Cohort profile: ‘Biomarkers of Personalised Medicine’ (BioPersMed): a single-centre prospective observational cohort study in Graz/Austria to evaluate novel biomarkers in cardiovascular and metabolic diseases

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ABSTRACT

Purpose Accumulating evidence points towards a close relationship between cardiovascular, endocrine and metabolic diseases. The BioPersMed Study (Biomarkers of Personalised Medicine) is a single-centre prospective observational cohort study with repetitive examination of participants in 2-year intervals. The aim is to evaluate the predictive impact of various traditional and novel biomarkers of cardiovascular, endocrine and metabolic pathways in asymptomatic individuals at risk for cardiovascular and/or metabolic disease.

Participants Between 2010 and 2016, we recruited 1022 regional individuals into the study. Subjects aged 45 years or older presenting with at least one traditional cardiovascular risk factors or manifest type 2 diabetes mellitus (T2DM) were enrolled. The mean age of the participants was 57±8 years, 55% were female, 18% had T2DM, 33% suffered from arterial hypertension, 15% were smokers, 42% had hyperlipidaemia, and only 26% were at low cardiovascular risk according to the Framingham ‘Systematic COronary Risk Evaluation’.

Findings to date Study procedures during screening and follow-up visits included a physical examination and comprehensive cardiovascular, endocrine, metabolic, ocular and laboratory workup with biobanking of blood and urine samples. The variety of assessed biomarkers allows a full phenotyping of individuals at cardiovascular and metabolic risk. Preliminary data from the cohort and relevant biomarker analyses were already used as control population for genomic studies in local and international research cooperation.

Future plans Participants will undergo comprehensive cardiovascular, endocrine and metabolic examinations for the next decades and clinical outcomes will be adjudicated prospectively.

INTRODUCTION

Cardiovascular (CVD) and metabolic diseases (MD) are globally representing the most important cause of disability and premature
death. Next to our genetic programming, modern lifestyle, including the use of tobacco, unhealthy nutritional habits, physical inactivity, and psychosocial stress are major risk factors of CVD and MD within different age groups not only promoting excess cardiovascular (CV) and metabolic morbidity but ultimately triggering excess mortality. In turn, primary prevention of these diseases has the potential to avoid many of related deaths. However, the initial euphoria about a decline of CVD prevalence at the beginning of this century gradually gives way to a sense of frustration. This comes in the light of increasing numbers of type 2 diabetes mellitus (T2DM), a very high lifetime-risk for the development of heart failure with stable incidence over the last decades, and a persistent high stroke mortality. In addition, the relevant underdiagnosing and/or undertreatment of patients at high risk for CVD related risk factors remains of utmost important. Therefore, early detection of asymptomatic CV and/or metabolic risk remains a crucial challenge in the prevention of both, onset and progression of CVD as well as of related complications.

Considering the multiplicity of risk pathophysiology, an integrative approach is needed to identify novel and to validate established CV and metabolic biomarkers for their scientific and clinical utility. Practical biomarkers are required to facilitate (1) the understanding of underlying mechanisms of disease development, (2) the detection of potential targets for specific preventive therapies, and finally (3) the precise estimation of individual risk. For this purpose, there is an unmet need for a cohort studies recruiting individuals at risk for CVD and/or MD well before clinical manifestation of the diseases.

