Cross-study validation for the assessment of prediction algorithms

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\textbf{ABSTRACT}

\textbf{Motivation:} Numerous competing algorithms for prediction in high-dimensional settings have been developed in the statistical and machine-learning literature. Learning algorithms and the prediction models they generate are typically evaluated on the basis of cross-validation error estimates in a few exemplary datasets. However, in most applications, the ultimate goal of prediction modeling is to provide accurate predictions for independent samples obtained in different settings. Cross-validation within exemplary datasets may not adequately reflect performance in the broader application context.

\textbf{Methods:} We develop and implement a systematic approach to ‘cross-study validation’, to replace or supplement conventional cross-validation when evaluating high-dimensional prediction models in independent datasets. We illustrate it via simulations and in a collection of eight estrogen-receptor positive breast cancer microarray gene-expression datasets, where the objective is predicting distant metastasis-free survival (DMFS). We computed the C-index for all pairwise combinations of training and validation datasets. We evaluate several alternatives for summarizing the pairwise validation statistics, and compare these to conventional cross-validation.

\textbf{Results:} Our data-driven simulations and our application to survival prediction with eight breast cancer microarray datasets, suggest that standard cross-validation produces inflated discrimination accuracy for all algorithms considered, when compared to cross-study validation. Furthermore, the ranking of learning algorithms differs, suggesting that algorithms performing best in cross-validation may be suboptimal when evaluated through independent validation.

\textbf{Availability:} The \texttt{survHD: Survival in High Dimensions} package (http://www.bitbucket.org/lewaldron/survhd) will be made available through Bioconductor.

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\textbf{Supplementary information:} Supplementary data are available at Bioinformatics online.

1 \textbf{INTRODUCTION}

Cross-validation and related resampling methods are de facto standard for ranking supervised learning algorithms. They allow estimation of prediction accuracy using subsets of data that have not been used to train the algorithms. This avoids over-optimistic accuracy estimates caused by ‘re-substitution’. This characteristic has been carefully discussed in Molinaro et al. (2005), Baek et al. (2009) and Simon et al. (2011). It is common to evaluate algorithms by estimating prediction accuracy via cross-validation for several datasets, with results summarized across datasets to rank algorithms (Boulesteix, 2013; Demšar, 2006). This approach recognizes possible variations in the relative performances of learning algorithms across studies or fields of application. However, it is not fully consistent with the ultimate goal, in the development of models with biomedical applications, of providing accurate predictions for fully independent samples, originating from institutions and processed by laboratories that did not generate the training datasets.

It has been observed that accuracy estimates of genomic prediction models based on independent validation data are often substantially inferior to cross-validation estimates (Castaldi et al., 2011). In some cases this has been attributed to incorrect application of cross-validation; however even strictly performed cross-validation may not avoid over-optimism resulting from potentially unknown sources of heterogeneity across datasets. These include differences in design, acquisition and ascertainment strategies (Simon et al., 2009), hidden biases, technologies used for measurements, and populations studied. In addition, many genomics studies are affected by experimental batch effects (Baggerly et al., 2008; Leek et al., 2010). Quantifying these heterogeneities and describing their impact on the performance of prediction algorithms is critical in the practical implementation of personalized medicine procedures that use genomic information.

There are potentially conflicting, but valid, perspectives on what constitutes a good learning algorithm. The first perspective is that a good learning algorithm should perform well when trained and applied to a single population and experimental setting, but it is not expected to perform well when the resulting model is applied to different populations and settings. We call such an algorithm specialist, in the sense that it can adapt and specialize to the population at hand. This is the mainstream perspective for assessing prediction algorithms and is consistent with validation procedures performed within studies (Baek et al., 2009; Molinaro et al., 2005; Simon et al., 2011). However, we argue that it does not reflect the reality that ‘samples of convenience’ and uncontrolled specimen collection are the norm in genomic biomarker studies (Simon et al., 2009).

We promote another perspective: a good learning algorithm should be generalist, in the sense that it yields models that may be suboptimal for the training population, or not fully representative of the dataset at hand, but that perform reasonably well...
across different populations and laboratories employing comparable but not identical methods. Generalist algorithms may be preferable in important settings, for instance when a researcher develops a model using samples from a highly controlled environment, but hopes the model to be applicable to other hospitals, labs, or more heterogeneous populations.

