Binary disease prediction using tail quantiles of the distribution of continuous biomarkers

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ABSTRACT
In the analysis of binary disease classification, numerous techniques exist, but they merely work well for mean differences in biomarkers between cases and controls. Biological processes are, however, much more heterogeneous, and differences could also occur in other distributional characteristics (e.g. variances, skewness). Many machine learning techniques are better capable of utilizing these higher-order distributional differences, sometimes at cost of explainability. In this study, we propose quantile based prediction (QBP), a binary classification method based on the selection of multiple continuous biomarkers and using the tail differences between biomarker distributions of cases and controls. The performance of QBP is compared to supervised learning methods using extensive simulation studies, and two case studies: major depression disorder (MDD) and trisomy. QBP outperformed alternative methods when biomarkers predominantly show variance differences between cases and controls, especially in the MDD case study. More research is needed to further optimise QBP.

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1. Introduction
Biomarker research has increased fastly due to the development of new molecular biotechnologies (Pepe, Feng, Janes, Bossuyt, and Potter 2008). A biomarker is defined as ‘any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease’ (World Health Organization et al. 2001). Biomarkers are developed for many different purposes: classification and prediction of diseases, as surrogate outcomes in clinical trials, as measures of toxic or preventive exposures, or as a guide to individual treatment choice (Halaris 2013).

For the classification and prediction of diseases, single biomarkers do often not have sufficient discriminating power to separate cases from controls (Calfee et al. 2011; Hsu, Chang, and Hsueh 2014; Jentsch et al. 2015). When analysing multiple biomarkers simultaneously, models might become harder to interpret, but could also face the problem of high dimensionality with respect to the available number of observations.

Firstly, to enhance the transparency of these classification or prediction models with numerous biomarkers, insight into the selected features and its importance is crucial.
Whereas classical statistical techniques hold the possibility to perform in-depth inference on present relations, many machine learning techniques do not allow for a similar degree of interpretability. Secondly, when the number of biomarkers \( p \) exceeds the number of observations \( n \) (\( p > n \) or \( p \gg n \)), it is key to reduce the dimensionality of the data and to select a sparse set of biomarkers with high discriminant power that can be used to produce reliable predictions.

Binary classification methods that reduce the dimensionality of the input variables can be categorised based on the relations between original input variables and new input variables (Ma and Huang 2008). (i) Dimension reduction methods that construct new input variables using linear combinations of all input variables (i.e. partial least squares (PLS) and principal component analysis (PCA)). (ii) Feature selection methods, which select a subset of the original input variables. Examples include likelihood functions for parametric models, such as penalised logistic regression (PLR) and linear discriminant analysis (LDA) by optimal scoring. (iii) Hybrid methods using (i) and (ii). These traditional methods focus mainly on mean differences in the biomarker distributions between cases and controls. However, differences may occur somewhere else, since a disease may affect the variation, skewness and kurtosis of the biomarker distribution (Just 2014).

Over time, a wide scale of classification tree based techniques is developed, from individual trees (CART) to an ensemble of individual trees with various modifications such as different sampling strategies like bootstrapping (Random Forest) or boosting (AdaBoost, XGBoost). Other machine learning techniques for classification include support vector machines (SVM) and the k-nearest neighbours (kNN) algorithm that does not require a model to be fit (Friedman, Hastie, and Tibshirani 2009).

In this paper, we introduce a new approach for binary classification that takes advantage of the tail differences of the biomarker distributions between cases and controls. The performance of this new method is compared with various traditional binary classification methods and machine learning techniques using simulation studies and two case studies. Logistic regression is applied with and without penalisation. The selected penalty functions are the lasso (Tibshirani 1996), elastic net (Zou and Hastie 2005) and the ridge (Hoerl and Kennard 1970). Alternatively, to address multicollinearity among the predictors, principal component logistic regression (PCLR) is included in the analysis (Aguilera, Escabias, and Valderrama 2006). Next to these LR based methods, also LDA and PLS with LDA (abbreviated as PLS-LDA) were used (Marigheto, Kemsley, Defernez, and Wilson 1998). The considered machine learning techniques include SVM, kNN, random forest (RF) and extreme gradient boosting (XGBoost).

The first case study describes data on patients with major depressive disorder (MDD), which is a disease with a lifetime prevalence of around 15%. It is a major cause of disability in the Western world (Sobocki, Jönsson, Angst, and Rehnberg 2006; Bromet et al. 2011) and the prediction of MDD with biomarkers can help physicians diagnose MDD better. The second case study, is on an ongoing Dutch population study on the prevalence of trisomy 13, 18 and 21, containing 4894 observations.

In this paper, the receiver operating characteristic (ROC) curve approach is considered as the primary performance measure for predicting cases and controls. We will use the area under the ROC curve (AUC). The AUC is a variant of the concordance (\( c \)) statistic for binary outcomes, that indicates the discriminative ability of a generalised linear model (Steyerberg et al. 2010). Advantages of this nonparametric statistic are that it does
not depend on a decision threshold and gives an indication of how well the negative and positive classes are separated (Bradley 1997). However, a disadvantage of the AUC is an inflation towards the class that is rare in case of severe imbalance in cases and controls (Berrar and Flach 2012). For this reason, Cook and Ramadas (2020) recommended changing the performance measure and use of the area under the precision recall curve (AUCPR). In contrast to changing the performance measure, Japkowicz (2000) proposed a random resampling strategy to regenerate a balanced setting. Both downsampling the majority class and oversampling the minority class were proven to be effective methods. As part of a sensitivity study, we evaluate the AUCPR for three unbalanced simulation scenarios and the Trisomy case study. In addition, we applied downsampling for the Trisomy dataset with a low number of cases.

To assess the predictive performance of all methods in terms of AUC, we use different cross-validation strategies. For the simulation scenarios, we apply k-fold cross-validation (CV) on the training dataset to determine the set of tunable parameters with the highest average AUC over all k folds. This set of parameters is used on an independently simulated validation dataset with 5000 observations to find a reliable estimate of the true prediction performance. In the case studies, we apply repeated double cross-validation (rdCV). This strategy, that is suitable for small datasets, selects the optimal parameter based on multiple repetitions instead of a single double cross-validation that can be optimistic or pessimistic (Filzmoser, Liebmann, and Varmuza 2009). Here, double (k-fold) cross-validation (dCV) is preferred above single k-fold CV, Monte Carlo CV (MCCV) or leave-one-out CV (LOOCV). Primarily because dCV is able to simultaneously provide an estimate for the prediction error and the tunable parameter, whereas single k-fold cross-validation only succeeds to perform one of these goals (Smit et al. 2007). Secondly, dCV has a reduced computational complexity compared to LOOCV.

The remainder of this article is structured as follows. In the next section, both the proposed and selected traditional classification methods are formulated mathematically. Moreover, a description on the applied performance measures and cross-validation techniques are presented. In the section ‘Simulation study’ a detailed description of the design of the simulation study is provided, followed by the corresponding results. In the section ‘Case studies’, the major depression disorder (MDD) dataset and trisomy dataset are presented. Here, we first describe the design of the study and then present the results of the different prediction methods. The last section contains the discussion.

2. Methods

In this section we assume that $y_i$ denotes the group (or disease) indicator for subject $i = 1, \ldots, n$ with $y_i = 0$ a healthy control and $y_i = 1$ a case. The (continuous) value of the $k$th biomarker for subject $i$ is denoted by $x_{i,k}$, where $k = 1, \ldots, r$ and $r$ the number of observed biomarkers.

2.1. Quantile based prediction

Quantile based prediction (QBP) is a binary prediction method for continuous biomarkers, that uses the left and right tails of the empirical biomarker distributions of two groups to discriminate between cases and controls. QBP is able to discriminate when the tails of
two groups are shifted with respect to each other (irrespective of mean differences or the remainder part of the distribution). The stronger the shift in the tails of a biomarker, the more likely it is that this shift is due to the disease. By combining multiple biomarkers a subject’s total disease score can be constructed. This disease score represents some likelihood of being a case or control.

The remainder of this paragraph follows the structure of QBP – that distinguishes the definition of its characteristics, the scoring mechanism based on these characteristics and the attribution of scores to individual subjects. An artificial example of a single biomarker \( k \) is presented to illustrate the construction of the QBP characteristics (Figure 1 and Table 1) and the scoring mechanism (Table 2). Lastly, the arbitrary situation in Table 3 exemplifies the attribution of scores to a set of individuals in case of multiple biomarkers.

2.1.1. QBP characteristics
The first step is to select a quantile (or percentile) \( q_p \), with corresponding proportion \( p \). For the left-tail percentile, we select proportion \( p_{L_0} < 0.50 \) and we select the right-tail percentile with proportion \( p_{R_0} > 0.5 \). Without loss of generality, we select the tail proportion \( p_{R_0} \) based on symmetry such that \( p_{R_0} = 1 - p_{L_0} \). The corresponding percentiles for the controls and cases for each biomarker \( k \) are used to determine the predominant group in the left tail \( D_{L,k} \in \{ 0, 1 \} \) and in the right tail \( D_{R,k} \in \{ 0, 1 \} \). For each biomarker this is defined by

\[
D_{L,k} = \begin{cases} 
0 & \text{if } q^{(0)}_{p_{L_0},k} < q^{(1)}_{p_{L_0},k} \\
1 & \text{if } q^{(0)}_{p_{L_0},k} > q^{(1)}_{p_{L_0},k} \\
\text{NA} & \text{if } q^{(0)}_{p_{L_0},k} = q^{(1)}_{p_{L_0},k}
\end{cases}
\]

\[
D_{R,k} = \begin{cases} 
0 & \text{if } q^{(0)}_{p_{R_0},k} > q^{(1)}_{p_{R_0},k} \\
1 & \text{if } q^{(0)}_{p_{R_0},k} < q^{(1)}_{p_{R_0},k} \\
\text{NA} & \text{if } q^{(0)}_{p_{R_0},k} = q^{(1)}_{p_{R_0},k}
\end{cases}
\]

with \( q^{(0)}_{p,k} \) and \( q^{(1)}_{p,k} \) the \( p \)th percentile (\( p \in \{ p_{L_0}, p_{R_0} \} \)) of group 0 (healthy control) and group 1 (cases) of biomarker \( k \), respectively. Thus, the predominant group has its percentile at proportion \( p_{L_0} \) or \( p_{R_0} \) more extreme than the other group. For example, in the illustration
Table 1. QBP characteristics on an arbitrary example using three \((m = 2)\) proportions per tail \((p_L = (p_{L0}, p_{L1}, p_{L2}) = (0.1, 0.05, 0.01))\) and \((p_R = (p_{R0}, p_{R1}, p_{R2}) = (0.9, 0.95, 0.99))\) for a single biomarker \(k\) (index \(k\) suppressed).

|        | \(q_{p_{L0}}\) | \(q_{p_{L1}}\) | \(q_{p_{L2}}\) | \(q_{p_{R0}}\) | \(q_{p_{R1}}\) | \(q_{p_{R2}}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| Percentiles \((y_i = 0)\) | 273 | 372 | 424 | 796 | 849 | 947 |
| Percentiles \((y_i = 1)\) | 357 | 380 | 396 | 644 | 713 | 880 |
| Predominant group | \(C_{p_{L0}}\) | \(C_{p_{L1}}\) | \(C_{p_{L2}}\) | \(C_{p_{R0}}\) | \(C_{p_{R1}}\) | \(C_{p_{R2}}\) |

|        | \(C_{p_{L0}}\) | \(C_{p_{L1}}\) | \(C_{p_{L2}}\) | \(C_{p_{R0}}\) | \(C_{p_{R1}}\) | \(C_{p_{R2}}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| Cutpoints | 273 | 372 | 424 | 644 | 713 | 880 |

\[ F(y_i)(C_{p_{Ls}}) = \frac{1 - F(y_i)(C_{p_{Rs}})}{\sum_{s} C_{p_{Rs}}(y_i)} \]

|        | \(\rho_{L2}\) | \(\rho_{L1}\) | \(\rho_{L0}\) | \(\rho_{R0}\) | \(\rho_{R1}\) | \(\rho_{R2}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| Tail area \((y_i = 0)\) | 0.01 | 0.05 | 0.1 | 0.407 | 0.240 | 0.03 |
| Tail area \((y_i = 1)\) | 0.00 | 0.031 | 0.225 | 0.1 | 0.05 | 0.01 |

|        | \(R_{p_{L2}}\) | \(R_{p_{L1}}\) | \(R_{p_{L0}}\) | \(R_{p_{R0}}\) | \(R_{p_{R1}}\) | \(R_{p_{R2}}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| Exceed ratio | 0 | 0.62 | 2.25 | 4.07 | 4.8 | 3 |

\[ l_3 \quad l_2 \quad l_1 \quad l_0 \quad l_{R1} \quad l_{R2} \quad l_{R3} \]

|        | \((-\infty, 273]\) | \((273, 372]\) | \((372, 424]\) | \((424, 644]\) | \((644, 713]\) | \((713, 880]\) | \([880, \infty)\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|

