WHAT MAKES A MARKER?

Marker Validation Initiative in Nutrition Research

Loek Pijls
Nestlé Research Center & Nestlé Health Science
A TALE OF THE MARKER AND THE MARKED
1. It is *not* about the marker, but about the marked (health)

2. Marker of *what*?

3. How do we know if a marker actually *marks*?
1. Some – more – ideas
2. ILSI Europe
   a) MARKERS History
   b) Marker Validation Initiative
1. Blood pressure -> risk stroke & CHD
2. Cholesterol -> cardiovascular risk
3. Body temperature -> fever
Marker - marked?
Lisbon Case study: Appetite

Endpoint = Appetite score
• Marker = hormone level
• Marker = food consumed

Endpoint = food consumed
• Marker = appetite score
Marker: principles

- No *face validity*; not relevant *itself*
- Something that *marks* something else that *is* relevant for health
- Health endpoint: impacts noticeably, now or later, on a person’s – quality of - life
Validity

• Has value
• Reflects something of interest
• We cannot validate a factor in sense of making it valid:
  • We can only assess whether or not it is valid
• If not, we look for other ones..
Not valid because e.g.:

1. One in complex system of compensation, homeostasis and abundance
2. Changing predictors does (not) change health risk
3. Small, meaningless changes
1. Risk, predictor, intermediate: express future

2. No such thing as intermediate endpoint

3. Independent: Specify, or don’t say it

4. Clinical (trial):
   1. Subjects: patients?
   2. Outcomes: as in clinic, hospital?
   3. Setting: clinic, hospital?
<table>
<thead>
<tr>
<th></th>
<th>Marker</th>
<th>Factor</th>
<th>Predictor</th>
<th>Endpoint</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bio-</strong></td>
<td>Biomarker</td>
<td>Biological factor</td>
<td>Biological predictor</td>
<td>Biological Endpoint</td>
<td>Biological outcome</td>
</tr>
<tr>
<td><strong>Risk</strong></td>
<td>Risk marker</td>
<td>Risk factor</td>
<td>Risk predictor</td>
<td>Risk endpoint</td>
<td>Risk outcome</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>Intermediate marker</td>
<td>Intermediate factor</td>
<td>Intermediate predictor</td>
<td>Intermediate endpoint</td>
<td>Intermediate outcome</td>
</tr>
<tr>
<td><strong>Independent</strong></td>
<td>Independent marker</td>
<td>Independent factor</td>
<td>Independent predictor</td>
<td>Independent endpoint</td>
<td>Independent outcome</td>
</tr>
<tr>
<td><strong>Surrogate</strong></td>
<td>Surrogate marker</td>
<td>Surrogate factor</td>
<td>Surrogate predictor</td>
<td>Surrogate endpoint</td>
<td>Surrogate outcome</td>
</tr>
<tr>
<td><strong>Predictive</strong></td>
<td>Predictive marker</td>
<td>Predictive factor</td>
<td>Predictive predictor</td>
<td>Predictive endpoint</td>
<td>Predictive outcome</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td>Clinical marker</td>
<td>Clinical factor</td>
<td>Clinical predictor</td>
<td>Clinical endpoint</td>
<td>Clinical outcome</td>
</tr>
</tbody>
</table>
Maintain health = keep disease out

1. maintain health = prevent disease = reduce risk of disease

2. If disease risk reduction is not maintaining health, then what is it?

3. If maintaining health is not reducing disease risk, then what is it?

4. Prevention = risk reduction, sometimes to 0, often not
1. Some – more – ideas

2. ILSI Europe
   a) MARKERS History
   b) Marker Validation Initiative
FUFOS: From evidence based on markers for functional foods to types of claims relevant to them

Consumption of functional food component

Markers of exposure to food component

Markers of target function / biological response

Markers of intermediate endpoint

Enhanced target function

Reduced risk of disease

TYPE A CLAIMS (enhanced function)

TYPE B CLAIMS (reduced risk of disease)
Assess scientific support for health claims on foods:

- Direct evidence of benefits to humans
- Usefulness of markers of intermediate effects
- Effects evaluated by the markers should be statistically and biologically meaningful.