In this article we systematically use independent validations for the comparison of learning algorithms, in the context of microarray data for disease-free survival of estrogen receptor-positive breast cancer patients. Although concern has been often expressed about the lack of independent validation of genomic prediction models (Michael et al., 2012; Subramanian and Simon, 2010), independent validation has not been systematically adopted in the comparison of learning algorithms. This deficiency cannot be addressed for prediction contexts where related, independent datasets are unavailable. For many cancer types, however, several micro-array studies have been performed to develop prognostic models. These datasets pave the way for a systematic approach based on independent validations. For instance, a recent meta-analysis of prognostic models for late-stage ovarian cancer provides a comparison of publicly available microarray datasets (Waldron et al., 2014). Furthermore, Riester et al. (2014) showed that combining training datasets can increase the accuracy of late-stage ovarian cancer risk models. Thus situations exist in genomic data analysis where comparable, independent datasets are available, and these present an opportunity to use independent validation as an explicit basis for assessing learning algorithms.

We propose what we term ‘leave-one-dataset-in’ cross-study validation (CSV) to formalize the use of independent validations in the evaluation of learning algorithms. Through data-driven simulations, and an example involving eight publicly available estrogen receptor-positive breast cancer microarray datasets, we assess established survival prediction algorithms using our ‘leave-one-dataset-in’ scheme and compare it to conventional cross-validation.

2 METHODS
2.1 Notation and setting
> We consider multiple datasets \( i = 1, \ldots, I \) with sample sizes \( N_1, \ldots, N_I \). Each observation \( s \) appears only in one dataset \( i \), i.e. datasets do not overlap, and the corresponding record includes a primary outcome \( Y \) and a vector of predictor variables \( X_i \); throughout this article \( X_i \) will be gene-expression measurements. Our goal is to compare the performance of different learning algorithms \( k = 1, \ldots, K \) that generates prediction models for the primary outcome using the vector of predictors. Throughout this article, the primary outcome is a possibly censored survival time.

We are interested in evaluating and ranking competing prediction methods \( k = 1, \ldots, K \). Since the ranking may depend on the application, the first step is to define the prediction task of interest. We focus on the prediction of metastasis-free survival time in breast cancer patients based on high-throughput gene-expression measurements. Our approach and the concept of CSV, however, can be applied to different types of response variables and any other prediction task.

2.2 Algorithms
We assess six learning algorithms \( (k = 1, \ldots, 6) \) appropriate for high-dimensional continuous predictors and possibly censored time-to-event outcomes: Lasso and Ridge regression (Goeman, 2010), CoxBoost (Binder and Schumacher, 2008), SuperPC (Blair and Tibshirani, 2004), Unicox (Tibshirani, 2009) and Phsminus (Zhao et al., 2013). All parameters were tuned either by default methods included in their implementation (Ridge and Lasso regression: R-package glmnet) or by testing a range of parameters in internal cross-validation. Our focus is not to provide a comprehensive array of algorithms, but simply to use a few popular, representative algorithms to investigate CSV.

2.3 CSV matrices
We refer in this article to \( m \)-fold cross-validation and related resampling methods collectively as cross-validation (CV). Our ranking procedure for learning algorithms is based on a square matrix \( Z^k \) of scores \((k = 1, \ldots, K)\). The \((i, j)\) element in the matrix measures how well the model produced by algorithm \( k \) trained on dataset \( i \) performs when validated on dataset \( j \). Since we consider \( K \) methods we end up with \( K \) method-specific square matrices \( Z^1, \ldots, Z^K \). We set the diagonal entries of the matrices equal to performance estimates obtained with 4-fold CV in each dataset. We will call \( Z^k \) the CSV matrix.

Possible definitions for the \( Z^k_{ij} \) scores include the concordance index in survival analysis (Harrell et al., 1996), the area under the operating characteristic curve in binary classification problems, or the mean squared error between predicted and observed values in regression problems. We use survival models and focus on a concordance index, the C-index, which is a correlation measure (Gini and Heller, 2005) between survival times and the risk scores, such as linear combinations of the predictors, provided by a prediction model. The heatmap in Figure 1A displays the CSV matrix of C-statistics obtained through validation of eight models trained on the studies in Table 1 with Ridge regression.