In the BioPersMed cohort (Biomarkers of Personalised Medicine), we enrolled community dwelling and asymptomatic individuals from the regional communities who were at risk for CVD or MD in order to evaluate the predictive value of various traditional and novel biomarkers and to observe disease courses starting in the preclinical, asymptomatic phase. The latter shed light on different pathways of CVD and MD development by use of cutting edge laboratory measurements, advanced imaging techniques, comprehensive genetic investigations, and state of the art functional tests. The BioPersMed project is designed as a single-centre, prospective, observational study. Only asymptomatic subjects without diagnosed CVD but with at least one traditional CV risk factor were eligible to participate. According to the published European Guidelines on CVD prevention in clinical practice, traditional CV risk factors besides of age and gender comprise (1) smoking, (2) elevated total cholesterol levels and (3) arterial hypertension. Moreover, sedentary lifestyle, obesity, social environment, T1DM or T2DM, low High-density lipoprotein (HDL) cholesterol, increased triglyceride levels, elevated fibrinogen, apolipoprotein B, lipoprotein(a), familial hypercholesterinaemia, increased high sensitivity C reactive protein, preclinical evidence of atherosclerosis and chronic kidney disease (glomerular filtration rate ≤60mL/min/1.73m²) were regarded as additional potential CV and MD risk factors. From October 2010 (first patient in) to February 2016 (last patient in), we enrolled a total of 1022 community dwelling adult men and women who live in the greater Graz area via an established recruitment network, consisting of general practitioners, peripheral hospitals, and in most cases through the outpatient clinics of the Departments of Cardiology as well as Endocrinology and Diabetology, respectively.

Subjects presenting with significant non-CVD or who were expected not to be able to complete study specific examinations, were excluded from participation. The BioPersMed study was conducted in compliance with the laws and guidelines of the Medical University of Graz and complies with the Declaration of Helsinki and the Austrian laws. All participants in the BioPersMed cohort were thoroughly checked for inclusion and exclusion criteria before the first phenotyping at baseline examination in order to avoid screening failures.

The baseline examinations have been repeated every 2years in addition to interim telephone visits, which take place between the on-site visits. A summary of all examinations is shown in [figure 1](#), and a more detailed description can be found in online supplemental table S1-S10.

According to the presented scheme, participants will be followed for the next decades and clinical outcomes are adjudicated prospectively. A total of 169 (17%) participants dropped-out for various reasons. Causes for premature unintended termination of the study ranged from a change in the place of residence (n=5) to limited personal time or lacking will for continuous study visits (n=136) or new onset of non-CV-related diseases (cancer: 4, accident: 3, other: 9). Twelve people have died so far (cancer: 7, sepsis: 3, CVD: 2). In summary, 1022 persons were included in the baseline examination and 799 persons attended the first follow-up 2years after baseline examination. With 1 September 2021, 628 persons have completed the second follow-up at 4years after baseline visit, 531 persons have completed the third follow-up at 6years after the baseline visit, and 225 persons have completed the follow-up of 8years after the baseline visit. A small number of participants skipped one follow-up
but decided to continue to participate in the study. This issue explains the discrepancy between the number of drop-outs and the number of missing follow-up visits. A detailed overview of the recruitment process and study protocol is presented in figure 2.

At baseline and at regular 2 years-follow-ups, an in-depth diagnostic CV and MD work-up was carried out, laying emphasis on standardisation and reproducibility of history taking, questionnaires for health, psychological and sleep issues, physical examination, ECG, laboratory/blood sampling with biobanking, exercise testing (6 min walking test, grip strength, spiroergometry), echocardiographic analysis of cardiac structure and function, pulmonary function testing, carotid intima/media-thickness measurement, pulse-wave analysis (PWA), and ophthalmologic examinations as well as body composition, bone density including bone and hormonal biomarkers, and oral glucose tolerance testing (OGTT). The number of examinations performed at each visit increased over time, due to additional new diagnostic tools (eg, non-mydriatic funduscopy). A detailed description of all methods used, as well as a concise overview of the assessed data can be found in the online supplemental file 1. Statistics have been calculated using RStudio V.1.2.5033 (RStudio, USA). Besides the already included packages others, namely ‘hmisc’ (version : 4.6), ‘ggplot2’ (3.3), ‘tidyverse’ (1.12), ‘readxl’ (1.3), ‘MatchIt’ (4.1), ‘data.table’ (1.13), ‘dplyr’ (1.0) and ‘lubridate’ (1.7), were needed for structuring of data, analysis and visual representation. Normal distribution of data was tested and, if positive, Pearson-correlation calculated. In case of a violation of normal distribution, a non-parametric equivalent was used. A description of the data is given in the corresponding tables. GCP trained and authorised independent persons from the Clinical Trials Unit at the Medical University Graz have carefully evaluated and monitored the data and relevant procedures around the cohort study. Based on the steering committees agreement, risk based monitoring of 100% of the baseline dataset and 80% of the follow-up dataset was performed. This was done to generate an adequate security of the structure and verification of the assessed data.