Table 2. Scoring mechanism for the arbitrary example in Table 1 using lower boundaries for the exceed ratios \(R^* = (2, 3, 5)\) and maximal interval scores \(v = (v_1, v_2, v_3) = (1, 2, 3)\) for a single biomarker \(k\) (index \(k\) suppressed).

|        | \(R_{p_{L2}}\) | \(R_{p_{L1}}\) | \(R_{p_{L0}}\) | \(R_{p_{R0}}\) | \(R_{p_{R1}}\) | \(R_{p_{R2}}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| Exceed ratio | 0 | 0.62 | 2.25 | 4.07 | 4.8 | 3 |
| Lower boundaries on exceed ratio \((R^*)\) | 5 | 3 | 2 | 2 | 3 | 5 |

|        | \(I_{L3}\) | \(I_{L2}\) | \(I_{L1}\) | \(I_0\) | \(I_{R1}\) | \(I_{R2}\) | \(I_{R3}\) |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Binary exceed scores | \(e_{L3}\) | \(e_{L2}\) | \(e_{L1}\) | \(e_{L0}\) | \(e_{R0}\) | \(e_{R1}\) | \(e_{R2}\) |
| Maximal interval scores \((v)\) | \(v_3\) | \(v_2\) | \(v_1\) | \(v_0\) | \(v_{R1}\) | \(v_{R2}\) | \(v_{R3}\) |
| Interval scores | \(V_{L3}\) | \(V_{L2}\) | \(V_{L1}\) | \(V_0\) | \(V_{R1}\) | \(V_{R2}\) | \(V_{R3}\) |

\[ 1 \quad 1 \quad 1 \quad 0 \quad -1 \quad -2 \quad -2 \]

Note that \(D_L = 1\) and \(D_R = 0\).

of QBP in Figure 1, the control group \((y_i = 0)\) is predominant in the right tail and the case group \((y_i = 1)\) is predominant in the left tail.

In the second step, the tails of the biomarkers that have a predominant group will be included in the discrimination of groups using scores. The tails having no predominant group \((D_{L,k} = NA\) or \(D_{R,k} = NA\)) are eliminated in the discrimination of groups by attributing a neutral score (value 0).

The third step is to define \(m\) additional percentiles that are located further in the tail. The left and right tail now contain \(m + 1\) percentiles, with proportions \(p_L = (p_{L0}, p_{L1}, \ldots, p_{Lm})\) in the left tail \((p_{Ls−1} > p_{Ls})\) and \(p_R = (p_{R0}, p_{R1}, \ldots, p_{Rm})\) in the right tail. Again, without loss of generality, we use symmetry of the tails and take \(p_{R1} = 1 − p_{Ls}\). The cutpoints \(C_{p,k}\)
Aiming to discriminate cases from controls, we will attribute the interval scores 2.1.2. Scoring mechanism
doing on biomarker \( k \) for proportions \( p \in \{p_L, p_R\} \) will be defined by the quantiles of the non-predominant group. In particular, for \( s = 1, \ldots, m \)

\[
C_{PLs,k} = q_{PLs,k}^{(1-D_{L,k})}, \quad C_{PRs,k} = q_{PRs,k}^{(1-D_{R,k})}.
\]

With these cutpoints, we define \( m + 1 \) intervals \( I_{s,k} \) in each tail that will later be used to attribute scores to subjects. We define the intervals \( I_{s,k} \) as follows

\[
I_{L,k} = (C_{PL_{s+1,k}}, C_{PLs,k}), \quad I_{0,k} = (C_{PL0,k}, C_{PR0,k}), \quad I_{R,k} = [C_{PRs,k}, C_{PR_{s+1,k}})
\]

with \( s = 1, \ldots, m \), \( C_{PL_{m+1,k}} = -\infty \), \( C_{PR_{m+1,k}} = \infty \). In Figure 1, the cutpoints and intervals of QBP are shown for an arbitrary biomarker.

The fourth step is to determine the exceed ratio \( R_{PL,k} \) based on the cutpoints. Here, an exceed ratio is a measure for the relative difference of mass in the tails of the predominant and non-predominant group. The higher the exceed ratio at a cutpoint, the higher the probability that a new subject contained in this tail belongs to the predominant group. Note that the predominant group may be different for the left and the right tail and the predominant group has more mass in the tail at the \( C_{PL0,k} \) and \( C_{PR0,k} \) than the non-predominant group. Thus the exceed ratio \( R_{PL0,k} \) is greater than 1 at the corresponding quantile \( q_{PL0,k}^{(1-D_{L,k})} \) and \( q_{PR0,k}^{(1-D_{L,k})} \). However, this may not necessarily be greater than 1 for the other percentiles further in the tails. For the left and the right tail, the exceed ratio is defined by

\[
R_{PL,k} = F_{(D_{L,k})(C_{PLs,k})/p_{L,k}}, \quad R_{PR,k} = (1 - F_{(D_{R,k})(C_{PRs,k})/p_{R,k}})
\]

with \( F_{(0,k)} \) and \( F_{(1,k)} \) the empirical cumulative distribution function of biomarker \( k \) for the controls and the cases, respectively.

2.1.2. Scoring mechanism
Aiming to discriminate cases from controls, we will attribute the interval scores \( V_{s,k} \in \{V_{0,k}, V_{L,k}, V_{R,k}\} \) to the different intervals \( I_{s,k} \in \{I_{0,k}, I_{L,k}, I_{R,k}\} \), that were defined in (1), respectively. The result of the scoring mechanism – as explained below – applied on the artificial example from Figure 1 is shown in Table 2.

Firstly, the predominant group in a tail will determine the sign of the interval scores. Whereas negative signs correspond to predominance of the healthy control group (\( D_{L,k} = 0 \) or \( D_{R,k} = 0 \)), positive signs belong to predominance of the cases (\( D_{L,k} = 1 \) or \( D_{R,k} = 1 \).

| Interval | 1  | 2  | 3  | 4  | 5  | Subject | TDS |
|----------|----|----|----|----|----|---------|-----|
| \( I_{L,k} \) | 1  | -1 | 2  | -3 | -3 | a       | 3   |
| \( I_{L,k} \) | 1  | -1 | 2  | -1 | -2 | b       | 0   |
| \( I_{L,k} \) | 1  | -1 | 2  | -1 | 0  | c       | -7  |
| \( I_{0,k} \) | 0  | 0  | 0  | 0  | 0  |         |     |
| \( I_{0,k} \) | -1 | 0  | 1  | 0  | 0  |         |     |
| \( I_{0,k} \) | -2 | 0  | 2  | 0  | 2  |         |     |
| \( I_{0,k} \) | -2 | 0  | 3  | 3  | 2  |         |     |

Table 3. Arbitrary example of the calculation of the summation of disease scores \( DS_{j,k} \) and the total disease score \( TDS \), for subjects \( i \in \{a, b, c\} \).
Secondly, to guarantee the predominant group has more mass in the tail for a certain percentile than the non-predominant group, and therefore a certain discriminating power, we introduce lower boundaries \( R_s^* = (R_s^1, \ldots, R_s^m) \) on the exceed ratios in (2) with \( R_s^* > 1, \forall s \in \{1, \ldots, m\} \). To indicate whether these lower boundaries – which we can choose ourselves – are met for biomarker \( k \), we apply binary exceed scores for the left-tail \( e_{L,k} = (e_{L0,k}, \ldots, e_{Lm,k}) \) and right-tail \( e_{R,k} = (e_{R0,k}, \ldots, e_{Rm,k}) \). Note that this can vary per tail (percentile) and biomarker, as can be seen in the artificial example in Table 2. The binary exceed scores are mathematically defined by

\[
e_{L,k} = 1(R_{pL,k} \geq R_s^*), \quad e_{R,k} = 1(R_{pR,k} \geq R_s^*),
\]

for \( s = 0, \ldots, m \) and with \( 1(A) \) an indicator value being 1 if \( A \) is true and zero otherwise. Note that for \( s = 1, \ldots, m \), the binary exceed scores \( e_{L-1,k} \) and \( e_{R-1,k} \) correspond to the intervals \( I_{L,k} \) and \( I_{R,k} \), respectively.

Thirdly, intending to put more emphasis on subjects having (extreme) values in tails, we introduce maximal interval scores \( v = (v_1, \ldots, v_m) \) such that \( v_1 \leq v_2 \leq \cdots \leq v_m \). By appending these maximal interval scores with the binary exceed scores, we will ensure that scores are only assigned in case of a certain discriminating power of a tail. For \( s = 1, \ldots, m \), we obtain the interval scores

\[
V_{L,k} = (-1)^{(1-D_{L,k})} \cdot \max\{v_1 \cdot e_{L0,k}, \ldots, v_s \cdot e_{Ls-1,k}\},
\]

\[
V_{R,k} = (-1)^{(1-D_{L,k})} \cdot \max\{v_1 \cdot e_{R0,k}, \ldots, v_s \cdot e_{Rs-1,k}\}.
\]

Note that for increasing \( s \), the functions \( \max\{v_1 \cdot e_{L0,k}, \ldots, v_s \cdot e_{Ls-1,k}\} \) and \( \max\{v_1 \cdot e_{R0,k}, \ldots, v_s \cdot e_{Rs-1,k}\} \) are non-decreasing and that the central interval \( I_{0,k} \) always obtains a neutral score \( V_{0,k} = 0 \).

### 2.1.3. Scoring and classifying individual subjects

Now all elements of the QBP are determined, the disease scores \( DS_{i,k} \) can be computed for each subject \( i \) per biomarker \( k \). The disease score \( DS_{i,k} \) is in essence a measure of the position of the biomarker value \( x_{i,k} \) with respect to the predominant group. In order to prioritise specific biomarkers above others, biomarker weights \( w = (w_1, \ldots, w_r) \) are introduced. The disease score \( DS_{i,k} \) defined by

\[
DS_{i,k} = \begin{cases} 
V_{L,k} \cdot w_k & \text{if } x_{i,k} \in I_{L,k}, \\
0 & \text{if } x_{i,k} \in I_{0,k}, \\
V_{R,k} \cdot w_k & \text{if } x_{i,k} \in I_{R,k},
\end{cases}
\]

with \( s = 1, \ldots, m \). Note that \( x_{i,k} \) will always fall in one of the intervals \( I_{k,m,k}, I_{k,m-1,k}, \ldots, I_{k,1,k}, I_{k,0,k}, I_{k,1,k}, \ldots, I_{k,m-1,k}, I_{k,m,k} \). By summing over all biomarkers, a total disease score \( TDS_i = \sum_{k=1}^{m} DS_{i,k} \) per subject \( i \) can be calculated. An extreme positive value for subject \( i \), indicates that the subject is most likely a case, while an extreme negative value means that subject \( i \) most likely a control. A value of zero would indicate that the subject is as likely a case as a control. This scoring procedure is applied on an arbitrary example in Table 3.

