers in various fields of nutrition research. Building a database of validated markers with input from 9 ILSI Europe task forces

ILSI Europe database of validated markers in various fields of nutrition research
PC Calder et al. Improving selection of markers in nutrition research: evaluation of the criteria proposed by the ILSI Europe Marker Validation Initiative Nutr. Res. Rev. 2017, in press

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ILSI Europe database of validated markers in various fields of nutrition research
Monitoring immune modulation by nutrition in the general population: identifying and substantiating effects on human health

Ruud Albers¹, Raphaëlle Bourdet-Sicard², Deborah Braun³, Philip C. Calder⁴, Udo Herz⁵, Claude Lambert⁶, Irene Lenoir-Wijnkoop⁷, Agnès Méheust⁸, Arthur Ouwehand⁹, Phoukham Phothirath¹⁰, Tomoyuki Sako¹¹, Seppo Salminen¹², André Siemensa¹³, Henk van Loveren¹⁴ and Ulrich Sack¹⁵

British Journal of Nutrition Vol. 110 Supplement No. 2 August 2013
<table>
<thead>
<tr>
<th>Levels</th>
<th>Clinical relevance</th>
<th>Biological sensitivity</th>
<th>Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical relevance</td>
<td>Clinical relevance</td>
<td>Feasibility</td>
</tr>
<tr>
<td>Proven (+++)</td>
<td>Reproducibly proven association of differential expression with differential risk</td>
<td>Proven explanation backed by human data</td>
<td>Marker is stable and validated and highly available assay and can easily be done repeatedly with high throughput (e.g., CRP)</td>
</tr>
<tr>
<td></td>
<td>Generally accepted as a risk factor (correlation with onset/resolution of the clinical endpoint)</td>
<td>High variation explainable (e.g., circadian cycle) and possible to correct it and relevant effects reproducibly superior to variation: effects likely to be observed between groups of fifties to hundreds of people</td>
<td>Service commercially available in accredited laboratories (e.g., LDT through CLIA laboratories in the USA)</td>
</tr>
<tr>
<td>Strong (+++)</td>
<td>Direct evidence linking differential response to differential risk (e.g., vaccination)</td>
<td>High variation explainable (e.g., age, sex, BMI, ethnicity and genotype) and possible to correct it with stratification and relevant effects reproducibly superior to variation: effects likely to be observed between groups of fifties to hundreds of people</td>
<td>High variation explainable (e.g., circadian cycle) and possible to correct it and relevant effects reproducibly close to variation: effects likely to be observed between groups of fifties to hundreds of people</td>
</tr>
<tr>
<td></td>
<td>Described as a cause and effect relationship, but not (yet) generally accepted as a risk factor, needs more studies or not specific</td>
<td>High variation explainable (e.g., circadian cycle) and possible to correct it and relevant effects reproducibly close to variation: effects likely to be observed between groups of fifties to hundreds of people</td>
<td>Sample can easily be made stable and limited processing (e.g., preparation of PBMC), or sample can be refrigerated for a limited time (e.g., ELISA of cytokines)</td>
</tr>
<tr>
<td>Medium (+)</td>
<td>Indirect evidence linking a change in function to a change in risk</td>
<td>Plausible mechanistic hypothesis backed by animal data</td>
<td>Sample needs to be frozen, or assay can only be done once (e.g., response to vaccination)</td>
</tr>
<tr>
<td></td>
<td>Body of evidence suggesting correlation, but cause and effect not established</td>
<td>High variation explainable (e.g., circadian cycle) and possible to correct it and relevant effects reproducibly close to variation: effects likely to be observed between groups of fifties to hundreds of people</td>
<td>Sample needs to be extensively processed or stored at (80°C or analysed fast (e.g., in-line functional assays)</td>
</tr>
<tr>
<td>Low (0)</td>
<td>Plausible hypothesis with supporting animal data</td>
<td>Plausible mechanistic hypothesis backed only by <em>in vitro</em> data</td>
<td>No commercially available LDT locally (in house) and validated/published protocols available</td>
</tr>
</tbody>
</table>

