Title: Providing the measurement infrastructure to allow quantitative diagnostic methods for biomarkers of coronary heart diseases

Abstract

Coronary heart disease is one of the main challenges for health care in the EU with 11.3 million new cases and 1.8 million deaths per year. Cardiac biomarkers help to confirm the diagnosis of coronary heart diseases, provide prognostic information and, thus, enable successful treatment. However, the results from mandatory inter-laboratory comparisons for the main biomarkers for coronary heart diseases, vary widely and because there is a lack of SI traceable reference measurement procedures for such biomarkers, inter-laboratory comparisons can only produce method specific consensus values (determined as the median value for a method). Therefore traceable reference methods for the main biomarkers for coronary heart diseases such as cardiac troponin (cTn), apolipoproteins and myoglobin (MYO) and N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) for heart failure, are needed. Furthermore, the structural heterogeneity of the most important biomarkers should be investigated as well as their limits of quantification (LOQ).

Keywords

Biomarker, coronary heart diseases, reference methods, quantitative diagnostics, structural heterogeneity

Background to the Metrological Challenges

In 2015, 49 million people were living with coronary heart disease, which meant their condition had to be monitored on a regular basis using, cardiac biomarkers with certified medical tests. Important health relevant parameters for such biomarkers e.g. cTn and their respective concentrations and permissible deviations are defined in directives such as the 'Richtlinie der Bundesärztekammer' (Directive of the German Medical Association). Clinical laboratories then have to prove their capability to measure these quantities in mandatory inter-laboratory comparisons. However, results from these mandatory comparisons are inconsistent, for example in the CM4/17 comparison organised by the Reference Institute for Bio-analytics there was an 85 % coefficient of variation for cTn. Currently, cTn, quantification methods are available, however no reference methods or reference values exist. The available methods are also neither standardised nor harmonised and in most cases, the target molecule has not been characterised in detail.

The European Society of Cardiology (ESC) has introduced guidelines on the prevention, diagnosis and treatment of coronary heart diseases such as acute and chronic heart failure. Biomarkers such as N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) are especially important for the assessment of the status for heart failure. For coronary heart diseases, the guidelines recommend amongst others MYO as an indicative biomarker besides standard cTn medical tests. MYO in blood is a marker for muscle damage, and is usually found in blood only in very low concentrations. Its function of binding oxygen in striated muscle tissue such as cardiac or skeletal muscles is similar to the function of haemoglobin in blood. Therefore with damage to the heart muscle, the MYO concentration in blood increases. However, as it is also released during other muscle damages, it can only serve as indicative marker. Therefore research is needed into concentration variations of MYO in heart diseases.

Apolipoproteins also play an important role in lipid metabolism and in atherosclerotic processes and they represent important biomarkers for coronary heart disease risk assessment. However, at present apolipoproteins are predominantly measured in expert clinical laboratories and are rarely used for diagnosis in routine clinical practice. Furthermore, these tests are matrix sensitive and are not reliable in some pathological states.

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The measurement of cardiac biomarkers with quantitative cardiac Magnetic Resonance Imaging (cMRI) parameters is a novel diagnostic approach. cMRI provides detailed quantifiable information on the patient's coronary ejection fraction, end diastolic volume, end systolic volume, heart-time volume and cardiac mass. For patients with coronary heart disease, cMRI can be used to quantify the extent of acute myocardial infarction by volumetric analysis which enables the calculation of the mass of perished myocardial tissue. Therefore, comparing the results of cMRI with different diagnostic techniques, is of great interest for estimating coronary heart disease risk.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The JRP shall focus on the characterisation of selected cardiac biomarkers and development of reference measurement procedures for the quantification of these biomarkers to provide traceability and comparability to the results of laboratory medicine.

The specific objectives are:

1. To develop and document reference measurement procedures for traceable quantification of proteins serving as biomarkers for coronary heart diseases such as cardiac troponin (cTn), myoglobin (MYO) and apolipoproteins using molecular and elemental mass spectrometry coupled to various separation techniques. This includes the preparation and characterisation of necessary isotopically enriched spike materials by either replacing the metal contained in the relevant proteins with metal ions enriched in one isotope or synthesising the analytes using amino acids enriched in ^{13}C, ^{15}N or ^{34}S. These procedures should target very low limits of quantification (LOQ) for these biomarkers (e.g. 10 ng/L to 100 ng/L for cTn) as well as ensuring the homogeneity of the samples and spike materials.

2. To develop a reference measurement procedure for quantification of biomarkers of heart failure such as the 1-32 brain natriuretic peptide and the N-terminal pro-Brain Natriuretic Peptide (NT-ProBNP) with the final aim of providing reference values to proficiency testing schemes and improving patient outcomes through standardisation of bioassays. This includes i) the development of mass spectrometry based methods to monitor the metabolites of brain natriuretic peptides in patient samples and of epitope mapping strategies to identify the source of variability of bioassays; ii) the development of reference measurement procedures; iii) the provision of reference values to proficiency testing schemes and standardisation of immunoassay methods.

3. To develop and apply complementary methods such as cardiac magnetic resonance imaging (cMRI) in combination with computed tomography for accurately ruling out heart failure and correlation with the results from the biomarker analysis.

4. To develop fast, selective and highly efficient enrichment methods to achieve the demanded low LOQs. Enrichment should be performed by selective binders (such as antibodies, nanobodies, aptamers, peptides, receptors, designed imprinted polymers) using any form of bio interaction. The development of fast and quasi-continuous monitoring of cardiac biomarkers should be performed via quick tests or biosensor probes. This enables a very early diagnosis of heart attacks by detecting an increase of the respective biomarkers, which is a much more reliable indicator than the absolute concentration of a biomarker.

5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain, IVD producers and relevant national clinical associations, standards developing organisations (including the Joint Committee on Traceability in Laboratory Medicine, ISO and CEN) and end users clinical reference laboratories.

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners, hospitals and industry is strongly recommended, both prior to and during methodology development.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this. In particular, proposers should outline the achievements of the EMRP project HLT05 Metallomics and how their proposal will build on those.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.8 M€, and has defined an upper limit of 2.1 M€ for this project.

EURAMET also expects the EU Contribution to the external funded partners to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the "end user" community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your JRP results are going to:
- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Transfer knowledge to the health sector.

You should detail other impacts of your proposed JRP as specified in the document "Guide 4: Writing Joint Research Projects (JRPs)"

You should also detail how your approach to realising the objectives will further the aim of EMPIR to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:
- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work

Time-scale

The project should be of up to 3 years duration.