In order to classify individual subjects, a cut-off value is introduced. Setting a cut-off value for QBP is in some way similar to logistic regression, where the default classification threshold is set on 0.5 (to label a case when \( P(Y_i = 1 \mid x_i) > 0.5 \)). Nevertheless, based
on a desired performance measure (e.g. minimal sensitivity and specificity) this logistic regression threshold could be adapted and take any value in-between 0 and 1. For QBP, this threshold is not expressed in a probability but in terms of a TDS. Each individual subject $i$ with a corresponding $TDS_i$ is classified as a control if $TDS_i \leq T$ and is marked as a case if $TDS_i > T$. Note that a threshold for TDS of $T = 0$ corresponds to a logistic regression threshold of 0.5. Moreover, the theoretical range of the TDS is determined by the multiplication of the number of biomarkers and the maximal interval scores.

Based on all individual classifications, a confusion matrix can be constructed. When applying QBP in practice, we recommend selecting a cut-off value such that the desired performance metric is optimised. In Paragraph 2.10, it is explained how the classification performance is evaluated in this study. Note that no single cut-off is selected for any of the methods, as we used cut-off independent performance measures.

### 2.2. (Penalised) Logistic Regression

As described in Hosmer and Lemeshow (2000), logistic regression considers $n$ independent observations $\{(y_i, x_i); \ i = 1, \ldots, n\}$, where $y_i$ corresponds to a disease ($y_i = 1$) or no disease ($y_i = 0$) and $x_i = (1, x_{i,1}, \ldots, x_{i,r})$ is the vector of independent predictor variables, which are the results of the $r$ biomarkers. The logistic regression model assumes that,

$$P(Y_i = 1 | x_i) = \pi(x_i) = 1 - P(Y_i = 0 | x_i),$$

with $Y_i$ Bernoulli($\pi(x_i)$) distributed and $\pi(x_i)$ given by

$$\pi(x_i) = \frac{\exp(\beta_0 + \sum_{k=1}^r x_{i,k} \beta_k)}{1 + \exp(\beta_0 + \sum_{k=1}^r x_{i,k} \beta_k)} \quad (3)$$

In case the number of events is large enough to be able to estimate all model parameters, maximum likelihood estimators can be used. The log-likelihood function for $y = (y_1, \ldots, y_n)$ is given by

$$l(\beta) = \sum_{i=1}^n [y_i \cdot \pi(x_i) + (1 - y_i) \cdot \log(1 - \pi(x_i))]. \quad (4)$$

In case the number of events is sparse, a penalised logistic regression can be used to determine the most promising or relevant biomarkers. The penalised logistic regression model maximises the log-likelihood function $l(\beta)$ in (4) with a penalty term $P(\beta)$, i.e. maximises $l_\lambda(\beta) = l(\beta) - \lambda P(\beta)$ over $\beta$ for a fixed value of $\lambda$ that determines the strength of the penalty. Three well known penalty functions are the lasso (Tibshirani 1996), elastic net (EN) (Zou and Hastie 2005) and the ridge (Hoerl and Kennard 1970) (see (5)).

- **Lasso**: $P(\beta) = \sum_{k=1}^r |\beta_k|$  
- **EN**: $P_\alpha(\beta) = \sum_{k=1}^r \left[ \frac{1 - \alpha}{2} \beta_k^2 + \alpha |\beta_k| \right]$  
- **Ridge**: $P(\beta) = \sum_{k=1}^r \beta_k^2 \quad (5)$

with $\alpha$ an additional parameter for the elastic net.
2.3. Principal components logistic regression

First of all, we briefly describe the concept of principal component analysis (PCA) in line with a more comprehensive description of this method in Aguilera et al. (2006). Let all observations be contained in matrix $X = (x_{i,k})_{n \times r}$, with column vectors $X_1, X_2, \ldots, X_r$. Furthermore, denote the sample covariance matrix $S = (s_{k,l})_{r \times r}$ with the elements $s_{k,l} = \frac{1}{n-1} \sum_{i=1}^{n} (x_{i,k} - \bar{x}_k)(x_{i,l} - \bar{x}_l)$, with sample means given by $\bar{x}_k = \frac{1}{n} \sum_{i=1}^{n} x_{i,k}$, with $k = 1, \ldots, r$. In order to simplify, without loss of generality, it is considered that the observations are centred, so that $\bar{x}_1 = \ldots = \bar{x}_r = 0$, and the sample covariance matrix $S = (s_{k,l})_{r \times r} = \frac{1}{n-1} X'X$.

The sample principal components (pc’s) are defined as orthogonal linear spans with maximum variance of the column matrix $X$, denoted by $Z_k = XV_k$ with $k = 1, \ldots, r$. The vectors $V_1, \ldots, V_r$ that define the pc’s, are the eigenvectors of the sample covariance matrix $S$ associated to their corresponding eigenvalues $\lambda_1 \geq \ldots \geq \lambda_r \geq 0$. These eigenvalues are again the variances of the corresponding pc’s. If we denote by $Z$ the matrix whose columns are the sample pc’s, it can be expressed as $Z = XV$, with $V = (v_{k,l})_{r \times r}$ being the matrix whose columns are the eigenvectors of the sample covariance matrix. Note that the sample variance can be decomposed as $S = V \Delta V'$, with $V$ orthogonal, $V'$ being the transposed of $V$ and $\Delta = diag(\lambda_1, \ldots, \lambda_r)$, so the matrix of observations is given by $X = ZV$. This pc decomposition has given us an approximate reconstruction of each original observation in terms of a reduced number of pc’s that was selected based on explained variance, namely

$$X_k = \sum_{i=1}^{s} Z_l v_{k,l}, \quad k = 1, \ldots, r, \text{ with } s \leq r.$$ 

The percentage of the variability that is accounted for by the model is given by

$$\frac{\sum_{k=1}^{s} \lambda_k \cdot 100}{\sum_{k=1}^{r} \lambda_k}, \quad \text{with } s \leq r.$$

Now that the pc’s are obtained, the logit model is applied, with (3) being replaced by

$$\pi(Z_i) = \frac{\exp\{\beta_0 + \sum_{k=1}^{r} z_{i,k} v_{k,l} \beta_l\}}{1 + \exp\{\beta_0 + \sum_{k=1}^{r} z_{i,k} v_{k,l} \beta_l\}} = \frac{\exp\{\beta_0 + \sum_{k=1}^{r} z_{i,k} \gamma_k\}}{1 + \exp\{\beta_0 + \sum_{k=1}^{r} z_{i,k} \gamma_k\}}$$

with $z_{i,l}$ being the elements of the pc’s matrix $Z = XV$ and $\gamma_l = \sum_{k=1}^{r} v_{k,l} \beta_k$, $k = 1, \ldots, r$. In the setting where the last $r-s$ pc’s are removed, we obtain

$$\pi_s(Z_i) = \frac{\exp\{\gamma_0 + \sum_{k=1}^{s} z_{i,k} \gamma_k\}}{1 + \exp\{\gamma_0 + \sum_{k=1}^{s} z_{i,k} \gamma_k\}}.$$ 

2.4. Linear discriminant analysis

In the search for a separating hyperplane using linear discriminant analysis (LDA), two approaches can be distinguished, namely LDA based on the Bayes’ rule and Fisher-LDA. We focus on Bayesian LDA, since it appears to be more suitable with a large number of covariates (Vera et al. 2011).
As extensively described in Friedman et al. (2009), Bayesian LDA assumes Gaussian class densities with a common covariance matrix for all classes. For the binary case, this comes down to observing the log-ratio of the cases \( y = 1 \) and the controls \( y = 0 \). This log-ratio is defined by

\[
\log \frac{P(Y = 1 \mid X = x)}{P(Y = 0 \mid X = x)} = \log \frac{\pi_1}{\pi_0} - \frac{1}{2} (\mu_1 + \mu_0)^T \Sigma^{-1} (\mu_1 - \mu_0) + x^T \Sigma^{-1} (\mu_1 - \mu_0),
\]

with the prior distributions \( \pi_1 \) and \( \pi_0 \) and the mean vectors of the multivariate Gaussian \( \mu_1 \) and \( \mu_0 \) of the cases and controls, respectively. In addition, \( \Sigma^{-1} \) denotes the common covariance matrix and \( x \) the vector of biomarker values of a subject.

2.5. Partial least squares – linear discriminant analysis

Partial least squares (PLS) (Wold 1985) was first introduced for a continuous response; however, later a two-step approach for binary classification was proposed, namely PLS-LDA (Nguyen and Rocke 2002). Here, PLS is used for dimension reduction and then (Fisher)-LDA is used on the PLS latent variables. The underlying idea of PLS regression is to find uncorrelated linear transformations of the original predictor variables which have high covariance with the response variables. In this case, the classes of cases and controls are represented as binary responses and treated as if they were continuous in the projection on the latent structure of PLS (Boulesteix 2004). Since the principle of LDA is already explained in Section 2.4, we will now explain the PLS dimension reduction using the SIMPLS algorithm (De Jong 1993).

Let us first recall that \( X \in \mathbb{R}^{n \times r} \) denotes the matrix containing all biomarker observations. Then, \( Z = XA \in \mathbb{R}^{n \times s} \) denotes the matrix of linear transformations, with the column vectors \( Z_1, \ldots, Z_s \) representing the PLS latent variables of \( Z \). Here, the matrix \( A \in \mathbb{R}^{r \times s} \) defines the linear transformation and contains the vectors \( a_1, \ldots, a_s \) as its columns. The SIMPLS algorithm determines the vector \( a_1, \ldots, a_s \) by computing linear transformations of \( X \) and linear transformations of \( y = (y_1, \ldots, y_n) \) which have maximal covariance, under the constraint that the linear transformations of \( X \) (the PLS latent variables) are mutually uncorrelated. In particular, we first determine the unit vector \( a_1 \) and scalar \( b_1 \) maximising the empirical covariance \( \hat{\text{COV}}(Xa_1, b_1y) \). Then for all \( l = 2, \ldots, s \), the unit vector \( a_l \) and scalar \( b_l \) maximise \( \hat{\text{COV}}(Xa_l, b_ly) \) subject to \( \hat{\text{COV}}(Xa_u, b uy) = 0 \) for all \( u = 1, \ldots, l - 1 \). Note that before applying the SIMPLS algorithm, \( y \) and the columns of \( X \) need to be centred.

Now we have obtained the matrix \( Z = XA \), Fisher LDA is applied using \( Z_1, \ldots, Z_s \) as predictor variables. In order to determine the optimal number of components \( s \) that results into the best classification performance, cross-validation (see 2.11) is performed.

2.6. Support vector machine

Support vector machine (SVM) is a generalisation of optimal separating hyperplanes to the non-separable case and creates non-linear decision boundaries for classification composed by taking linear combinations of a largely transformed (sometimes infinite) version of the feature space (Boser, Guyon, and Vapnik 1992; Cortes and Vapnik 1995).
In both the separable and non-separable situations, we have $n$ independent observations \{(yi, xi); i = 1, \ldots, n\}, where $y_i$ corresponds to a disease ($y_i = 1$) or no disease ($y_i = -1$) and $x_i = (1, x_{i,1}, \ldots, x_{i,r})$. Here, we can define a hyperplane by

$$\{x : f(x) = x^T \beta + \beta_0 = 0\}$$

and the classification rule $\text{sign}[x^T \beta + \beta_0]$ to distinguish between cases and controls.

As extensively described in Friedman et al. (2009), we can find the optimal separating hyperplane that maximises the margin ($M$) between the cases and controls, by solving the following optimisation problem

$$\max_{\beta, \beta_0, \|\beta\| = 1} M$$

subject to $y_i(x_i^T \beta + \beta_0) > M, \ i = 1, \ldots, n.$

(6)

Note that the problem (6) cannot be solved for the non-separable case. To allow for overlap in the feature space between cases and controls, a slack variable $\xi_i$ that allows for points on the wrong side of the decision boundary was introduced. This concept of accepting errors in the training set is called soft margin. When extending (6) with this slack variable that is proportional to the margin, we the following optimisation problem

$$\min_{\beta, \beta_0} \frac{1}{2} \|\beta\|^2 + C \sum_{i=1}^{n} \xi_i$$

subject to $\xi_i \geq 0, \ y_i(x_i^T \beta + \beta_0) > 1 - \xi_i, \ \forall i,$

where the parameter $C$ is a cost parameter that can be used for regularisation (Cortes and Vapnik 1995). Note that for $C = \infty$, we obtain the separable case again.