Biological and technical outliers were manually identified and corrected. This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)-ME recommendations for reporting cohort studies.
Health status
At each visit, a careful assessment of the participants’ health status was performed. Data collection documents anthropometric, biochemical, metabolic, hormonal, dietary, physical activity, socioeconomic, medical and other variables.

Questionnaires
Questionnaires at all clinical visits include the Short-Form 36 Health Survey and the Hospital Anxiety and Depression Scale, as well as other questionnaires to assess depressive or disease-related symptoms and sleep qualities.16–21 Raw data of these questionnaires have been collected and are available for further analysis.

Echocardiography
Cardiac chamber geometry and function were assessed via state-of-the-art transthoracic echocardiography. Two-dimensional, Doppler-mode and M-mode echocardiography were performed following standardised protocols according to current guidelines and are digitally archived.

Physical functioning and exercise capacity spiroergometry
For the assessment and interaction of vascular disease, CV risk and biomarkers, an assessment of physical functioning/exercise capacity was essential. Complementary to questionnaires on physical activity, physical fitness and symptom-limited cardiopulmonary exercise capacity was assessed by spiroergometry, 6 min walk test22 and handgrip measurements.23 24

Pulse wave analysis and ECG
PWA and pulse wave velocity measurements were performed according to the expert consensus published by Laurent et al.25 Additionally, a 12-lead ECG was performed in every patient.

Ultrasound of the abdomen
Routine abdominal ultrasound examinations with special attention to liver and spleen characteristics (dimensions, texture, abnormalities) were carried out at each visit.

Carotid ultrasound examinations
Carotid ultrasound examinations were performed in supine position on the right and left side. The intima/media thickness of the left and right common carotid artery is measured.26

Bone density and body composition measurements
Regular dual energy X-ray absorptiometry measurements of bone density at the spine, hip and whole-body density include body composition and Trabecular Bone Score.27

Laboratory and functional metabolic measurements
Sample acquisition includes serum, plasma, saliva and urinary as well as stool samples. Routine tests include liver and kidney function and electrolytes, blood counts, hormonal and metabolic data including lipid profiles and urinary analyses. A standardised OGTT (including insulin and c-peptide) was performed. These materials were collected at each visit for immediate analysis as well as biobanking (including samples of function tests).

Biobanking
Serum, EDTA and citrate plasma, whole blood and cell pellets, spot urine, and saliva were collected at each study visit. They were immediately aliquoted and stored at the biobank of the Medical University of Graz (Austria) at –80°C.28 Biobanking guarantees an accurate description of the sample collection and sample handling according to the STROBE-ME recommendations.15 Biospecimen-derived measurements adhere to the European guidelines.29
Autoantibody phenotyping
Routine measurement of thyroid autoantibodies and autoimmune parameters for coeliac disease were performed in all participants. Further exploratory autoantibody detection (endocrine receptors, various epitopes) was done using newly developed luciferase-based fusion protein assays together with the Charité-Universitätsmedizin Berlin, Germany.30

Metabolic phenotyping by nuclear MR
(Un)targeted metabolomic analysis of 1012 baseline serum and urine samples was performed by nuclear MR spectroscopy and state-of-the art chemoinformatics.31–33 Metabolite and lipoprotein concentrations were determined using Topspin and the Pre-Clinical Screening and In Vitro Diagnostics Research package of Bruker.34–36

Genome-wide characterisation of cohort
A whole genome global screening array (Illumina bead chip (Infinium Global Screening Array-24 V.2; Illumina, USA)) with nearly 700k specific single nucleotide polymorphisms (Illumina, in cooperation with the Human Genotyping Facility at the Erasmus University Rotterdam, NL) is available from all cohort participants.

