Cardiac murmurs in children: Predictive value of cardiac markers (CAMUS)

Statistical analyses plan

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Statistical analysis plan

1. DESIGN

Design overview

Cardiac murmurs in children: Predictive value of cardiac markers (CAMUS) is an observational study on spontaneously discovered cardiac murmurs in children. The primary aim is to establish the predictive value of cardiac markers for identification of cardiac disease in children with heart murmurs. NT-proBNP and hs-troponins are considered the most relevant cardiac markers. Secondary aims are: a) To establish age-adjusted reference range for cardiac markers in children, and b) To explore aspects of cardiovascular physiology in children.

In part 1 of the project, a total of ~500 individuals aged 4 weeks to 10 years will be consecutively recruited among those referred to the Dept. of Paediatrics and Adolescent Medicine, Akershus University Hospital (Ahus) for assessment of heart murmurs. The protocolized investigational program of the study includes clinical examination, symptom assessment, pulse oximetry, blood sampling, ECG recording, and echocardiography. A definite diagnosis of cardiac disease or not will be based upon the echocardiographic examination, hence serving as the "gold standard".

In part 2 of the project, a total of ~125 individuals aged 4 weeks to 10 years with confirmed congenital heart disease (CHD) and scheduled for intervention at OUS Rikshospitalet will be included. They will undergo the same investigational program as in part 1. A main purpose of part 2 is to validate the prediction model developed in part 1.

Table 1. Criteria for inclusion and exclusion

Inclusion criteria	*Exclusion criteria*
Spontaneously discovered heart murmur at general practitioner's office (part 1)	Known cardiac disease (part 1 only)
Confirmed cardiac anomaly and scheduled for intervention at OUS Rikshospitalet (part 2)	Other chronic or acute disorder (part 1 only)
Age > 4 weeks, < 10 years	Medications (part 1 only)

2. ENDPOINT

Outcome variable

The primary outcome variable in part 1 is a diagnosis of cardiac disease based upon echocardiographic assessment. The diagnostic process will adhere to international consensus guidelines on paediatric cardiology (Doshi & Chikkabyrappa, 2018; Sachdeva et al., 2020; Writing Committee et al., 2021). Thereafter, all participants will be assigned to either of two groups: a) A cardiac diagnosis that needs intervention/treatment and/or further clinical follow-up (cases); b) No cardiac diagnosis or a cardiac

diagnosis that does not need intervention/treatment and/or further clinical follow-up (controls). Hence, the primary outcome variable is dichotomous. The assignment to cases vs. controls will follow pre-defined criteria pertaining to each cardiac diagnosis/diagnostic group (Table 2).
In part 2, all participants are assigned as cases, as per the inclusion criterium of a confirmed cardiac anomaly in need of intervention.

Table 2. Criteria for assignment of cases (need of intervention and/or clinical follow-up) vs. controls (no need of intervention nor clinical follow-up)

Primary cardiac diagnosis	*Criteria for caseness (only one criterium needs to be fulfilled per diagnosis)*
Persistent foramen ovale (PFO)	N/A (never assigned as case)
Atrial septal defect (ASD), all subgroups	Case if: - Ostium premium defect - Sinus venosus defect - Ostium secundum defect diameter > 3 mm OR with signs of right atrium volume load.
Persistent ductus arteriosus (PDA)	Case if: - Ductus diameter >1.5 mm. - LA/Ao ratio >1.5 - Retrograde diastolic flow in the abdominal aorta. - Continuous murmur
Ventricular septum defect (VSD), all subgroups	Case if - Perimembranous location - Inlet location - Subpulmonary outlet/supracristal location - Muscular location with diameter >5 mm OR left ventricular dilatation OR Qp/Qs ≥1.5. - Multiple defects
Aortic valve pathology (stenosis (AS), regurgitation (AR), bicuspid valve)	Case if: - Flow velocity ≥ 2.0 m/s. - Hypertrophy of left ventricle. - Aortic regurgitation (more than trivial)
Pulmonary valve pathology (stenosis (PS), regurgitation (PR))	Case if: - Flow velocity ≥ 2.5 m/s (valvular, subvalvular or supravalvular) - More than a trivial regurgitant jet (vena contracta < 2 mm) with dilatation of right ventricle OR diastolic flow reversal in the main pulmonary artery OR rapid flow deceleration during the entire diastole OR pressure half time < 100 ms.
Mitral valve pathology (stenosis (MS), regurgitation (MR))	Case if: - MS: dilatation of left atrium with pressure half time of E wave > 140 ms OR mean pressure gradient > 5 mm Hg. - MR: o More than a small central jet filling more than 20 % of left atrium. OR eccentric jet reaching the posterior wall of left atrium. o Dilatation of left atrium and left ventricle OR pulmonary vein systolic flow reversal. o Other structural/functional abnormalities of the mitral apparatus (leaflet prolapse, leaflet split, etc.)
Tricuspid valve pathology (stenosis (TS), regurgitation (TR))	Case if: - TS defined as mean pressure gradient > 5 mmHg AND pressure half time >190 ms. - TR with dilatation of right atrium OR flow velocity > 2.8 m/s
Peripheral pulmonary stenosis (PPS)	Case if: - Flow velocity > 2.5 m/s.
Coarctation of the aorta (CoA)	Case if: - Flow velocity ≥ 2.0 m/s AND diastolic continuation of flow across the suspected coarctation region. - Abdominal aorta doppler pattern with low systolic waveform amplitude and antegrade diastolic flow.