The quadratic optimisation problem can be rewritten as a dual SVM problem, such that it only depends on inner products. We obtain

$$\max_{\alpha} \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i=1}^{n} \sum_{j=1}^{n} \alpha_i \alpha_j y_i x_i^T x_j$$

subject to $0 \leq \alpha_i \leq C$

This form makes it possible to apply the kernel trick, in which the inner product $x_i^T x_j$ is replaced by $K(x_i, x_j)$ representing a kernel that enlarges the original feature space using polynomials or splines. The main advantage of this enlarged space is the enhanced training-class separation. To avoid over-fitting, one can make a trade-off between model complexity and error frequency by changing the soft margin cost parameter $C$ (Cortes and Vapnik 1995).

In this study, we apply two types of kernels, namely the linear $K(x_i, x_j) = \langle h(x_i), h(x_j) \rangle$ and the radial base function (RBF) with $K(x_i, x_j) = \exp(-\gamma \|x_i - x_j\|^2), \gamma > 0$.

2.7. Random forest

A random forest algorithm is an ensemble of individual regression (or decision) trees, that can be used for both regression or classification problems.
Each individual tree is grown by recursively selecting a number of random features from
the training sets composed of bootstrap samples from the original data, and consequently
creating two daughter nodes at the feature that provides the best split. Here, the best split
is defined such that the response can be predicted in the best possible way. This parti-
tioning at nodes continues until a stopping criterion has been met. In the end, each tree
provides a tree-structured classifier \( \hat{C}_b(x) \). Since individual trees have a relatively low bias
but are noisy, it is beneficial to average individual trees to reduce the variance (Friedman
et al. 2009).

The random forest classifier consists of a majority vote of the collection of all individual
tree classifiers. In specific,

\[
\hat{C}_{rf}^B(x) = \text{majority vote}\{\hat{C}_b(x)\}_1^B.
\]

By combining all votes of \( x \) for which \( x \) is not contained in the training set, we obtain the
out-of-bag classifier of input \( x \) (Friedman et al. 2009). The proportion of these out-of-bag
votes is used to determine the classification performance in terms of AUC, as explained in
Section 2.10.

2.8. k-nearest neighbours

The philosophy behind k-nearest neighbours (kNN) is that observations that show a high
degree of similarity are likely to share the same class label. Here, the distance between data
points is considered a measure for similarity. The (kNN) technique searches, for each point
in the validation dataset, the \( k \) datapoints from the training set that are closest in terms of
Euclidean distance.

The classification is decided by majority vote, with ties broken at random. If there are ties
for the kth nearest vector, all candidates are included in the vote (Friedman et al. 2009). In
the case of skewed class distributions, this majority voting might be somewhat problematic,
since one class is dominant by default (Coomans and Massart 1982).

Generally, larger values of \( k \) make the classification less susceptible to the effect of noise
(Everitt, Landau, Leese, and Stahl 2011). The value of value \( k \) is based on cross-validation,
as explained in Section 2.10. Moreover, in this study, we normalise all input variables before
applying kNN.

2.9. XGBoost

EXtreme Gradient Boosting (XGBoost) is a variant of the Gradient Boosting Machines
(GBM) algorithm that includes regularisation and dedicates its name due to its highly
efficient algorithmic implementation (Chen and Guestrin 2016). XGBoost is a machine
learning technique that uses the boosting principle by combining weakly performing indivi-
dual trees into an ensemble of trees representing a strong classifier. The primary purpose
of boosting is to reduce bias, but also suitable for reducing variance (Zhou 2012).

XGBoost evaluates the classification performance in each iteration and aims to correct
for the errors in each consequent step by adding a new tree. This new tree is trained on
the gradient, that is determined by deriving the negative gradient of the loss function with
respect to the predictions. The algorithm repeats this process for pre-specified number of
iterations. Regularisation is applied to avoid overly complex models. The predictions of the

final ensemble of trees are the weighted sum of the predictions on the log odds scale from the individual tree models.

As extensively described in Chen and Guestrin (2016), XGBoost aims to minimise the regularised objective function

\[ \mathcal{L}^{(t)} = \sum_{i=1}^{n} l \left( y_i, \hat{y}_i^{(t-1)} + f_t(x_i) \right) + \Omega(f_t), \]  

where \( \hat{y}_i^{(t)} \) is the prediction of the ith instance at the tth iteration, \( \Omega(f) \) the regularisation term. In each iteration, a new tree \( f_t \) is added aiming to minimise (7). Given the convex nature of the loss function \( l \), a second-order approximation of \( \mathcal{L}^{(t)} \) is applied.

In addition to the regularisation of leaf weights, shrinkage is implemented in the XGBoost algorithm by scaling newly added weights with a factor \( \nu \), with \( 0 < \nu \leq 1 \). Here, the lower the value for \( \nu \), the higher the computation time. Empirically, it was found that small values (\( \nu < 0.1 \)) lead to much better generalisation error (Friedman 1999).

\[ \text{Sensitivity} = \frac{TP}{TP + FN}, \quad \text{Specificity} = \frac{TN}{TN + FP}. \]

With \( TP = \text{true positives} \), \( TN = \text{true negatives} \), \( FP = \text{false positives} \), \( FN = \text{false negatives} \). In fact, the area under the ROC curve (AUC) represents the probability that a randomly chosen positive example is correctly rated (ranked) with greater likelihood than a randomly chosen negative example. Moreover, this probability of correct ranking is the same quantity estimated by the nonparametric Wilcoxon statistic (Bradley 1997). Thus, the higher the AUC the better the classification. Here, a perfect separation of cases and controls is denoted by \( AUC = 1 \) and the baseline setting where separation is not better than random is denoted by \( AUC = 0.5 \).

The performance of the biomarker inclusion is evaluated with the sensitivity, specificity and accuracy. Here, the accuracy defined by

\[ \text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}. \]
The closer the accuracy is to one the better the classification. For the imbalanced simulation scenarios and the trisomy case study, the area under the precision recall curve (AUCPR) is evaluated as well. Where recall is defined in (8), precision is described by

$$\text{Precision} = \frac{TP}{TP + FP}.$$ 

In the perfect situation, a model gives an AUCPR of 1, which means that all positive examples are correctly labelled (recall = 1), without falsely labelling a negative example as positive (precision = 1). In contrast to the AUC, the baseline for the AUCPR is denoted by the percentage of positive examples. To determine the AUC and the AUCPR, the trapezoidal integration method is used, which is implemented by the [R] software package ‘ROCR’ (Sing, Sander, Beerenwinkel, and Lengauer 2005).

### 2.11. Cross-validation

A major difference between the simulation scenarios and the case studies is the (dis-)ability to generate datasets of an arbitrary size. Therefore, we choose to apply different cross-validation (CV) strategies for the simulation scenarios and the case studies.

For all simulation scenarios, we generate a total number of 500 repetitions, each with a separate training set of size $n$ and new validation set with 5000 subjects. Note that the training set size depends per simulation scenario, and is defined in Table 4. For every single repetition, we apply 6-fold CV on the training set to determine the optimal set of tunable parameters for a particular method. Here, the parameter settings with the highest mean AUC over all 6 folds are selected as the optimal set of tunable parameter $t_{opt}$. So $t_{opt} = \text{argmax}_t\{\text{mean}(\text{AUC}(t))\}$. Then the predictive performance of each method is assessed on the validation set using the optimal parameters obtained via CV on the training data.

In the case studies, we apply repeated double CV (rdCV) with a total number of 500 repetitions. We use repetitions, each with different splits to obtain an unbiased estimate of the predictive performance. This way of cross-validation is especially useful when limited data or just one dataset is available, as one particular split of the outer CV could skew the results positively or negatively (Filzmoser et al. 2009). In addition, the prediction error is representative for new samples (Westerhuis et al. 2008). For each repetition, 6-fold outer CV is applied to assess the predictive performance. Here, the dataset is divided into a training and validation (also called test) set, according to a 5:1 ratio. For all 6 permutations of the outer CV with five folds for training and one fold for validation, $t_{opt}$ is determined using 6-fold inner CV on the outer CV training set with five folds. Note that each permutation could result in a different parameter $t_{opt}$, that is consequently used to assess the predictive performance on the outer CV validation set. By applying rdCV, the train and test set remain separated.

### 2.12. Tunable parameters

In this study, the considered methods vary in the number of tunable parameters. Where LR and LDA have no tunable parameters, the methods PLR, PLS-LDA, PCLR and kNN just have a single tunable parameter. Lastly, QBP, RF, SVM and XGBoost use numerous tunable parameters.
Table 4. Full design simulation study: All characteristics of all 9 datasets.

| Dataset 1 to 4 | Dataset 5 | Dataset 6 to 7 | Dataset 8 |
|---------------|-----------|---------------|-----------|
| ($\Psi_k = \ell$) | ($\Psi_k \not\in \{\ell^*, \text{exp}\}$) | ($\Psi_k = \text{exp}$) | ($\Psi_k \in \{\ell, \text{exp}\}$) |
| $\beta_k = 0$ | $\beta_k = 0$ | $\beta_k = 0$ | $\beta_k = 0$ |
| $\nu_k = 0$ | $\nu_k = 0$ | $\nu_k = 0$ | $\nu_k = 0$ |
| $\forall_k$ | $\forall_k$ | $\forall_k$ | $\forall_k$ |

Values of $\alpha_k$ and $\nu_k$ per transformation

| $\Psi_k = \ell$ | $\Psi_k = \text{exp}$ | $\Psi_k = \ell^*$ |
|----------------|----------------|----------------|
| $k$ | $\alpha_k$ | $\eta_k$ | $\alpha_k$ | $\eta_k$ | $\alpha_k$ | $\eta_k$ | $\beta_k$ | $\beta_k$ | $\nu_k$ | $\Psi_{k_{0}}$ | $\Psi_{k_{1}}$ | $\beta_k$ | $\nu_k$ | $\Psi_k$ | $\beta_k$ | $\nu_k$ |
| 1 | 617.8 | 509.7 | 6.19 | 0.65 | 604.4 | 439.2 | exp | exp | exp |
| 2 | 276.9 | 296.3 | 5.33 | 0.87 | 301.1 | 322.4 | exp | exp | exp |
| 3 | 2.61 | 14.94 | -1.86 | 1.53 | 0.50 | 1.55 | exp | exp | exp |
| 4 | 6.94 | 4.81 | 1.62 | 0.95 | 7.90 | 9.52 | exp | exp | exp |
| 5 | 72.08 | 16.72 | 4.25 | 0.23 | 72.13 | 17.02 | exp | exp | exp |
| 6 | 16.69 | 17.28 | 2.27 | 1.21 | 20.23 | 36.99 | exp | exp | exp |
| 7 | 3.25 | 1.28 | 1.11 | 0.38 | 3.27 | 1.30 | exp | exp | exp |
| 8 | 5.94 | 2.73 | 1.69 | 0.42 | 5.94 | 2.63 | exp | exp | exp |
| 9 | 11.66 | 13.59 | 1.84 | 1.22 | 13.29 | 24.78 | exp | exp | exp |
| 10 | 1.41 | 0.38 | 0.31 | 0.26 | 1.42 | 0.38 | exp | exp | exp |
| 11 | 62.29 | 20.64 | 4.07 | 0.37 | 62.73 | 23.78 | exp | exp | exp |
| 12 | 592.1 | 1395 | 5.90 | 0.86 | 526.6 | 549.8 | exp | exp | exp |
| 13 | 103.1 | 129.9 | 3.88 | 1.36 | 121.7 | 279.9 | exp | exp | exp |
| 14 | 177.4 | 61.28 | 5.13 | 0.31 | 177.0 | 55.50 | exp | exp | exp |
| 15 | 53.88 | 29.79 | 3.87 | 0.47 | 53.74 | 26.80 | exp | exp | exp |
| 16 | 8.55 | 0.76 | 2.14 | 0.09 | 8.56 | 0.78 | exp | exp | exp |
| 17 | 12.97 | 11.29 | 2.30 | 0.69 | 12.62 | 9.84 | exp | exp | exp |
| 18 | 0.71 | 0.48 | -0.47 | 0.51 | 0.71 | 0.39 | exp | exp | exp |
| 19 | 0.37 | 1.78 | 1.47 | 0.78 | 5.93 | 5.45 | exp | exp | exp |
| 20 | 0.78 | 1.11 | -1.54 | 2.01 | 1.63 | 12.27 | exp | exp | exp |
| 21 | 33.24 | 19.59 | 3.37 | 0.51 | 33.17 | 18.05 | exp | exp | exp |
| 22 | 0.31 | 0.20 | -1.30 | 0.58 | 0.32 | 0.21 | exp | exp | exp |
| 23 | 0.34 | 0.23 | -1.29 | 0.71 | 0.35 | 0.29 | exp | exp | exp |
| 24 | 0.22 | 0.29 | -1.87 | 0.80 | 0.21 | 0.20 | exp | exp | exp |