Areas reviewed:
- CVD
- bone health,
- physical performance
- body weight & diabetes
- diet-related cancer,
- gut and immunity
- mental state
1. Some – more - ideas

2. ILSI Europe
   a) MARKERS History
   b) Marker Validation Initiative
• 3 years
• 3 steps
• 9 Task Forces
Marker Validation Initiative in Nutrition Research

Review of existing criteria

Approach A

Review of existing criteria for selecting markers

Done by a selected EG through a literature search covering all fields of nutrition research
Results A

- Analytics: well measurable
- Marker’s effect on endpoint: biologically plausible
- Association marker & endpoint
- In response to intervention
  - Marker changes consistently with endpoint
  - $\Delta$ marker explains substantial part of $\Delta$ endpoint
The practical approach:

Approach B
Identification of criteria for selecting markers based on broadly used markers in the various fields of nutrition research

- Probiotics
- Dietary Carbohydrates
- Nutrient Requirements
- Eating behaviour and Energy Balance
- Functional Foods
- Nutrition and Mental Performance
- Metabolic Imprinting
- Nutrition and Immunity
- Addition of Nutrients to Food
## Approach B: questions

### Validation

<table>
<thead>
<tr>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>When was the marker accepted as a valid marker? Reference is needed: is it an article, a review, a meta-analysis, a consensus… ? Why was it accepted as a valid marker?</td>
</tr>
<tr>
<td>Has the marker replaced a former one used to explore the same target? What were the arguments to agree on using the new marker?</td>
</tr>
<tr>
<td>Is there a relationship between the value of the marker and the intensity of the function/status…? Is it a linear one? Are there threshold values when the relationship between the marker and the function/status change?</td>
</tr>
<tr>
<td>What is a biological relevant change in the level of the marker? How was that determined?</td>
</tr>
<tr>
<td>Which method(s) was/were used to validate the marker?</td>
</tr>
<tr>
<td>How is the analytical variability (intra and inter laboratory variations) determined?</td>
</tr>
<tr>
<td>What are the limitations of the marker? Should we expect a better marker to replace that new marker? If yes, why?</td>
</tr>
</tbody>
</table>
## Approach B: Results

Table 19: Combined criteria identified by the Approach B expert groups

<table>
<thead>
<tr>
<th>Technical feasibility/usability</th>
<th>Adequacy to nutritional interventions</th>
<th>Predictive and prognostic value, and surrogacy or Biological relevance and plausibility</th>
<th>Adequacy of interpretation</th>
<th>Body of evidence vs. innovation or Scientific excellence</th>
</tr>
</thead>
<tbody>
<tr>
<td>The methodology used to measure a marker should be calibrated</td>
<td>A biomarker value should change as a result of an intervention</td>
<td>In assessing the risk prediction of a biomarker, the marker should demonstrate added value to this risk beyond traditional and conventional risk factors.</td>
<td>Researchers should carry out their studies using a panel of the disease risk biomarkers</td>
<td>Existence of a solid body of cohorts/studies in which the marker has been tested</td>
</tr>
<tr>
<td>The methodology used to measure a marker should be technically standardized, meaning that the methodology is generally approved</td>
<td>Sensitivity to change</td>
<td>A biomarker change should reflect in the underlying pathophysiology and eventually should reflect a change in hard endpoints such as disease event rate or mortality</td>
<td>The results of a marker measurement should be analysed and interpreted in a statistically valid way</td>
<td>Gold standard (same as for widespread use; process on how to get to a validated marker)</td>
</tr>
<tr>
<td>The methodology used to measure a marker, like the sampling, should practical and easy to use</td>
<td>The marker should be sensitive to intervention or manipulation with micro- or macronutrients</td>
<td>Researchers should use a marker that is strongly related to the mechanism of the targeted endpoint</td>
<td>Good statistical correlation with other standard tests/markers in the same domain</td>
<td>Demonstration of biomarker value in several independently performed studies, ideally studies by independently working research groups.</td>
</tr>
<tr>
<td>Construct validity</td>
<td>The marker should be influenced by nutrition</td>
<td>The results of a marker measurement should be analysed and interpreted in a biologically relevant way</td>
<td>The marker should have good psychometric properties such as test-retest reliability, construct validity</td>
<td>Good scientific literature to back-up the use of the marker</td>
</tr>
<tr>
<td>Reasonable within subject variation</td>
<td>The marker should show sensitivity to nutritional interventions.</td>
<td>The marker should be validated against specific populations (e.g. discrimination between healthy and impaired groups)</td>
<td>The marker should consistently correlate with other markers measuring the same biological function with a different methodology</td>
<td>Large prospective studies should establish this predictive value and/or link.</td>
</tr>
<tr>
<td>Reasonable/ explainable between subjects variation</td>
<td>Evidence that the marker can be modulated by a dietary constituent in a nourished healthy population</td>
<td>The marker should be related to tissue function and structure as measured with other techniques</td>
<td>Correlates with relevant clinical endpoint</td>
<td>Proper clinical trials are needed to investigate the effect of nutritional intervention on the marker</td>
</tr>
</tbody>
</table>
Marker Validation Initiative: Step 2 in Nutrition Research