Optometric phenotyping
In addition, a large part of the cohort has been assessed by non-mydriatic retinal photography since 2015. Ophthalmologic examinations comprised undiluted fundus photography and optical coherence tomography including the assessment of retinal vessel diameters.

Data monitoring and quality assurance
All incoming data are checked by the study staff for completeness and plausibility and are entered into an electronic data capture system (OpenClinica; www.openclinica.com), specifically adapted for this project. Additional validation processes such as cross-validation with that is, external independent validation in samples were regularly performed. External monitoring by certified clinical monitors has been done in 100% of the baseline study records. Adequate external monitoring of the follow-up data is regularly ongoing.

Patient and public involvement
No patient involved.

FINDINGS TO DATE
A descriptive overview of the cohort including the CV, endocrine and metabolic risk profile is given in table 1. More female (55%) than male (45%) persons were included in the study. The mean age of the participants is 57±8 years and the mean body mass index (BMI) is 26.5±4.5 kg/m². A majority of 59% of the examined persons has a BMI greater than 25 kg/m². Although asymptomatic, only 26% of the study population is considered to be at low CV risk according to the Framingham ‘Systematic COronary Risk Evaluation’,37 while 38% show

| Table 1 Cardiovascular, endocrine, and metabolic risk profile of the BioPersMed cohort (N=1022) |
|---|
| **Sex** |
| Men | 455 | 45 |
| Women | 567 | 55 |
| **Smoking** |
| Active smoker | 154 | 15 |
| Former smoker | 326 | 32 |
| Non-smoker | 506 | 49 |
| Unknown | 36 | 4 |
| **Framingham SCORE** |
| Low risk (<3 %) | 263 | 26 |
| Intermediate risk (3%–4%) | 390 | 38 |
| High risk (5%–9%) | 267 | 26 |
| Very high risk (≥10%) | 9 | 1 |
| Unknown | 93 | 9 |
| **Diabetes status†** |
| NG | 390 | 38 |
| PreD | 315 | 31 |
| T2DM | 181 | 18 |
| Unknown | 136 | 13 |
| **Medical history‡** |
| Arterial hypertension | 341 | 33 |
| Hyperlipidaemia | 434 | 42 |
| Stroke/TIA | 7 | 1 |
| **Antihypertensive drugs per patient** |
| No antihypertensive drug | 670 | 65 |
| One antihypertensive drug | 154 | 15 |
| Two antihypertensive drugs | 118 | 12 |
| More than two antihypertensive drugs | 80 | 8 |
| **Lipid-lowering drugs per patient** |
| No lipid-lowering drug | 876 | 85 |
| One lipid-lowering drug | 139 | 14 |
| More than one lipid-lowering drugs | 7 | 1 |
| **Antidiabetic drugs per patient** |
| No antidiabetic drug | 844 | 82 |
| Dietetic treatment | 119 | 12 |
| One antidiabetic drug | 54 | 5 |
| More than one antidiabetic drugs | 5 | 1 |
| **Age** |
| <55 years | 437 | 43 |
| 55–65 years | 382 | 38 |

Continued
intermediate risk, 26% show high risk and 1% show very high risk. Due to some missing biomarkers at the baseline visit, we were not able to calculate the CV risk in 9% of the study population using the Framingham Score. The most common risk factor is hyperlipidaemia (42% of the population), followed by arterial hypertension (33% of the population), T2DM (18% of the population) and active smoking (15% of the population). A rather high number, nearly a third of the study population, has been identified as pre-diabetics based on oGTT data.