(continued)
### Table 4. Continued.

| Dataset 1 to 4  | Dataset 5  | Dataset 6 to 7 | Dataset 8  |
|-----------------|------------|---------------|------------|
| $\Psi_k = l$    | $\Psi_k \in [l^*, \exp]$ | $\Psi_k = \exp$ | $\Psi_k \in [l^*, \exp]$ |
| 1               | 2          | 3             | 4          |
| $\beta_k = 0$   | $\beta_k = 0$ | $\beta_k = 0$ | $\beta_k = 0$ |
| $\nu_k = 0$     | $\nu_k = 0$ | $\nu_k = 0$   | $\nu_k = 0$ |
| $\forall_k$     | $\forall_k$ | $\forall_k$   | $\forall_k$ |
| $\sigma_{27}$   | $\sigma_{27}$ | $\sigma_{27}$ | $\sigma_{27}$ |
| $\exp$          | $\exp$    | $\nu_k = 0$   | $\exp$ |
| $\psi_k = l^*$  | $\psi_k = \exp$ | $\forall_k$   | $\forall_k$ |
| 5               | 6          | 7             | 8          |
| $\beta_k = 0$   | $\beta_k = 0$ | $\beta_k = 0$ | $\beta_k = 0$ |
| $\nu_k = 0$     | $\nu_k = 0$ | $\nu_k = 0$   | $\nu_k = 0$ |
| $\forall_k$     | $\forall_k$ | $\forall_k$   | $\forall_k$ |
| $\exp$          | $\exp$    | $\exp$       | $\exp$ |
| $\sigma_{30}$   | $\exp$    | $\exp$       | $\exp$ |
| $\psi_k = l^*$  | $\psi_k = \exp$ | $\forall_k$   | $\forall_k$ |

**Values of $\alpha_k$ and $\nu_k$ per transformation**

| $\Psi_k = l$ | $\Psi_k = \exp$ | $\Psi_k = l^*$ |
|--------------|-----------------|----------------|
| $k$ | $\alpha_k$ | $\eta_k$ | $\alpha_k$ | $\eta_k$ | $\alpha_k$ | $\eta_k$ | $\beta_k$ | $\beta_k$ | $\nu_k$ | $\psi_{k0}$ | $\psi_{k1}$ | $\beta_k$ | $\nu_k$ | $\psi_{k0}$ | $\psi_{k1}$ |
| 25 | 0.07 | 0.10 | -2.82 | 0.64 | 0.07 | 0.05 | exp | exp | exp | 0.32 | exp | 0.32 |
| 26 | 3.64 | 2.12 | 1.04 | 1.01 | 4.72 | 6.29 | $\sigma_{27}$ | $\sigma_{27}$ | $\sigma_{27}$ | exp | exp | 0.10 |
| 27 | 66.95 | 82.64 | 3.37 | 1.82 | 153.1 | 794.2 | 0.10 | exp | exp | exp | 0.10 | exp |
| 28 | 4.98 | 2.34 | 1.39 | 0.92 | 6.10 | 7.02 | $\sigma_{30}$ | exp | exp | 0.26 | exp | 0.26 |
| 29 | 21.40 | 29.97 | 2.64 | 0.81 | 19.47 | 18.87 | exp | $\exp$ | 0.31 | exp | 0.31 |
| 30 | 13.09 | 24.77 | 1.71 | 1.36 | 14.03 | 32.74 | exp | exp | exp | exp |
| 31 | 14.69 | 12.06 | 2.39 | 0.82 | 15.23 | 14.84 | exp | exp | exp | 0.31 | exp |
| 32 | 7.28 | 5.65 | 1.77 | 0.65 | 7.25 | 5.26 | exp | exp | exp | exp |
| 33 | 15.37 | 37.54 | 1.67 | 1.38 | 13.69 | 32.66 | exp | exp | exp | exp |
| 34 | 0.13 | 0.20 | -2.64 | 1.21 | 0.15 | 0.27 | $\sigma_{34}$ | $\sigma_{34}$ | $\sigma_{34}$ | exp | exp |
| 35 | 22.53 | 37.47 | 2.62 | 0.94 | 21.27 | 25.29 | $\exp$ | $\exp$ | $\exp$ |

**Nr. relevant biomarkers**

| Dataset 1 to 4  | Dataset 5  | Dataset 6 to 7 | Dataset 8  |
|-----------------|------------|---------------|------------|
| 0               | 5          | 10            | 9          |
| 100             | 100        | 100           | 100        |
| 100             | 100        | 100           | 100        |

**Nr. of participants (n)**

| Dataset 1 to 4  | Dataset 5  | Dataset 6 to 7 | Dataset 8  |
|-----------------|------------|---------------|------------|
| a: 100          | a: 100     | a: 100        | a: 100     |
| b: 400          | b: 400     | b: 400        | b: 400     |
| c: 250          | c: 250     | c: 250        | c: 250     |

**Proportion of cases ($\phi$)**

| Dataset 1 to 4  | Dataset 5  | Dataset 6 to 7 | Dataset 8  |
|-----------------|------------|---------------|------------|
| 1/2             | 1/2        | 1/2           | 1/2        |
| 1/2             | 1/2        | 1/2           | 1/2        |
| 1/2             | 1/2        | 1/2           | 1/2        |
| 1/2             | 1/2        | 1/2           | 1/2        |

Note that the transformation $\Psi_k = l$ equals $\Psi_k(x) = x$ and $\Psi_k = \exp$ equals $\Psi_k(x) = \exp(x)$. Moreover, $\alpha_k$ and $\eta_k$ denote the applied mean and standard variance derived from the MDD case study. Lastly, $\beta_k$ and $\nu_k$ denote the shift in mean and standard deviation. Lastly, empty cells correspond to a value of 0.
In specific, we define the penalty term for PLR, the number of principal components for PLS-LDA and PCLR, and the number of neighbours for kNN. Here, the penalty term of PLR was obtained using the automated cross-validation procedure of the \textit{glmnet} package (Friedman, Hastie, and Tibshirani 2010) of [R]. For both PLS-LDA and PCLR we selected the optimal number of sparse components via CV $ncomp \in \{1, \ldots, p\}$, with $p$ the number of covariates. For kNN, the optimal number of neighbours was selected from a set of candidates with step size from 1 to 20 and an increasing step size above 20 neighbours.

QBP has in principle many tunable parameters, but we made some decisions upfront. We fix both the number of percentiles and the corresponding proportion choice – obtaining $\{q_1, q_3, q_{10}, q_{90}, q_9, q_9\}$ – and keep all biomarker weights equal. The settings that are determined by cross-validation are the lower boundaries of the exceed ratios and the maximal interval scores, which are defined by the sets $R^\ast = (R_1^\ast, R_2^\ast, R_3^\ast) \in \{(1.5, 2, 3), (1.5, 2, 5), (1.5, 2.5, 5), (1.4, 2.5, 8), (2, 3, 6), (2, 3, 10)\}$ and $\nu = (\nu_1, \nu_2, \nu_3) \in \{(1, 2, 3), (1, 4, 9)\}$, respectively. Eventually, the optimal setting is selected from $R^\ast \times \nu$.

To reduce the computational complexity for RF, XGBoost and SVM in the final simulation study, we have selected a subset of a larger grid of candidate tunable parameters. Each combination of tunable parameters was used to fit a model on a training dataset of 5000 subjects, after which the performance was evaluated on the corresponding validation datasets with 5000 subjects. To determine the final subset, we considered all scenarios and selected the most relevant tuning parameters using a regression approach.

For RF, checking the convergence of the out-of-bag error resulted in a total number of trees $ntree$ of 3000. In addition, we chose number of variables sampled randomly at each split to be $mtry \in \{6, 9, 12, 15, 18\}$. For XGBoost, the final set of tunable parameters is $nrounds = 300$, $eta \in \{0.05, 0.15, 0.3\}$, $max_depth \in \{2, 4\}$, $colsample_bytree = 0.75$, $min_child_weight = 2$, $gamma = 0$ and $subsample = 1$.

### 3. Simulation study

#### 3.1. Model and settings

The group indicator $y_i \in \{0, 1\}$ was divided such that we obtain $\phi \cdot n$ cases ($y_i = 1$) and $(1 - \phi) \cdot n$ controls ($y_i = 0$), where $\phi$ denotes the proportion of cases and $n$ the total number of participants $n$. Then the variables $z_{i, 1}, \ldots, z_{i, r}$ were drawn from a multivariate distribution with mean 0 and variance–covariance matrix $R$. In the statistical software [R], we used the \textit{mvnrnorm} function of the ‘MASS’ package to create the variables $z$ (Venables and Ripley 2002). Then the variables $v_{i, 1}, \ldots, v_{i, r}$ were created equal to

$$v_{i, k} = \mu_{i, k} + \sigma_{i, k} z_{i, k},$$

with $\mu_{i, k} = \alpha_k + \beta_k y_i$ and $\sigma_{i, k} = \eta_k \cdot (1 + v_k - 2v_k y_i)$ for all $i = 1, \ldots, n$ and $k = 1, \ldots, r$. When $\beta_k = 0$ and $v_k = 0$, cases and controls are drawn from the same distribution and the variable $v_{i, k}$ does not contribute directly to the classification of cases and controls. Moreover, the variables $\alpha_k$ and $\eta_k$ differ per dataset and are based on (a transformation of) the MDD case study and correspond to its mean and standard deviation, respectively. Note that positivity of $\sigma_{i, k}$ is ensured in the simulation study by positivity of $\eta_k$ and selecting $v_k$ such that $-0.5 < v_k < 0.5$ for all $k$. Finally, we take a transformation of the variables $v_{i, k}$
to have non-normally distributed variables that can be skewed. Thus, \( x_{i,k} = \Psi_k(v_{i,k}) \) with \( \Psi_k \), the transformation that can be unique for each variable \( k = 1, \ldots, r \).

In total, nine different types of datasets are simulated, with varying transformations, sample sizes and number of relevant biomarkers (\( \beta_k \neq 0 \) and/or \( \nu_k \neq 0 \)). We distinguish two types of transformations, namely

\[
\Psi_k(x) = x, \quad \Psi_k(x) = \exp(x),
\]

where the former one results into normally distributed data and the latter in log-normally distributed data. We select only one type of transformation per dataset and biomarker, except for dataset 5, where for some covariates the biomarker distributions of the controls are normally distributed and those of the cases log-normally distributed, to create differences in terms of skewness. Here, the values for \( \alpha_k \) and \( \nu_k \) of \( \Psi_k = I^* \) of the control group are chosen such its expected average and variance are similar to those of the distribution of the cases with \( \Psi_k = \exp \).