2.4 Summarization of a CSV matrix
In order to rank learning algorithms \( k = 1, \ldots, K \), we summarize each matrix \( Z^k \) by a single score. We consider following two candidate approaches.

(1) The Simple Average of all non-diagonal elements of the \( Z^k \) matrix:

\[
\text{CSV} = \frac{\sum_{i \neq j} Z^k_{ij}}{(I-1)(I-2)}
\]

(2) The Median or more generally a quantile of the non-diagonal entries of \( Z^k \). Quantiles offer robustness to outlier values, and the possibility to reduce the influence of those studies that are consistently associated with poor validation scores, both when used for training and validation, and independently of the learning algorithm.

2.5 True global ranking
Throughout our analyses the score \( Z^k_{ij} \) is a random variable. First, studies \( i \) and \( j \) can be seen as randomly drawn from a population of studies. Second, observations within each study can be considered as randomly drawn from the unknown and possibly different distributions \( F_i \) and \( F_j \) underlying studies \( i \) and \( j \). With this view of \( Z^k_{ij} \) as random variable, we consider the theoretical counterparts of the empirical aggregating scores (simple average and quantiles) described in Section 2.4 to summarize \( Z^k \). The theoretical counterparts are the expected value or quantiles of each \( Z^k_{ij} \) score, \( i \neq j \), obtained by integrating the two levels of randomness that we described. The true global ranking of the learning algorithms \( k = 1, \ldots, K \) is then defined by these expected values (or quantiles), one for each algorithm. We will call the ranking global because it depends on the super-population (Hartley and Siekken, 1975) and not which populations were sampled by the available datasets.
The true global ranking can be considered as the estimation target of evaluation procedures such as CV or CSV. In Section 2.7 we present the design of a data-driven simulation study in which we can compute the true ranking through Monte Carlo integration. This allows us to evaluate and compare the ability of CV and CSV to recover the true global ranking.

2.6 Datasets

We used a compendium of breast cancer microarray studies curated for the meta-analysis of Haibe-Kains et al. (2012) and available as supplement to their article. We selected all eight datasets (Table 1) for which distant metastasis-free survival (DMFS), the most commonly available time to event endpoint, as well as Estrogen Receptor (ER) status, were available. These studies were generated with Affymetrix HGU GeneChips HG-U133A, HG-U133B and HG-U133PLUS2. We considered exclusively ER-positive tumors. Of these datasets, only one originated from a population-based cohort (Schmidt et al., 2008). Four studies considered only patients who did not receive hormone therapy or adjuvant chemotherapy. Only four provided date ranges of patient recruitment (Chin et al., 2006; Desmedt et al., 2007; Foekens et al., 2006; Schmidt et al., 2008). Table 1 points also to important differences in survival (for instance 3Q survival) that are not easily explicable based on known characteristics of these studies. This variability in design strategies, reporting, as well as outcomes, highlights the prevalence of ‘samples of convenience’ in biomarker studies discussed by Simon et al. (2009). Samples from dataset ST1 duplicated in dataset VDX were removed. Expression of each gene was summarized using the probeset with

Table 1. Breast cancer microarray datasets curated by Haibe-Kains et al. (2012)