**Methodology:**

- Compare criteria from approach A with criteria resulting from approach B
- Analyse similarities and differences
- Identify consensus criteria applicable to all fields in nutrition research
- Identify specific criteria for specific fields, if relevant
- Can we create a grading system (e.g. GRADE)?
Lisbon Criteria

- Analytics: well measurable
- Association:
  - marker & health endpoint
  - $\Delta$ marker & $\Delta$ health endpoint within & across studies
- Plausibility
Next

• Elaborate Lisbon criteria
• Guide & evaluate their use
• Immunity approach → apply generically?
• Apply to current & future markers:
  • let go of non-markers
  • identify new markers
<table>
<thead>
<tr>
<th>Level</th>
<th>Biological relevance</th>
<th>Biological sensitivity</th>
<th>Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>differentially expressed</td>
<td>correlated with clinical endpoint</td>
<td>within subject variation</td>
</tr>
<tr>
<td></td>
<td>differentially expressed</td>
<td>linked to causal pathway</td>
<td>between subjects variation</td>
</tr>
<tr>
<td></td>
<td>reproducibly proven association of differential expression with differential risk</td>
<td>general accepted as a risk factor (correlation with onset/resolution of the clinical endpoint)</td>
<td>minimal variation, relevant effects highly superior to variation: effects likely to be seen between groups of 10's of people</td>
</tr>
<tr>
<td></td>
<td>strong (++)</td>
<td>described cause and effect relationship but not (yet) generally accepted as risk factor, need more studies or not specific</td>
<td>high variation explainable (e.g. circadian cycle...) possible to correct it, relevant effects reproducibly superior to variation: effects likely to be seen between groups of 50-100's of people</td>
</tr>
<tr>
<td></td>
<td>medium (+)</td>
<td>body of evidence suggesting correlation but cause and effect not established</td>
<td>high variation explainable (e.g. circadian cycle...) possible to correct it, relevant effects reproducibly close to variation: effects may be seen between groups of 50-100's of people</td>
</tr>
<tr>
<td></td>
<td>low (0)</td>
<td>plausible hypothesis with supporting animal data</td>
<td>high and unexplained variation on a short time span, relevant effects likely to be seen between groups of 1000's of people</td>
</tr>
</tbody>
</table>

1 Table 18. Criteria for evaluation of immune markers
## Applied criteria to example markers of immunity
(context: general population, resistance to infections)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Response to vaccination</th>
<th>Ex vivo NK cell activity (PBMC)</th>
<th>CD4/CD8 (HIV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Analytical aspects</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2 Reflect /mark endpoint</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 association</td>
<td>+++</td>
<td>++</td>
<td>0 (+++</td>
</tr>
<tr>
<td>2.ii association of changes</td>
<td>+++</td>
<td>++</td>
<td>0 (+++</td>
</tr>
<tr>
<td>2.iii relevant target population</td>
<td>+++</td>
<td>++</td>
<td>0 (+++</td>
</tr>
<tr>
<td>2.iv plausibility, causal relationship, Mechanism</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3 must respond to dietary intervention?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. It is not about the marker, but about the marked (health)
2. Marker of what?
3. How do we know if a marker actually marks?