The variance--covariance matrix \( R \) was always the same and based on the MDD case study in this paper. The full specification of \( R \) and the settings for \( \alpha_k \) and \( \eta_k \) are given in Table 4. The relevant biomarkers varied in number and in the way that they were different between cases and controls. Some varied only in mean (\( \beta_k \neq 0 \)), some varied only in variance (\( \nu_k \neq 0 \)) and other varied in both means and variances. A full overview of the choices is given in Table 4. Each dataset type is simulated 500 times.

Dataset 1, 2 and 3 have the identity biomarker transformation and therefore obey a normal distribution. The datasets differ in terms of number of relevant biomarkers. Moreover, the applied linear transformation is a shift in mean \( \beta_k \) of one standard deviation of that particular biomarker \( \sigma_k \). Dataset 4 is also normally distributed with a shift in standard deviation \( \nu_k \). A difference in skewness for some of the biomarkers is simulated in dataset 5. Whereas datasets 6 and 7 solely have log-normally distributed biomarkers, dataset 8 has a mixture of normally and log-normally distributed biomarkers. Dataset 6 to 8 show a fixed shift in mean \( \beta_k \) and/or shift in standard deviation \( \nu_k \). Besides that these datasets vary in the total number of participants \( n \), where both a balanced and unbalanced number of cases and controls are considered. Except datasets 6c, 7c and 8c that consider an unbalanced setting with \( \phi = 1/5 \), all other datasets are balanced (\( \phi = 1/2 \)).

### 3.2. Results

For all binary classification techniques and datasets, the predictive performance in terms of AUC is presented in Table 5. In Figures 2 and 3, we present the density plots and confidence intervals of the predictive performance, respectively. These graphs only contain a subset of the techniques, namely PLR.Lasso, LDA, SVM.Radial, RF, kNN, XGB and QBP. This selection is based on superior performance in at least one of the simulated datasets. As part of a sensitivity analysis, the predictive performance in terms of AUCPR is presented for all unbalanced datasets (6c, 7c and 8c) in Table 6.

Moreover, in Table 7 the number of used biomarkers in the final model is presented as well as the applied number of sparse components for the PCLR and PLS-LDA and the number of neighbours for kNN. The effect of a sample size on the sensitivity, specificity and accuracy of the biomarker selection is presented in Table 8. Here, only the methods PLR.Lasso, PLR.EN and QBP are included in the overview, since all the other methods
always include all biomarkers and therefore apply no selection. Finally, in Table 9, the average computation times are listed for the datasets with a sample size of \( n = 100 \), \( n = 250 \) and \( n = 400 \).

**Figure 2.** Performance (in AUC) of all 500 simulations with each a different training set to tune the parameters and validation set of 5000 subjects to assess the performance.
Figure 3. Confidence intervals of the classification performances (in AUC) based on 500 simulations per dataset type.
Table 5. Performance (in AUC) of all considered techniques of all simulated datasets on validation data.

| Dataset | PLR | SVM |
|---------|-----|-----|
|         | LR  | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
| 1       | Mean 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
|         | SD 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| 2       | Mean 0.943 | 0.971 | 0.972 | 0.973 | 0.977 | 0.977 | 0.971 | 0.973 | 0.919 | 0.853 | 0.915 | 0.915 | 0.854 |
|         | SD 0.022 | 0.022 | 0.011 | 0.029 | 0.009 | 0.009 | 0.010 | 0.010 | 0.015 | 0.026 | 0.014 | 0.034 |
| 3       | Mean 0.984 | 0.988 | 0.992 | 0.980 | 0.995 | 0.996 | 0.994 | 0.993 | 0.968 | 0.957 | 0.953 | 0.948 |
|         | SD 0.009 | 0.007 | 0.006 | 0.013 | 0.003 | 0.002 | 0.004 | 0.005 | 0.008 | 0.013 | 0.017 | 0.017 |
| 4       | Mean 0.499 | 0.499 | 0.499 | 0.500 | 0.499 | 0.499 | 0.500 | 0.527 | 0.629 | 0.530 | 0.584 | 0.652 |
|         | SD 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.017 | 0.029 | 0.011 | 0.031 | 0.034 |
| 5       | Mean 0.943 | 0.971 | 0.972 | 0.973 | 0.977 | 0.977 | 0.971 | 0.973 | 0.919 | 0.853 | 0.915 | 0.915 | 0.854 |
|         | SD 0.022 | 0.022 | 0.011 | 0.029 | 0.009 | 0.009 | 0.010 | 0.010 | 0.015 | 0.026 | 0.014 | 0.034 |
| 6       | Mean 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
|         | SD 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| 7       | Mean 0.943 | 0.971 | 0.972 | 0.973 | 0.977 | 0.977 | 0.971 | 0.973 | 0.919 | 0.853 | 0.915 | 0.915 | 0.854 |
|         | SD 0.022 | 0.022 | 0.011 | 0.029 | 0.009 | 0.009 | 0.010 | 0.010 | 0.015 | 0.026 | 0.014 | 0.034 |
| 8       | Mean 0.499 | 0.499 | 0.499 | 0.500 | 0.499 | 0.499 | 0.500 | 0.527 | 0.629 | 0.530 | 0.584 | 0.652 |
|         | SD 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.017 | 0.029 | 0.011 | 0.031 | 0.034 |

Except the unbalanced datasets 6c, 7c and 8c ($\phi = 1/5$), all datasets are balanced ($\phi = 1/2$). Moreover, dataset 1 to 5, 6a, 7a, 8a have $n = 100$ and dataset 6b, 7b, 8b have $n = 400$.

Table 6. Sensitivity analysis for all simulation scenarios with a class imbalance ($\phi = 1/5, n = 250$): Performance (in AUCPR) of all considered techniques on validation data.

| Dataset | PLR | SVM |
|---------|-----|-----|
|         | LR  | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
| 6c      | Mean 0.551 | 0.580 | 0.570 | 0.552 | 0.539 | 0.517 | 0.523 | 0.438 | 0.457 | 0.506 | 0.350 | 0.560 | 0.423 |
|         | SD 0.042 | 0.056 | 0.043 | 0.044 | 0.048 | 0.045 | 0.042 | 0.055 | 0.053 | 0.031 | 0.034 | 0.032 | 0.053 |
| 7c      | Mean 0.623 | 0.626 | 0.627 | 0.629 | 0.618 | 0.623 | 0.618 | 0.603 | 0.621 | 0.705 | 0.588 | 0.663 | 0.704 |
|         | SD 0.032 | 0.038 | 0.036 | 0.030 | 0.038 | 0.030 | 0.031 | 0.032 | 0.03 | 0.028 | 0.026 | 0.029 |
| 8c      | Mean 0.666 | 0.656 | 0.654 | 0.655 | 0.652 | 0.647 | 0.650 | 0.633 | 0.671 | 0.729 | 0.607 | 0.725 | 0.726 |
|         | SD 0.024 | 0.034 | 0.034 | 0.026 | 0.034 | 0.026 | 0.024 | 0.023 | 0.027 | 0.019 | 0.027 | 0.024 |

4. Case study

4.1. Major depression disorder

4.1.1. Design of the study

The MDD study contains 102 subjects in total of which 51 cases and 51 controls (van Buel et al. 2019). Five subjects with missing values were excluded from the analysis to make a fair comparison between the methods and avoid the effect of imputations on the
Table 7. Number of included biomarkers, components for sparse methods derived from training data and applied in final model and neighbours included for kNN.

| Dataset | Nr. relevant biomarkers | Biomarkers | Sparse components |
|---------|-------------------------|------------|-------------------|
|         |                         | PLR        |                   |
|         |                         | Lasso EN   | QBP PCLR PLS-LDA kNN |
| 1 (n = 100, φ = 1/2) | 0          | Mean 16.0 16.5 26.0 15.0 5.4 12.0 | |
|         | SD 13.6 13.8 6.9 11.5 5.6 9.2 |           |                   |
| 2 (n = 100, φ = 1/2) | 5          | Mean 18.5 23.0 21.8 29.1 6.1 19.8 | |
|         | SD 5.9 7.4 5.0 4.6 3.0 6.6 |           |                   |
| 3 (n = 100, φ = 1/2) | 10         | Mean 18.8 23.5 25.0 26.3 4.7 19.4 | |
|         | SD 4.5 6.4 4.6 6.4 3.1 6.7 |           |                   |
| 4 (n = 100, φ = 1/2) | 9          | Mean 16.4 16.8 27.2 14.6 5.4 10.4 | |
|         | SD 13.6 13.9 6.0 11.5 5.3 8.4 |           |                   |
| 5 (n = 100, φ = 1/2) | 9          | Mean 14.2 14.8 22.9 16 4.8 9.7 | |
|         | SD 13.4 13.6 4.8 11.6 4.6 8.2 |           |                   |
| 6a (n = 100, φ = 1/2) | 7          | Mean 11.7 12.5 24.2 24.4 5.4 18.9 | |
|         | SD 9.6 10.9 6.3 6.8 3.8 7.7 |           |                   |
| 6b (n = 400, φ = 1/2) | 7          | Mean 24.3 26.2 6.8 33.6 6.4 47.7 | |
|         | SD 5.6 5.7 4.2 2.1 2.9 9.6 |           |                   |
| 6c (n = 250, φ = 1/5) | 7          | Mean 18.5 20.4 15.7 31.8 6.0 32.9 | |
|         | SD 9.2 10.3 7.3 3.1 3.0 10.9 |           |                   |
| 7a (n = 100, φ = 1/2) | 9          | Mean 20.3 20.7 27.4 20.2 5.8 11.8 | |
|         | SD 13.4 13.6 5.8 11.7 5.0 8.6 |           |                   |
| 7b (n = 400, φ = 1/2) | 9          | Mean 28.1 28.6 16.9 30.2 7.2 21.9 | |
|         | SD 9.6 9.8 4.4 9 3.9 15.1 |           |                   |
| 7c (n = 250, φ = 1/5) | 9          | Mean 22.1 22.8 23.2 21.2 5.4 19.0 | |
|         | SD 12.8 13.0 7.5 12.9 4.1 13.4 |           |                   |
| 8a (n = 100, φ = 1/2) | 14         | Mean 17.5 18.4 28.7 21.3 3.9 13.4 | |
|         | SD 12.3 12.5 5.3 9.1 3.6 8.5 |           |                   |
| 8b (n = 400, φ = 1/2) | 14         | Mean 20.8 21.2 19.7 29.9 3.7 34 | |
|         | SD 10.2 10.7 3.9 4.9 2.6 14.9 |           |                   |
| 8c (n = 250, φ = 1/5) | 14         | Mean 21.7 22.7 24.5 27.1 3.9 21.9 | |
|         | SD 11.2 11.7 6.8 7.5 2.9 13.1 |           |                   |

Note that LR.LOGIT, PLR.Ridge, PCLR, PLS-LDA, LDA, SVM, RF, kNN & XGB use all biomarkers: mean = 35 and SD = 0.

Performance. This results in a dataset with 47 cases (MDD patients) and 50 controls, for which the predictive performance of all methods is assessed using rdCV.

For each subject, a total of 35 biomarkers is included, of which 16 are serum-based biomarkers and 19 are urine-based biomarkers. This set of serum and first morning urine biomarkers was selected based on a thorough literature search, combined with a biomarker selection based on a pilot study with 24 participants (12 MDD patients and their sex, age and ethnic matched non-MDD controls). An overview of all biomarker types is presented in Table 10.