	- Presence of posterior shelf as sign of ductal tissue involvement in aortic wall
Complex congenital heart disease	N/A (always assigned as case)
Cardiomyopathies, all subgroups	N/A (always assigned as case)

3. OTHER VARIABLES

The main analysis is aimed to construct a prediction model for CHD that can be applied in general practice. The analysis will entail bivariate logistic regression between potential predictor (independent) variables and the primary outcome variable (cf. above), followed by multivariable logistic regression modelling and construction of receiver operating curves (ROC).

Relevant predictor (independent) variables include (but are not restricted to):

- *Background*: Sex, age, ethnicity, weight/height
- *Clinical symptoms*: Breathlessness, exertion intolerance/low physical fitness, sweating, reduced general condition
- *Routine clinical findings*: Heart rate, respiratory rate, SaO_2, murmur characteristics, blood haemoglobin,
- *ECG characteristics:* PR interval, QRS interval, QRS axis, Sokolow-index
- *Blood biomarkers:* NT-proBNP, troponin T, hsCRP, IL-6

4. SAMPLE SIZE CONSIDERATIONS

The value of possible predictors will be assessed through the construction of Receiver Operating Characteristics (ROC) curves, and calculation of negative predictive value. Based upon register data from the outpatient clinical at Ahus, the prevalence of cardiac disorder in the population under study is estimated at 2 %. The area under the ROC-curve for NT-proBNP in a previous study with similar methodology has been established at 0.85 (Law et al, 2009). The possible clinical application of the results from the present study requires a very low risk of false negatives (ie. no patients with cardiac disorders should be deemed healthy based upon a certain cardiac marker). Therefore, the level of significance (α) is set to 0.01. Given a test power $(1 - \beta) = 0.8$, sample size is estimated at 400 patients. A drop-out rate of up to 20 % might be assumed (technical problems, refusal of blood sampling, etc), therefore a total of 500 patients should be included in the study.

In part 2, when a population of 125 patients with well-defined CHD is added to the population of about 500 individuals previously recruited, the ratio of cases:controls increases to about 0.25. This will enable an α-value less than 1×10^{-10}, and a power $(1-\beta)$ of comparable magnitude, which we find satisfactory.

5. ANALYSIS SETS

Full analysis set

The 'full analysis set' is defined as all participants who were subsequently included in the two parts of the study. Missing values will be imputed based on the principle of 'mean substitution'.

Per protocol analysis set
The 'per protocol analysis set' is defined as all patients in the 'full analysis set' that completed the investigational program without any of the following protocol deviations:

- Primary outcome missing

Missing data will not be imputed in the per protocol analysis set.

6. STATISTICAL METHODS

General considerations
Continuous variables will be reported with parametric (mean/standard deviation) or non-parametric (median, quartiles) descriptive statistics, depending on the distribution. Ordinal/nominal variables will be reported as frequency tabulation. All statistical tests will be carried out two-sided. A p-value ≤ 0.05 is considered statistically significant throughout.

Predictor analysis
In part 1, the relationship between each potential predictor variable and the primary outcome variable will first be explored in univariate logistic regression analyses. Collinearity between predictor variables will be assessed by correlation analyses, and dimensionality reduction by PCA will eventually be considered. Then, all variables with a p-value ≤ 0.25 in the univariable analyses will be included in the first modelling step. In order to obtain a more parsimonious model, the combination of variables that gives the lowest Akaike Information Criteria (AIC) will constitute the final model. The value of predictor(s) in the final multivariable model will be assessed through the construction of Receiver Operating Characteristics (ROC) curves, and calculation of negative predictive value.

In part 2, the prediction model from part one will be validated by including a total of n~125 individuals with known CHD in the study population. The prediction model should correctly classify all these cases; if not, the model will be adjusted. We aim for zero false negatives, i.e. all cases with cardiac abnormalities in need of intervention and/or hospital follow-up should be identified. To explore the potential of the prediction model to classify all cases based upon this premise, we will apply different combinations of levels of the identified predictor variables.

Cross-sectional analyses
For the secondary study aims, cross-sectional analyses across cases and controls are feasible. Such analyses will feature parametric tests (Student t), non-parametric tests (Mann-Whitney), regression analyses or table analyses (Chi-square, Fisher) as appropriate.

Sensitivity analyses
Throughout, sensitivity analyses applying a different classification of caseness in the primary outcome variable will be carried out:

1. Caseness defined as all abnormalities identified by echocardiography, independent of intervention/follow-up needs
2. Caseness defined as all abnormalities in need of surgical or catheter-based intervention

7. REFERENCES

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