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Adjuvant therapy</th>
<th>Number of patients</th>
<th>Number of ER+</th>
<th>3Q survival [mo.]</th>
<th>Median follow-up [mo.]</th>
<th>Original identifiers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAL</td>
<td>Chemo, hormonal</td>
<td>118</td>
<td>75</td>
<td>42</td>
<td>82</td>
<td>CAL</td>
<td>Chin et al. (2006)</td>
</tr>
<tr>
<td>2</td>
<td>MNZ</td>
<td>none</td>
<td>200</td>
<td>162</td>
<td>120</td>
<td>94</td>
<td>MAINZ</td>
<td>Schmidt et al. (2008)</td>
</tr>
<tr>
<td>3</td>
<td>MSK</td>
<td>combination</td>
<td>99</td>
<td>57</td>
<td>76</td>
<td>82</td>
<td>MSK</td>
<td>Minn et al. (2005)</td>
</tr>
<tr>
<td>4</td>
<td>ST1</td>
<td>hormonal</td>
<td>517</td>
<td>507</td>
<td>114</td>
<td>106</td>
<td>MDA5, TAM, VDX3</td>
<td>Foekens et al. (2006)</td>
</tr>
<tr>
<td>5</td>
<td>ST2</td>
<td>hormonal</td>
<td>517</td>
<td>325</td>
<td>126</td>
<td>121</td>
<td>EXPO, TAM</td>
<td>Symmans et al. (2010)</td>
</tr>
<tr>
<td>6</td>
<td>TRB</td>
<td>none</td>
<td>198</td>
<td>134</td>
<td>143</td>
<td>171</td>
<td>TRANSBIG</td>
<td>Desmedt et al. (2007)</td>
</tr>
<tr>
<td>7</td>
<td>UNT</td>
<td>none</td>
<td>133</td>
<td>86</td>
<td>151</td>
<td>105</td>
<td>UNT</td>
<td>Sotiriou et al. (2006)</td>
</tr>
<tr>
<td>8</td>
<td>VDX</td>
<td>none</td>
<td>344</td>
<td>209</td>
<td>44</td>
<td>107</td>
<td>VDX</td>
<td>Minn et al. (2007)</td>
</tr>
</tbody>
</table>

Datasets acronyms: CAL, University of California, San Francisco and the California Pacific Medical Center (USA); MNZ, Mainz hospital (Germany); MSK, Memorial Sloan-Kettering (United States). ST1 and ST2 are meta-datasets provided by Haibe-Kains et al. (2012), TRB denotes the TransBIG consortium dataset (Europe), UNT denotes the cohort of untreated patients from the Oxford Radcliffe Hospital (UK), VDX = Veridex (the Netherlands). Number of ER+ is the number of patients classified as Estrogen Receptor positive. 3Q survival indicates the empirical estimate of the 75-th percentile of the distribution of the survival times (in months). Median follow-up (in months) is computed using the reverse Kaplan-Meier estimate to avoid under-estimation due to early deaths (Schemper and Smith, 1996). *Numbers shown are after removal of samples duplicated in the dataset VDX. †Dataset identifiers specified in Haibe-Kains et al. (2012).
maximum mean (Miller et al., 2011). The 50% of genes with lowest variance were removed. Subsequently, gene-expression values were scaled by linear scaling of the 2.5 and 97.5% quantiles as described by Haibe-Kains et al. (2012).

2.7 Simulation design
We simulate heterogeneous datasets with gene-expression profiles and time to event outcomes from a joint probability model. We define the model through a resampling procedure that we apply to the eight breast cancer datasets in Table 1. The resampling scheme is a combination of parametric and nonparametric bootstrap (Efron and Tibshirani, 1993).

The goal of our simulation study is to compare CV and CSV when used for ranking and evaluation of competing learning algorithms. Here we use resampling methods to iteratively simulate realistic ensembles of breast cancer datasets from a hierarchical probability model that we define using the actual datasets in Table 1. CV and CSV are then assessed with respect to their ability to recover the true global ranking, which we compute through Monte-Carlo integration.

We will quantify the ability to recover the ranking by using the Kendall correlation between the true global ranking and the estimates obtained with CV or CSV. For $b = 1, \ldots, B = 1000$ iterations, we generate a collection of $I = 8$ datasets as follows. First, we sample eight study labels with replacement from the list of breast cancer studies in Table 1. This step only involves simulations from a multinomial Mult$(8, [1/8, \ldots, 1/8])$ distribution. We resample the collection of study labels to capture variability in study availability, and heterogeneity of study characteristics. Second, for each of the eight randomly drawn labels, we sample $N = 150$ patients from the corresponding original dataset, with replacement. If a study is randomly assigned to the $j$-th label, then each of the $N = 150$ vectors of predictive variables is directly sampled from the empirical distribution of the $j$-th study in Table 1. Finally, we simulate the corresponding times to event using a proportional hazards model (parametric bootstrap) fitted to the $j$-th dataset:

$$M_{true}(t) = \lambda(t) = \lambda^0(t) \times \exp \left( \sum_{j=1}^{p} \beta_j x_j \right),$$  

where $\lambda(t)$ is the individual hazard function when the vector of predictors is equal to $x$ and $\beta_j$ denotes a vector of regression coefficients. We combine the truncated inversion method in Bender et al. (2005) and the Nelson–Aalen estimator for cumulative hazard functions to simulate survival times that reflect survival distributions and follow-up of the real studies. We set the vector $\beta_j$ to be the coefficients fitted in study $j = 1, \ldots, I$ using the CoxBoost method (Binder and Schumacher, 2008). A different regression method could have been used at this stage. The collections of simulated datasets are then used both (i) to compute by Monte-Carlo integration the vector of regression coefficients $\beta_j$ (i.e., $\beta_j$ obtained with CV or CSV).