4.1.2. Results
For all binary classification techniques, the predictive performance expressed in the mean and its standard error are shown in Table 11. Besides, this table contains the number of biomarkers that were used on the validation dataset. The density plots of the predictive performance of a subset of the techniques (LR, PLR.Lasso, SVM.Radial, RF, kNN, XGB and QBP) are shown in Figure 4.
Table 8. Effect of sample size on inclusion performance of the relevant biomarkers.

| Dataset     | Nr. relevant biomarkers | Measure | Lasso | EN | QBP |
|-------------|-------------------------|---------|-------|----|-----|
| 6a (n = 100, φ = 1/2) | 7 | Accuracy | 0.689 | 0.676 | 0.459 |
|             |             | Sensitivity | 0.557 | 0.58 | 0.878 |
|             |             | Specificity | 0.721 | 0.7 | 0.355 |
| 6b (n = 400, φ = 1/2) | 7 | Accuracy | 0.467 | 0.42 | 0.885 |
|             |             | Sensitivity | 0.903 | 0.922 | 0.696 |
|             |             | Specificity | 0.358 | 0.295 | 0.932 |
| 6c (n = 250, φ = 1/5) | 7 | Accuracy | 0.58 | 0.537 | 0.667 |
|             |             | Sensitivity | 0.771 | 0.797 | 0.790 |
|             |             | Specificity | 0.532 | 0.472 | 0.636 |
| 7a (n = 100, φ = 1/2) | 9 | Accuracy | 0.481 | 0.475 | 0.434 |
|             |             | Sensitivity | 0.618 | 0.628 | 0.922 |
|             |             | Specificity | 0.434 | 0.422 | 0.266 |
| 7b (n = 400, φ = 1/2) | 9 | Accuracy | 0.395 | 0.383 | 0.744 |
|             |             | Sensitivity | 0.883 | 0.887 | 0.942 |
|             |             | Specificity | 0.226 | 0.208 | 0.676 |
| 7c (n = 250, φ = 1/5) | 9 | Accuracy | 0.472 | 0.458 | 0.545 |
|             |             | Sensitivity | 0.701 | 0.715 | 0.903 |
|             |             | Specificity | 0.393 | 0.369 | 0.420 |
| 8a (n = 100, φ = 1/2) | 14 | Accuracy | 0.556 | 0.552 | 0.513 |
|             |             | Sensitivity | 0.569 | 0.599 | 0.917 |
|             |             | Specificity | 0.548 | 0.522 | 0.244 |
| 8b (n = 400, φ = 1/2) | 14 | Accuracy | 0.59 | 0.586 | 0.787 |
|             |             | Sensitivity | 0.732 | 0.738 | 0.939 |
|             |             | Specificity | 0.495 | 0.485 | 0.687 |
| 8c (n = 250, φ = 1/5) | 14 | Accuracy | 0.545 | 0.534 | 0.617 |
|             |             | Sensitivity | 0.705 | 0.729 | 0.895 |
|             |             | Specificity | 0.438 | 0.404 | 0.431 |

Note that the methods LR.LOGIT, PLR.Ridge, PCLR, PLS-LDA, LDA, SVM, RF, kNN & XGB use all biomarkers and are not included in this overview.

Table 9. Average computation times (in seconds) for a training (6-fold inner cross-validation) and validation (model fit and evaluation) cycle for a single datasets with a sample size of $n = 100$ (dataset 1,2,3,4,5,6a,7a,8a), $n = 400$ (dataset 6b,7b,8b) and $n = 250$ (dataset 6c,7c,8c).

| Sample size | LR | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
|-------------|----|-------|----|-------|------|---------|-----|--------|--------|----|-----|-----|-----|
| 100         | 0.06 | 0.81  | 0.46 | 0.98  | 9.09 | 8.03    | 0.01 | 81.1   | 5.64   | 18.7 | 94.5 | 80.5 | 22.6 |
| 400         | 0.06 | 0.39  | 0.40 | 0.67  | 9.71 | 9.64    | 0.02 | 363.9  | 31.6   | 94.9 | 135.6 | 86.5 | 25.4 |
| 250         | 0.06 | 0.48  | 0.43 | 0.70  | 9.51 | 8.89    | 0.01 | 367.2  | 14.0   | 51.4 | 125.8 | 82.4 | 24.3 |

Note that LR.LOGIT and LDA do not include a tunable parameter optimisation.

4.2. Trisomy

4.2.1. Design of the study

The trisomy dataset is provided by the Foundation of Prenatal Screening of the Northern Netherlands and consists of a first-trimester combined test screening program in the Netherlands in a multi-centre routine clinical setting. Whereas earlier evaluations have taken place based on data in the period of July 2002 to May 2004, as published in Schielen et al. (2006), this study only includes subjects after of July 1, 2010. From this moment, risks at trisomy were calculated by the Dutch National Institute for Public Health and the Environment (RIVM) according to the Astraia/Fetal Medicine Foundation (FMF) risk software.
Table 10. Included biomarkers in MDD data, where the numbers are aligned with the biomarker numbers $k$.

| Serum biomarkers | Urine biomarkers |
|------------------|------------------|
| 1. BDNF          | 9. Thromboxane   |
| 2. Midkine       | 10. Endothelin    |
| 3. Nitrotyrosine | 11. Lipocalin     |
| 4. EGF           | 12. NPY          |
| 5. TNFR2         | 13. Leptin       |
| 6. LTB4          | 14. HVEM         |
| 7. Cortisol      | 15. Vit-D        |
| 8. Calprotectin  | 16. Zonulin      |
| 17. cAMP         | 18. cGMP         |
| 21. LTB4         | 22. Cortisol     |
| 23. Thromboxane  | 24. Isoprostane  |
| 25. Endothelin   | 26. Aldosteron   |
| 27. Adiponectin  | 28. HVEM         |
| 29. Midkine      | 30. EGF          |
| 31. SubstanceP   | 32. TNFR2        |
| 33. Lipocalin    | 34. Pregnancylon |
| 35. NPY          |                  |

Figure 4. Performance (in AUC) measured on validation dataset: 500 repeats, 6-fold outer CV, 6-fold inner CV.

Table 11. Summary statistics of the performance of all methods on MDD data: AUC validation data and included number of components.

|                   | PLR | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
|-------------------|-----|-------|----|-------|------|---------|-----|--------|--------|----|-----|-----|-----|
|                   | AUC | VAL   |    |       |      |         |     |        |        |    |     |     |     |
| Mean              | 0.518|       |    |       | 0.486| 0.485   | 0.512| 0.492  | 0.505  | 0.523| 0.516| 0.501| 0.635| 0.495| 0.606| 0.680|
| SD                | 0.130|       |    |       | 0.131| 0.130   | 0.135| 0.131  | 0.131  | 0.134| 0.133| 0.134| 0.136| 0.131| 0.150| 0.132|
| NCOMP             | Mean | 35    |    |       | 22.2 | 22.7    | 35   | 35     | (19.3) | 35  | 35   | 35   | 35   | 35   | 27.6 |
|                   | SD   | 13.2  |    |       | 13.2 | 0       | 0    | (10.9) | (5.4)  | 0   | 0    | 0    | 0    | 0    | 4.5  |

For PCLR, PLS-LDA the number of sparse components is given by (…) and for kNN the number of neighbours $k$ is represented by (…).

The first-trimester combined test is composed of three elements: (1) assay of the serum concentrations of pregnancy-associated plasma protein A ($PAPP-A$) and the free $\beta$ subunit of human chorion gonadotrophin ($f\beta-hCG$) between 8–14 weeks of the pregnancy, (2) ultrasound measurement of the nuchal translucency ($NT$) subcutaneous oedema in the foetal neck, to be measured at a gestational age (GA) between 10–11 and 14 weeks, and (3) maternal age. Accompanied with this test, the crown-rump length ($CRL$) that was used to determine the GA was recorded, the age of the mother, parity and gravidity.

In the late 90s, with the introduction of maternal serum biochemistry and ultrasound screening for chromosomal defects at different stages of pregnancy, it has become necessary
to establish maternal and gestational age-specific risks for chromosomal defect (Nicolaiades 2003). Since the GA affects the biochemical parameters \(PAPP-A\) and \(f\beta-hCG\), we use the multiple of median (MoM) versions \(PAPP-A\) and \(f\beta-hCG\) in the analysis.

The method that RIVM uses to determine the risk on trisomy per subject, namely the FMF risk, takes into account women’s a priori risk, based on her maternal age and gestational age, and multiply this by a series likelihood ratios of \(MoM-f\beta-hCG\), \(MoM-PAPP-A\), \(NT\). This likelihood ratio is obtained by dividing the percentage of cases by the percentage of controls with that measurement. The probability on having Down Syndrome is defined in terms of an odds-ratio (Shiefa, Amargandhi, Bhupendra, Moulali, and Kristine 2013).

In the dataset provided by RIVM, the FMF risk is determined on a dataset with \(n = 3784\) observations (53 cases and 3731 controls) and derived using the biomarkers maternal age, \(NT\), \(MoM-f\beta-hCG\) and \(MoM-PAPP-A\). Note that for some subjects in this dataset a single biomarker value is missing. For these missing values of a certain combination of subject and biomarker, QBP imputes a disease score of 0, making that the biomarker distribution remains unaffected. As the classification performance of the FMF risk was assessed by training and validating on the full dataset, we do the same for QBP.

For the comparison of QBP with the selected alternative methods we use a smaller dataset with only complete observations to make sure that the comparison is not influenced by any imputation procedure. This dataset has \(n = 3514\) observations (48 cases and 3466 controls) and utilises the biomarkers maternal age, parity, gravidity, \(MoM-f\beta-hCG\), \(MoM-PAPP-A\), \(NT\) and \(CRL\). Here, the predictive performance is assessed using rdCV. Here, QBP uses the optimal tunable parameter setting of the maximal interval score and lower boundary on the exceed ratio. For the sensitivity analysis, downsampling is applied on the majority class (controls). In each repetition of the rdCV process, a different sample of size 48 is taken from the total set of 3466 controls.

### 4.2.2. Results

The predictive performance and number of biomarkers of all considered techniques is presented in Table 12. In Figure 5, the corresponding density plots of the AUC are provided for subset of the techniques – namely LR, PLR.Lasso, SVM.Radial, RF, kNN, XGB and QBP.
Table 12. Summary statistics of the performance of all methods on Trisomy data: AUC validation data and included number of components.

| Method | PLR | LR | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
|--------|-----|----|-------|----|-------|------|---------|-----|--------|--------|----|-----|-----|-----|
| AUC VAL | Mean | 0.914 | 0.834 | 0.854 | 0.727 | 0.909 | 0.881 | 0.886 | 0.909 | 0.886 | 0.903 | 0.896 | 0.908 | 0.908 |
|        | SD   | 0.058 | 0.176 | 0.155 | 0.209 | 0.062 | 0.076 | 0.073 | 0.058 | 0.075 | 0.074 | 0.070 | 0.070 | 0.066 |
| NCOMP | Mean | 7 | 5.0 | 6.4 | 7 | 7(6.8) | 7(4.6) | 7 | 7 | 7 | 7 | 143.2 | 7 | 5.8 |
|        | SD   | 0 | 2.1 | 0.8 | 0 | 0(0.7) | 0(2.0) | 0 | 0 | 0 | 0 | 0 (24.8) | 0 | 0.6 |

For PCLR, PLS-LDA the number of sparse components is given by \((\ldots)\) and for kNN the number of neighbours \(k\) is represented by \((\ldots)\).