**Table 1. Criteria for the evaluation of markers**
<table>
<thead>
<tr>
<th>Classification</th>
<th>Indicative of clinical relevance and involvement of immune function(s)</th>
<th>Defence against pathogens</th>
<th>Avoidance or mitigation of hypersensitivity (e.g. allergy)</th>
<th>Inflammation control (reduction of low-grade metabolic inflammation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A marker</td>
<td>Indicative of clinical relevance and involvement of immune function(s)</td>
<td>Pathogen-specific immune response (specific antibodies and specific T-cell response after natural or experimental infection) Vaccine-specific immune response (seroprotection, seroconversion, specific antibodies and specific T cells) Specific DTH or CHS response Mucosal IgA (in saliva, tears, etc)</td>
<td>Specific response or symptoms after an experimental allergen challenge (skin, labial, respiratory or oral provocation tests) Basophil activation test</td>
<td>NA</td>
</tr>
<tr>
<td>Group B marker</td>
<td>Indicative of clinical relevance but not necessarily of the involvement of immune function(s) (i.e. clinical symptom)</td>
<td>Symptoms of infection (incidence, duration and severity after natural or experimental infection) Pathogen load</td>
<td>Tryptase in plasma Allergen-specific IgE (sIgE)* Symptoms of allergy (rhinitis, asthma, urticaria, eczema, GI manifestations, etc.)</td>
<td>Symptoms associated with low-grade inflammation (e.g. insulin resistance and blood pressure)</td>
</tr>
<tr>
<td>Group C marker</td>
<td>Indicative of the involvement of immune function(s) and associated with clinical relevance in specific (sub)populations</td>
<td>Ex vivo (pathogen-specific) B-cell function</td>
<td>Response to general food provocation Allergen-specific IgE (sIgE)*</td>
<td>In vivo response to a pro-inflammatory challenge</td>
</tr>
<tr>
<td>Group D marker</td>
<td>Provides mechanistic insights but not necessarily into clinical relevance (non-exhaustive list of examples)</td>
<td>Ex vivo (pathogen-specific) T-cell function Ex vivo phagocyte function Ex vivo NK-cell function Migration of Langerhans cells</td>
<td>Ex vivo (allergen-specific) Th1/Th2-cell function Ex vivo (allergen-induced) production of Th1/Th2 mediators Ex vivo Treg-cell function Ex vivo APC function</td>
<td>Markers of acute-phase response (CRP, TNF, IL-1, IL-6 and blood sedimentation) (Ratio of) pro- and anti-inflammatory mediators</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma adiponectin and leptin</td>
</tr>
</tbody>
</table>

(Ratio of) pro- and anti-inflammatory mediators Percentage of subsets including CD4:CD8 ratio Circulating or ex vivo-produced antibodies (not antigen- or vaccine-specific) Circulating or ex vivo-produced cytokines | (Ratio of) pro- and anti-inflammatory mediators Total IgE | Plasma and faecal calprotectin |

Plasma endotoxin (LPS) Plasma and faecal calprotectin |
The diagram illustrates the variation of biomarker values over time. It shows different reference ranges for a less favourable (sub)population and a generally healthy population. The figure is labeled with points 1 to 5, indicating specific time intervals or biomarker values.
Brain imaging and human nutrition: which measures to use in intervention studies?

Stéphane V. Sizonenko¹, Claudio Babiloni²,³, Eveline A. de Bruin⁴, Elizabeth B. Isaacs⁵, Lena S. Jönsson⁶, David O. Kennedy⁷, Marie E. Latulippe⁶, M. Hasan Mohajeri⁸, Judith Moreines⁹, Pietro Pietrini¹⁰, Kristine B. Walhovd¹¹, Robert J. Winwood¹² and John W. Sijben¹³

British Journal of Nutrition Vol. 110 Supplement No. 1 August 2013
Towards microbial fermentation metabolites as markers for health benefits of prebiotics

Kristin A. Verbeke¹, Alan R. Boobis², Alessandro Chiodini³*, Christine A. Edwards⁴, Anne Franck⁵, Michiel Kleerebezem⁶, Arjen Nauta⁷, Jeroen Raes⁸, Eric A. F. van Töl⁹ and Kieran M. Tuohy¹⁰ on behalf of the ILSI Europe Prebiotics Task Force Expert Group ‘Microbial metabolism and fermentation’
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J. de Vries et al. Markers for nutrition studies: review of criteria for the evaluation of markers. EJCN 2013; 52(7):1685-1699

ILSI Europe database of validated markers in various fields of nutrition research
Improving selection of markers in nutrition research: evaluation of the criteria proposed by the ILSI Europe Marker Validation Initiative

Philip C. Calder¹*, Alan Boobis², Deborah Braun³, Claire L. Champ⁴, Louise Dye⁴, Suzanne Einöther⁵, Arno Greyling⁵, Christophe Matthys⁶, Peter Putz⁷, Suzan Wopereis⁸, Jayne V. Woodside⁹ and Jean-Michel Antoine¹⁰
Starting criteria for marker validation (for nutrition research)

1) the marker should be validated according to recognised methods
2) the marker should reflect an endpoint (there should be a significant association between the marker and an endpoint in a target population and the marker should change consistently with a change in the endpoint)
3) the marker must respond to a dietary intervention
Aims

• To assess the use of these criteria, using a range of different possible markers reflecting the breadth of nutrition research possibilities in order to test whether the criteria were fit-for-purpose, and, if not, to propose alternatives

• To consider the development of methods for scoring markers against the pre-specified criteria, and to develop a template for this purpose
How to robustly test the criteria?