The purpose of this manuscript is to describe the study cohort and to give an overview of the baseline characteristics. These are reported in detail including additional information on used materials and methods in online supplemental tables S1–S10. The central figure 3 shows an R-correlation plot of most measured biomarkers at baseline. In this plot, biomarkers are not grouped (eg, in organ systems); instead, clusters of high correlations were formed (blue indicates a positive correlation, red indicates a negative correlation and white indicates no correlation). Thereby, associations between different biomarkers of different organ systems can be revealed which may further serve as a basis for a multidisciplinary in-depth analysis.

Such analyses already identified the so far unknown correlation between IGF1 receptor autoantibodies with body composition and height as presented at the European Congress of Endocrinology in Barcelona, Spain 2018. Another preliminary finding revealed a correlation between diabetes status and echocardiographic parameters of the diastolic heart function as presented at the Congress of the European Association for the Study of Diabetes in Barcelona, Spain 2019. These observations were in line with previous findings of another research group. Furthermore, genetic data from the genome-wide association study and optometric phenotyping of the

**Table 1** Continued

| Health status | No (N=1022) | % |
|---------------|-------------|---|
| 66–75 years   | 178         | 17|
| >75 years     | 25          | 2 |
| Body mass index |            |   |
| <18.5 kg/m²   | 6           | 1 |
| 18.5–25.0 kg/m² | 408       | 40|
| >25.0 kg/m²   | 608         | 59|

Health status—abbreviations.
*Whenever present, a direct categorisation due to comorbidities was performed.
†Diabetes status based on oGTT results.
‡Hyperlipidaemia was assumed based on total cholesterol >200 mg/dL or the use of a lipid-lowering drug.
.NG, normoglycaemia; OGGT, oral glucose tolerance test; PreD, pre-diabetes; SCORE, Systematic COronary Risk Evaluation; T2DM, type 2 diabetes mellitus; TIA, transient ischaemic attack.

Figure 3  R-plot of the analysed data set. Potential biomarkers are not grouped; instead, random clusters of correlations are formed. blue zones indicate positive correlations, red zones indicate negative correlations. As an example, zones of strong correlations are zoomed and potential biomarkers are depicted.
BioPersMed cohort were already used as control data for a large keratoconus genomic study in cooperation with researchers from the UK and the Netherlands, 42 for the analysis of allelic determinants with a reported association to 25(OH)D levels and their influence on vitamin D, 43 and to identify novel biomarkers for non-alcoholic fatty liver disease 44 in cooperation with local researchers. Data on bone morphology from the BioPersMed cohort were used to link Sarcopenia with increased risk of falls, osteoporosis and mortality. 45 In addition, miRNA profiles were linked to Hashimoto’s Disease and Thyroid Antibodies. 46

**Strengths and limitations**

The main strength of the BioPersMed cohort is the joint evaluation of both CV and metabolic phenotyping including a broad spectrum of diagnostic, imaging, and functional tools. This assures comprehensive biomedical and scientific dimensions of the project within high-end diagnostic and analytical parameters and biomarkers. Second, biobanking with a large number of samples including serum/plasma and blood cells, urine, saliva and stool at each visit that have been aliquoted and stored on high-quality certification rules enabling a prospective view on candidate biomarker in the context of a large longitudinal cohort, where specific approaches can be predefined. 47 48 In addition, a specifically adapted large electronic database (OpenClinica; www.openclinica.com) assures the quality of data entry and delivery as well as the validity and reliability of biomarkers analyses in the BioPersMed cohort together with a continuous data monitoring.

A potential weakness of this study is the wide time range of a prolonged recruitment due to logistic reasons between 2010 and 2016. This results in a prolonged follow-up period of study participants between 4 and 10 years. Second, after a thorough standardised baseline follow-up period of study participants between 4 and 10 years. This results in a prolonged monitoring.

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**Patient consent for publication** Consent obtained directly from patient(s)

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