Subsequently, gene-expression values were removed. We set the vector $\mathbf{v}_j$ of survival times that reflect survival distributions and follow-up of the real studies. We will quantify the ability to recover the ranking by using the Kendall correlation between the true global ranking and the estimates obtained with CV or CSV.

3.1 Simulated data
Our focus in the simulation study is on differences between the rankings and performance estimates obtained by using CV and CSV. We will use CV and CSV to denote the means of the diagonal and non-diagonal elements of a CSV matrix, respectively. Recall that we compute the diagonal elements through CV.

Figure 2A shows, for $K = 6$ algorithms, the distributions of CSV and CV, and Figure 2B shows the distribution of the rankings estimates, across 1000 simulated collections of eight datasets. Table 2 compares the medians of the distributions in Figure 2B with the true global rankings that we obtained using the criteria in Section 2.4. The rank of method $k$ is 1 if it from real data. This scatterplot shows a clear two-cluster structure: the yellow dots display the 30 training and validation combinations within studies MNZ, ST1, ST2, TRP, UNT and VDX. We will return to this cluster structure in the discussion.

2.8 Evaluation criteria for simulations
In simulation studies we can assess and rank algorithms based on their ability to recover the true underlying models $M_{true}$, $i = 1, \ldots, I$. In this subsection, we introduce a criterion that reflects the degree of similarity between the true regression coefficients $\beta_j$ that were used to simulate the $j$-th in silico dataset and the coefficients $\hat{\beta}_{ij}^{(k)}$ fitted through algorithm $k$ on the $j$-th simulated dataset. We consider the $i = j$ and $i \neq j$ cases separately. Similarity between vectors is usually quantified by computing the Euclidean distance between them. However, since our focus is on prediction, we use the correlation $\text{cor}(\mathbf{X}_i, \mathbf{X}_j^{(k)})$, the correlation between true and estimated patient-specific prognostic scores, to measure the similarity between the true $\beta_j$ and estimated regression coefficients $\hat{\beta}_{ij}^{(k)}$. Here $\mathbf{X}_i$ is the matrix of predictors of dataset $i$ and $\text{cor}$ denotes Pearson's correlation. The average $S_{self}^{(k)} = (1/I) \cdot \frac{1}{I} \sum_{i} \text{cor}(\mathbf{X}_i, \mathbf{X}_j^{(k)})$ over the $I$ studies, provides a measure of the ability of learning algorithm $k$ to recover the model that has generated the training dataset, hence the index self.

Another criterion of interest is the ability of a learning algorithm $k$ to recover the vector of regression coefficients $\beta_j$ when it is trained on a separate dataset $j \neq i$ and the unknown models underlying datasets $i$ and $j$ might differ from each other.

This can be quantified with $S_{across}^{(k)} = (1/(I(I-1))) \cdot \frac{1}{I(I-1)} \sum_{i} \sum_{j \neq i} \text{cor}(\mathbf{X}_i, \mathbf{X}_j^{(k)})$, where the index across emphasizes the focus on cross-study similarity, i.e. on the ability of algorithm $k$ to recover the coefficients $\beta_j$ when fitted on dataset $j$, with $j \neq i$.