• Carefully evaluate the wording of each criterion

• Test the criteria by completing a template based on the criteria using examples of markers that reflect:
  - a broad range of interests in nutrition research
  - the use of different tools, including both questionnaires and laboratory tests
  - both long established and newer markers and tools
  - commonly used and not commonly used markers
Hence, the markers selected are not necessarily well validated or widely accepted. Markers covering the fields of nutrient intake, nutrient status, physiological function, metabolism, cognitive function, and disease risk were all evaluated.
<table>
<thead>
<tr>
<th>Specific field of application</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional epidemiology</td>
<td>Vitamin C intake determined by FFQ</td>
</tr>
<tr>
<td>Nutritional epidemiology</td>
<td>Serum/plasma vitamin $B_{12}$ as a status marker</td>
</tr>
<tr>
<td>Immune function</td>
<td>Response to vaccination</td>
</tr>
<tr>
<td>Cognitive function</td>
<td>Verbal memory</td>
</tr>
<tr>
<td>Cognitive function</td>
<td>Sustained attention</td>
</tr>
<tr>
<td>CVD/chronic inflammation</td>
<td>C-reactive protein concentration in plasma/serum</td>
</tr>
<tr>
<td>CVD/vascular function</td>
<td>Flow-mediated dilation</td>
</tr>
<tr>
<td>CVD/oxidative stress</td>
<td>$F_2$-isoprostane concentrations in urine</td>
</tr>
<tr>
<td>CVD – blood pressure</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>Metabolism and metabolic dysfunction</td>
<td>Branched-chain amino acids and their derivatives in plasma</td>
</tr>
<tr>
<td>Metabolism and metabolic dysfunction</td>
<td>FADS1 genetic polymorphisms</td>
</tr>
<tr>
<td>Intestinal barrier function/ intestinal permeability</td>
<td>Lactulose:mannitol ratio in urine</td>
</tr>
<tr>
<td>Energetics/obesity</td>
<td>Energy expenditure measured by doubly labelled water</td>
</tr>
</tbody>
</table>
Observations

Not all core components that form part of the criteria were clearly defined

In the absence of clear definitions, different individuals interpret the meaning of these terms or criteria differently

Although some markers are widely used, they fail to meet some of the criteria

Some “markers” are often used as endpoints rather than as markers (e.g. most measures of cognitive function)

There is a certain level of “turnover” of markers. This has been hastened by the emergence of new technologies, typically “omics”-based, that have enabled the simultaneous measurement of clusters or patterns of markers.
Many markers are evaluated in a static setting, for example in fasting blood samples. This separates the sample and its component markers from the reality of human physiology, which is the need to respond appropriately to “daily stressors” which may be metabolic, immune, physical (e.g. exercise, temperature), or psychological

A marker may not be equally useful across different applications

There is a challenge when considering statistical significance and clinical or biological significance

The proposed criteria could be improved upon
<table>
<thead>
<tr>
<th>Criteria to evaluate markers</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific field and related marker</strong></td>
<td></td>
</tr>
<tr>
<td><strong>SCORING CRITERIA</strong></td>
<td></td>
</tr>
<tr>
<td>Methodological aspects excluding study design</td>
<td></td>
</tr>
<tr>
<td>(Relevance of criteria can differ between different types and applications of markers)</td>
<td></td>
</tr>
<tr>
<td>Method should be validated according to recognised guidelines (please cite)</td>
<td></td>
</tr>
<tr>
<td>Appropriate* sensitivity</td>
<td></td>
</tr>
<tr>
<td>Appropriate* specificity</td>
<td></td>
</tr>
<tr>
<td>Reproducibility, accuracy, standardisation, stability (quality of the sample) and technical variation</td>
<td></td>
</tr>
<tr>
<td>Biological variation</td>
<td></td>
</tr>
<tr>
<td>Reflect/mark the study objective</td>
<td></td>
</tr>
<tr>
<td>A change in the marker is linked with a change in the endpoint in one or more target population(s)</td>
<td></td>
</tr>
<tr>
<td>Method should be validated according to recognised guidelines (please cite)</td>
<td></td>
</tr>
<tr>
<td><strong>ADDITIONAL INFORMATION</strong></td>
<td></td>
</tr>
<tr>
<td>Relevance to nutrition research</td>
<td></td>
</tr>
<tr>
<td>What is considered as a normal range for healthy people?</td>
<td></td>
</tr>
<tr>
<td>What is a significant change (consider both biological and statistical)?</td>
<td></td>
</tr>
<tr>
<td>(might vary for different applications, e.g. epidemiological studies v. individual level)</td>
<td></td>
</tr>
<tr>
<td>Is there evidence that nutrition influences the marker? If so, what is the size of the effect reported?</td>
<td></td>
</tr>
<tr>
<td>Which other factors also have an effect on the marker? (if any)</td>
<td></td>
</tr>
<tr>
<td>Other relevant information</td>
<td></td>
</tr>
<tr>
<td>Are there experimental data where dietary intervention has not resulted in an anticipated change?</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