Table 13. Sensitivity analysis on the classification performance of all methods on Trisomy data when using the full size unbalanced dataset compared to the downsampling strategy: AUC and AUCPR on validation data.

| Method | PLR | LR | Lasso | EN | Ridge | PCLR | PLS-LDA | LDA | Linear | Radial | RF | kNN | XGB | QBP |
|--------|-----|----|-------|----|-------|------|---------|-----|--------|--------|----|-----|-----|-----|
| AUC VAL | Mean | 0.914 | 0.834 | 0.854 | 0.727 | 0.909 | 0.881 | 0.886 | 0.909 | 0.886 | 0.903 | 0.896 | 0.908 | 0.908 |
|        | SD   | 0.058 | 0.176 | 0.155 | 0.209 | 0.062 | 0.076 | 0.073 | 0.058 | 0.075 | 0.074 | 0.070 | 0.070 | 0.066 |
| AUCPR VAL | Mean | 0.552 | 0.467 | 0.486 | 0.318 | 0.544 | 0.437 | 0.438 | 0.487 | 0.560 | 0.571 | 0.413 | 0.595 | 0.416 |
|        | SD   | 0.162 | 0.241 | 0.225 | 0.241 | 0.164 | 0.151 | 0.150 | 0.151 | 0.164 | 0.159 | 0.143 | 0.162 | 0.153 |

Regarding the FMF risk, we obtain a performance of the classification of cases and controls of \(AUC = 0.9151\). For QBP, we have \(AUC = 0.9249\) with the maximal interval score \(v = (1, 2, 3)\) and lower boundaries for the exceed ratios \(R^* = (2, 3, 6)\) as optimal tunable parameter combination.

In Table 13, the results of the sensitivity analysis are presented. Here, the AUC and the AUCPR are listed for both the original analysis using the full dataset as well as the scenario using downsampling of the number of controls.

5. Discussion

In this study, we have performed an extensive comparative study between supervised binary disease prediction methods, focusing on all sorts of differences in distributions between cases and controls that appear in reality caused by biological processes and the complexity of diseases. Inspired by the situation in which using simple location measures are failing to discriminate between cases and controls, and using only tail information may better capture differences in biomarker distributions, we proposed a novel method called QBP. Our method, that uses the quantiles of the continuous biomarker distributions, was compared with traditional statistical classification methods such as LR, PLR, PCLR, LDA and PLS-LDA, as well as more novel machine learning techniques such as kNN, RF, SVM and XGB. We studied the predictive performance of QBP compared to
the alternative methods, but also other features, e.g. effect of sample size and number of selected biomarkers/components in the final model.

In a simulation study, differences in means, variance and skewness between cases and controls were simulated for certain biomarkers. When cases and controls were drawn from the same distribution (dataset 1), it was demonstrated that QBP is unbiased (average \( AUC = 0.5 \)) just like all other methods. In the two datasets with biomarkers having only systematic shifts in the mean with a size of one times the standard deviation, LDA tends to be superior (\( AUC = 0.977 \) in dataset 2 and \( AUC = 0.996 \) in dataset 3). Compared to LDA, QBP has a worse predictive performance in terms of AUC (\( AUC = 0.854 \ (-12.6\%) \) and \( AUC = 0.948 \ (-5\%) \), for dataset 2 and 3, respectively). In contrast to the performance gap with LDA, PLR, PLS-LDA and SVM, QBP performs just slightly worse compared to RF and XGB. In case of normally distributed data with a shift in standard deviation (dataset 4), QBP is superior to all methods (\( AUC = 0.652 \)). Whereas RF and XGB seem to come relatively close (\( AUC = 0.629 \) and \( AUC = 0.584 \) respectively), all logistic regression and LDA-based techniques and SVM fail to discriminate better than random (\( AUC = 0.5 \)).

In order to create a mixture of skewed and not skewed biomarker distributions, both normal and log-normal biomarkers are simulated. When simulating a shift in skewness, while remaining the mean and variance constant (dataset 5), RF and XGB were superior (\( AUC = 0.963 \) and \( AUC = 0.917 \), respectively), followed by QBP (\( AUC = 0.860 \)). All other techniques show a very weak classification performance (\( AUC < 0.569 \)). Compared to dataset 2 and 3, it seems that changing the biomarker distribution from normally distributed biomarkers to log-normally distributed biomarkers – while maintaining the shifts in mean parameter for some biomarkers (dataset 6a, 6b and 6c) – just slightly changes the relative differences in performance between the techniques. In specific, QBP demonstrated an inferior performance (\( AUC = 0.688 \ (-14.4\%) \), \( AUC = 0.785 \ (-9.3\%) \) and \( AUC = 0.724 \ (-13\%) \) for datasets 6a, 6b and 6c, respectively) relative to the best in class Lasso. Simultaneously, the gap between Lasso and the machine learning techniques RF and XGB has shrunk. In the datasets with only changes in the variances for some biomarkers and log-normal biomarker distributions (datasets 7a, 7b and 7c), the predictive performance of QBP (\( AUC = 0.652 \), \( AUC = 0.751 \) and \( AUC = 0.674 \) for datasets 7a, 7b and 7c, respectively) was better or equal compared to its successor RF. This conclusion is also in line with dataset 4, where the data was normally distributed. Note that the difference in performance between QBP and RF decreased with increasing sample size. In the datasets where biomarkers may change in means, in variance or in both (dataset 8a, 8b and 8c), QBP performed equal compared to RF and XGB and was superior in relation to the other methods in terms of prediction. Thus, in the most realistic setting – where cases and controls do not just differ in mean – QBP truly competes with XGB and RF and does substantially better than more classical methods.

For the imbalanced simulation scenarios (dataset 6c, 7c and 8c), the relative performance between methods in terms area under the precision recall curve (AUCPR) is more or less the same compared to the relative performance in terms of AUC. Whereas the AUC has a fixed baseline performance of \( AUC = 0.5 \), the baseline of the imbalanced scenarios is depending on the percentage of cases – \( AUCPR = 0.2 \) in this case. When simulating a shift in mean on the log-normal scale (dataset 6c), QBP (\( AUCPR = 0.423 \)) shows a gap with traditional methods with Lasso (\( AUCPR = 0.580 \)) as the best in class. For shifts in
variances on the log-normal scale (dataset 7c), QBP and RF are superior ($AUCPR = 0.368$ and $AUCPR = 0.367$, respectively) compared to alternative methods. For combinations of shifts in means and variances on the log-normal scale (dataset 8c) QBP and RF are again superior ($AUCPR = 0.432$ and $AUCPR = 0.447$, respectively). For both dataset 7c and 8c, XGB shows a small gap with QBP and RF, but certainly outperforms traditional methods.

The simulation study also showed for all methods that an increase in sample size tends to increase the predictive performance and decrease the standard deviation. In particular, a balanced increase of the number of cases and controls appeared to be most effective. A primary cause of this increased performance is the fact that the standard error of the quantiles decreases when increasing the sample size. For QBP, this directly results into more precise estimates for the quantiles and estimates of the exceed ratios. As a consequence, the probability of falsely including biomarkers decreases. This sample size effect was mainly visible for QBP in the lower number of selected biomarkers and the increased specificity and accuracy of the biomarker selection for the balanced datasets with $n = 400$ compared to $n = 100$. For the PLR methods on the other hand, the specificity and accuracy decreased with increasing sample size, except for datasets 8a, 8b and 8c where the accuracy increased with increasing sample size. Whenever, a relative number of biomarkers is involved with different variances between cases and controls, QBP has a better sensitivity than traditional methods, although not always a better specificity when the number of cases and/or controls is low. This was observed in the balanced datasets with $n = 100$.

Apart from the simulation study, two case studies were analysed: a major depression disorder dataset and a trisomy dataset. Whereas the traditional methods barely detected any difference between cases and controls in the MDD dataset ($AUC \approx 0.5$), QBP reached an area under the curve of 0.680, which is more than 7.1% and 12.2% higher than the two successors RF and XGB, respectively. This superior performance can mainly be ascribed to the fact that most relevant biomarkers in this dataset show differences in distributional characteristics other than just differences in means between cases and controls. When considering the predictive performance in terms of $AUC$ of the methods on the trisomy dataset using all biomarkers, it can be concluded that QBP ($AUC = 0.908$) performs equally well as LR, PCLR, SVM.Linear and XGB, and significantly better than the other methods. A comparison of QBP and the FMF risk that is used by RIVM to predict trisomy was performed on a larger dataset with a lower number of biomarkers. It was shown that the classification performance of QBP in terms of the $AUC$ is slightly better than the FMF risk ($AUC = 0.9249$ and $AUC = 0.9151$ for QBP and FMF risk, respectively).

A sensitivity analysis was performed for the Trisomy dataset to assess the impact of the severe class imbalance on the performance to predict the minority class. Two strategies were considered: (1) changing the performance measure to the $AUCPR$ and (2) evaluation by means of AUC after performing downsampling on the majority class. The results of this sensitivity study are presented in Table 13. With respect to the former, we obtain the baseline performance in terms of $AUCPR$ of 0.014, equal to the percentage of cases (1.4%). Where QBP demonstrated to be competitive in terms of $AUC$, it surprisingly shows a gap in terms of $AUCPR$ compared to other methods. In contrast to RF and XGB ($AUCPR = 0.571$ and $AUCPR = 0.595$, respectively), QBP only reaches an $AUCPR$ of 0.416. When executing the downsampling strategy, it appears that all techniques are basically comparable in terms of $AUC$ and that QBP reaches an $AUC$ that is the same as for the unbalanced data.
In our simulation study, we only applied normal and log-normal distributions but did not use other statistical distributions. However, QBP can easily be translated to other continuous statistical distributions, most likely without losing its strength in detecting tail differences. Moreover, note that in the implementation of PCLR, the principal components are selected in the natural order given by their explained variances. Although an alternative method using a stepwise procedure of selecting principal components based on the conditional likelihood-ratio test is described to be superior (Aguilera et al. 2006), we do not expect the conclusions of this study to change in this case. We, however, used PLS-LDA as well, which creates a sparse representation of the data before applying LDA. Finally, although we currently did not include interactions or other higher-order terms, these could be easily constructed.

Additional research on the QBP should be conducted as the complete set of possible tunable parameters and corresponding settings have not been studied or explored in its full potential. This can be in terms of the number of percentiles and the corresponding proportions, where one could focus on its relation with the sample size. Note that the proportions should be selected with care, especially when dealing with small sample sizes, as this will result in less robust percentiles. Furthermore, it could be investigated whether the weights of biomarkers should be equal for all biomarkers or it should depend on a certain statistic. For example, biomarkers that vary in variation between cases and controls may receive larger weights that could be proportional to Levene’s test of homogeneity. When studying a wider range of tunable parameters we recommend to focus on both the balanced and unbalanced setting. Altogether it is not unlikely that the QBP can become even better in predicting cases and selecting relevant biomarkers.

Another point of attention is the topic of collinearity, since it could easily inflate the disease scores of QBP. A simple precaution could be to reduce the biomarker weights of biomarker scores in case of confounding; however, more sophisticated measures could be developed. At the moment, QBP is limited to binary outcomes and continuous biomarkers. If one wants to include binary covariates such as gender or use multiple outcome levels this is not straightforward. For binary covariates, we could for example apply location-scale transformations. Especially in datasets that are too small for separate QBP analyses this might be useful. For discrete covariates – which we treated as continuous covariates in the trisomy dataset – a more sophisticated rule based on proportions could be established to improve the performance of QBP. From a computational perspective, QBP algorithm is currently more computationally intensive than other classical statistical methods – especially in comparison to (P)LR or LDA. Relative to machine learning techniques, QBP seems to perform comparable or better. Note that the processing times are particularly high for the techniques that require CV to select the optimal set of tunable parameters. This CV was performed such that each method received exactly the same split of the training data, and with that ensuring a fair comparison by giving each method the same information to fit a model. Besides that the computational efficiency could still be improved, a mathematical or theoretical underpinning of QBP is needed to demonstrate its capability.

Summarizing, QBP outperforms the observed traditional methods in discriminating cases from controls if the predictor variables show differences in variances between cases and controls. In case only systematic shifts in the mean of normally or log-normally distributed predictor variables are present, QBP is inferior to the traditional methods. For situations with mixtures of shifts in means, variances or other distributional differences, as
expected in real life due to complex biological processes, QBP was superior to all methods in the MDD case study and was amongst the best-performing methods in the simulation study – together with RF and XGB. There are still numerous settings for which the performance of QBP should be assessed, but we demonstrated its potential on predicting diseases. Although QBP is currently applied on disease classification, it can be used in all fields involving binary classification with continuous covariates, such as economics, marketing, engineering and social sciences.

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No potential conflict of interest was reported by the author(s).

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