In alternative to averaging across studies, or pairs of datasets, as in Equations (2–3) one can also use different summaries, e.g. quantiles, as we do in Section 2.4. Both $S_{self}^{(k)}$ and $S_{across}^{(k)}$ are summary statistics to assess and compare learning algorithms. We denote the ranking obtained by ordering the algorithms according to $S_{self}(S_{across})$ by $R_{self}(R_{across})$. Both $S_{self}^{(k)}$ and $S_{across}^{(k)}$ vary across simulations of the datasets ensembles, although the hierarchical simulation model remains fixed and their computations involve the vectors $\beta_j$, $i = 1, \ldots, I$. We will therefore call the rankings $R_{self}$ and $R_{across}$ local because they are specific to the collection of datasets at hand.
outperforms the remaining $K-1$ training algorithms. We observe large differences in the distributions of CSV and CV across simulations (Fig. 1A): the average of the CSV scores, under all the algorithms we considered, is close to 0.65, while the CSV scores are centered at ~0.55. The variability of CV and CSV across simulations, however, is comparable.

Performance differences across algorithms, whether estimated by CV or CSV, are relatively small compared to the overall difference between CV and CSV performance estimates. We also observe differences between the rank distributions produced by CV and CSV. Accordingly, to both CV and CSV, in most of the simulations, *Lasso regression* is ranked as one of the worst performing algorithms, while *Ridge regression* and *Plusminus* are ranked first or second. However, the CSV summaries suggest an advantage of *Ridge regression over Plusminus* across most of the simulations while CSV rank *Plusminus* as the best performing algorithm in ~50% of the simulations. The median rank of *CoxBoost* across simulations has an improvement of two positions when it is estimated through CV and compared to the CSV summaries; in this case CSV results are more consistent with the true global rankings (Table 2). When we consider the criteria described in Section 2.4, *Ridge regression* and *Plusminus* exchange the top-two positions of the true global rankings (see Table 2), although for these two algorithms the *Z*_ij distributions under our simulation scenario are nearly identical.

The local rankings $R_{\text{across}}$ and $R_{\text{self}}$ of the $K=6$ algorithms defined by $S_{\text{across}}^k$ and $S_{\text{self}}^k$ in Section 2.8 vary across the 1000 simulated collections of studies. The median Kendall’s correlation between $R_{\text{across}}$ and $R_{\text{self}}$ across simulations is ~0.5, i.e. the performance measures $S_{\text{across}}^k$ and $S_{\text{self}}^k$ tend to define distinct rankings of the competing algorithms, see also the Supplementary Figure S1. We illustrate the extent to which CV and CSV recover the unknown rankings $R_{\text{across}}$ and $R_{\text{self}}$.

### Table 2. True global rankings and estimates with CV and CSV on simulated data

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>True global ranking</th>
<th>CSV (median ranks)</th>
<th>CV (median ranks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Medium</td>
<td>Average</td>
</tr>
<tr>
<td>Ridge</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Plusminus</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Superpc</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Unicox</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Lasso</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CoxBoost</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Median estimates across 1000 simulations are displayed for CV and CSV; individual columns refer to summarization of the *Z*_ij statistics by using the mean or the median as discussed in Section 2.4. We also computed the true global ranking as well as CV and CSV estimates by using the third quartile of the *Z*_ij summaries, and obtained results identical to those displayed for the rankings obtained by summarizing validation results through their median. Both CV and CSV tend to rank *Ridge regression* and *Plusminus* as best performing algorithms. Variability of CV and CSV rank estimates across simulations is shown in Figure 2B.

Finally, CSV tends to be more correlated with the true global ranking than CV. This suggests that CSV is more suitable for recovering the true global ranking. When we removed the two outlier studies (CAL and MSK) and repeated the simulation study, the advantage of CV over CSV in recovering the true global ranking was confirmed (median Kendall’s correlation 0.8 versus 0.6, see also Supplementary Figs S2–S4), moreover after their removal Kendall’s correlations between $R_{\text{self}}$ and the CSV estimates tend to be larger than those between $R_{\text{self}}$ and the CV estimates. Overall, as displayed by the Supplementary Figure S3, it appears that, after outlier studies are removed, CSV outperforms substantially CV when used for ranking algorithms.