**References**

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* Appropriate is used here to indicate that the required sensitivity and specificity of measurement may differ between study contexts, for example between a large epidemiological study and a much smaller randomised controlled trial.
Suggested a generic scoring template

<table>
<thead>
<tr>
<th>Levels</th>
<th>Methodological aspects (excluding study design)</th>
<th>Reflect/mark the study objective</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Methodological aspects (excluding study design)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levels</td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td>Appropriate sensitivity</td>
<td></td>
</tr>
<tr>
<td>(++++)</td>
<td>False negative rate (β) is well documented to</td>
<td>Minimal variation and relevant</td>
</tr>
<tr>
<td></td>
<td>be less than 1%</td>
<td>effects likely to be observed</td>
</tr>
<tr>
<td>Strong</td>
<td>Appropriate specificity</td>
<td></td>
</tr>
<tr>
<td>(+++)</td>
<td>False positive rate (α) is well documented to</td>
<td>Generally accepted marker</td>
</tr>
<tr>
<td></td>
<td>be less than 5%</td>
<td>(marker changes consistently</td>
</tr>
<tr>
<td>Medium</td>
<td>False negative rate (β) is well documented to</td>
<td>linked with a change in the</td>
</tr>
<tr>
<td>(+++)</td>
<td>be less than 10%</td>
<td>endpoint in one or more target</td>
</tr>
<tr>
<td>Low</td>
<td>False positive rate (α) is not well documented</td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>or shown to be at least 10%</td>
<td></td>
</tr>
</tbody>
</table>

Marker is reproducible (ICC>0.9) and accurate (less than 1% deviation from “correct” value), assay is highly standardised, sample is stable or can easily be made stable.

Marker is reproducible and accurate enough to detect biologically meaningful changes, assay is standardised, sample is stable or can be made stable.

Marker is reproducible and accurate enough for specific applications, assay is somewhat standardised and sample needs to be extensively processed or analysed fast.

Reproducibility and accuracy of the marker, standardisation of the assay and stability of the sample are either poor or not properly documented.

High and unexplained variation in a short time span and relevant effects likely to be observed between groups of thousands of people.

Plausible hypothesis, in use as an exploratory marker, but no substantial body of evidence (yet).

A change in the marker is linked with a change in the endpoint in one or more target population(s).

Generally accepted marker (marker changes consistently linked with a change in the endpoint).

Described as a cause and effect relationship, but not (yet) generally accepted as marker, due to a lack of (specific) studies.

Body of evidence suggesting correlation, but cause and effect not established.
Summary of this activity

• Proposed criteria for evaluating markers were tested using a total of 13 markers selected from a breadth of fields of nutrition research.
• The result of this testing was a modified list of criteria and a template.
• Subsequently a system for scoring a marker and an associated template were developed.
• This system will enable researchers to evaluate and to compare different candidate markers within the same field of nutrition research in order to identify their relative usefulness.
• It is anticipated that defining the scoring system and then using this to score possible markers would be done by researchers as a part of their study planning.
Comments and next steps

The ranking criteria of proven, strong, medium, or low are likely to vary according to research setting, research field and the type of tool used to assess the marker and therefore the criteria need to be determined in a setting-, field- and tool-specific manner.

The next step is to use the evaluation criteria and scoring system to evaluate markers.

It is anticipated that ILSI Europe will hold an open access “library” of completed evaluations, using the templates developed in this activity that will be available to the nutrition community for use, comment, modification, and updating.
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