### 3.2 Application to breast cancer prognostic modeling

We apply CV and CSV to the $I=8$ breast cancer studies described in Section 2. Generally, the results resemble those obtained on simulated data. The top panel in Figure 4 illustrates the distributions of the diagonal and off-diagonal validation statistics in *Z*_ij for each of the $K=6$ algorithms. Except for the distinctly larger interquartile ranges of the box-plots we observe several similarities with Figure 2. Note that each box-plot
CV estimates are ~0.06 higher than CSV estimates on the C-index scale. To interpret the magnitude of this shift on the C-index scale consider a population with two groups of patients, high and low risk patients, covering identical proportions 0.5 of the population. A perfect discrimination model that correctly recognizes the subpopulation of each individual, when the hazard ratio between high versus low risk patients is 2.7, achieves on average a C-index of 0.62. It is necessary to double the hazard ratio to 5.4 to increase the average C-index of the perfect discrimination model to 0.68. Thus, it is fair to say that the CV results are considerably more optimistic than the CSV estimates.

- The ranking defined by CSV, using median summaries of the $Z^k_{ij}$ scores, is nearly identical to the global ranking in our simulation study (see Supplementary Table ST1 and Table 2). With both, median and third quartile aggregation of the $Z^k_{ij}$ statistics, the rankings defined by CV and CSV differ substantially (Kendall’s correlations 0.6 and 0.07). This is consistent with the results of the simulation study, where median correlation of the rankings estimated through CSV and CV was ~0.4 (see Supplementary Fig. S1).

- The presence of outlier studies (CAL and MSK) has a strong effect on the ranking estimates when we use the mean to summarize the $Z^k$ matrices. After aggregating the validation statistics by averaging, both CSV and CV rank Superpc first. This result might be due to the high variability, ~0.5, of the $Z^k_{ij}$ validation scores corresponding to models trained by outlier studies. In particular, Superpc and Unicox are the only algorithms that produce models with substantial prediction performances when trained on the MSK study. With median summarization the ranking estimates are less influenced by the presence or absence of outlier studies. We therefore recommend the use of the median to summarize $Z^k$ matrices.

- Figure 4B illustrates lack of agreement between CSV and CV performance estimates. The black digits contrast, for each dataset $i$, the CSV summary $\sum_{j=1}^{K} (1 - I) Z^k_{ij}$ with the CV summary $Z^k_{ij}$. Similarly, with gray digits it contrasts the CSV summary $\sum_{j=1}^{K} (1 - I) Z^k_{ij}$ with the CV summary $Z^k_{ij}$. This panel shows results for the learning algorithm Ridge regression and the displayed numbers refer to Table 1 (outliers CAL and MSK were removed). Cross-validation statistics on the y-axis are moderately correlated to the CSV summaries on the x-axis; identical considerations hold for all $K = 6$ algorithms that we used Superpc first. This result might be due to the high variability, ~0.5, of the $Z^k_{ij}$ validation scores corresponding to models trained by outlier studies. In particular, Superpc and Unicox are the only algorithms that produce models with substantial prediction performances when trained on the MSK study. With median summarization the ranking estimates are less influenced by the presence or absence of outlier studies. We therefore recommend the use of the median to summarize $Z^k$ matrices.

- Figure 4B illustrates lack of agreement between CSV and CV performance estimates. The black digits contrast, for each dataset $i$, the CSV summary $\sum_{j=1}^{K} (1 - I) Z^k_{ij}$ with the CV summary $Z^k_{ij}$. Similarly, with gray digits it contrasts the CSV summary $\sum_{j=1}^{K} (1 - I) Z^k_{ij}$ with the CV summary $Z^k_{ij}$. This panel shows results for the learning algorithm Ridge regression and the displayed numbers refer to Table 1 (outliers CAL and MSK were removed). Cross-validation statistics on the y-axis are moderately correlated to the CSV summaries on the x-axis; identical considerations hold for all $K = 6$ algorithms that we used

### 3.3 CV and CSV summaries

Correlation between CSV and CV summary statistics, as displayed in Figure 4B, suggests that cross- and within-study performances are less redundant than one might expect. In Figure 4B study specific CSV summaries are plotted against CV for Ridge regression. For each study we have a single CV statistic and two CSV statistics obtained by averaging the
In this article we provide a conceptual framework, statistical approaches and software tools for this quantification. The conceptual framework is based on the long-standing idea that finite populations of interest can be viewed as samples from an infinite ‘super-population’ (Hartley and Siekken, 1975). This concept is especially relevant for heterogeneous clinical studies originating from hospitals that sample local populations, but where researchers hope to make generalizations to other populations.

As an illustrating example, we demonstrate CSV on eight independent microarray studies of ER-positive breast cancer, with metastasis-free survival as the endpoint of interest. We also develop a simulation procedure involving two levels of non-parametric bootstrap (sampling of studies and sampling of observations within studies) in combination with parametric bootstrap, to simulate a compendium of independent datasets with characteristics of predictor variables, censoring, baseline hazards, prediction accuracy and between-dataset heterogeneity realistically based on available experimental datasets.

Cross-validation is the dominant paradigm for assessment of prediction performance and comparison of prediction algorithms. The perils of inflated prediction-accuracy estimations by incorrectly or incompletely performed cross-validation are well known (Molinaro et al., 2005; Subramanian and Simon, 2010; Simon et al., 2011; Varma and Simon, 2006). However, we show that even strictly performed cross-validation can provide optimistic estimates relative to CSV performance. All algorithms, in simulation and example, showed distinctly decreased performance in CSV compared to cross-validation. Although it would be possible to further reduce between-study heterogeneity, for example by stricter filtering on clinical prognostic factors, we believe this degree of heterogeneity reflects the reality of clinical genomic studies and likely other applications. Some sources of biological heterogeneity are unknown, and it is impossible to ensure consistent application of new technologies in laboratory settings. Prediction models are used in presence of unknown sources of variation. Formal CSV provides a means to assess the impact of unknown or unobserved confounders that vary across studies.

In simulations, the ranking of algorithms by CSV was closer to the true rankings defined by cross-study prediction, both when we considered $R_{\text{across}}$ and the global true ranking. Surprisingly, CSV was also competitive with CV for recovering true rankings based on within-study prediction, such as $R_{\text{eff}}$. Although the performance differences we observed between algorithms were smaller than the difference between CV and CSV, Lasso consistently compared poorly with most of the competing algorithms, both under CV and CSV evaluations. Lasso, and other algorithms that ensure sparsity have been shown to guarantee poor prediction performances in previous comparative studies (Bøvelstad et al., 2007; Waldron et al., 2011).

Systematic CSV provides a means to identify relevant sources of heterogeneity within the context of the prediction problem of interest. By simple inspection of the CSV matrix we identified two outlier studies that yielded prediction models no better than random guessing in new studies. This may be related to known differences in these studies: smaller numbers of observations, higher proportions of node positive patients, different treatments and larger tumors (Supplementary Figs S6–S9). Conversely, other known between-study differences do not seem to have created outlier studies or clusters of studies as seen in the $Z$-matrix column- and row-wise. In the column-wise case correlations, between CSV and CV summaries, vary across algorithms ~0.5, while in the row-wise case all the correlations are negative. Overall, we can consider cross- and within-study prediction as two related but distinct problems.

We also noted that CV is less suitable for detection of outlier studies than CSV, in particular CV can estimate encouraging prediction performances even on studies associated, under each training algorithm, with poor CSV summaries $P_{i,k}$. For instance, with the SuperPC algorithm all but one C-index estimates obtained with CV are above 0.6.

### 3.4 Specialist and generalist algorithms

Our analyses lead to the question of whether some algorithms can be considered as generalist or specialist procedures according to our definitions. Our examples are not exhaustive and additional comparisons, within the development of new prognostic models, are necessary in order to determine ‘specialist’ or ‘generalist’ tendencies of these algorithms. However, the fact that Ridge regression, Lasso regression and CoxBoost are ranked distinctly better accordingly to CV than CSV, in most iterations of our simulation study, suggests that these algorithms might be specialist procedures and adapt to the specific properties of the individual dataset. The status of generalist versus specialist, for each algorithm, can be discussed using the local performance criteria $S_{\text{eff}}$ and $S_{\text{across}}$ which are conceived to measure within-single-studies and generalizable prediction performances. We note that CoxBoost and Ridge regression tend to achieve better ranks in $R_{\text{eff}}$ than in $R_{\text{across}}$. In particular CoxBoost improves its position by 1 or 2 ranks in most simulations, which is similar to what we observed comparing CoxBoost’s CSV and CV rankings. In summary, in our study, these two algorithms seem to have—accordingly to all the criteria that we considered—a tendency to specialize to the dataset at hand. We mention that, as one can expect, for all the algorithms $S_{\text{eff}}$ is consistently higher than $S_{\text{across}}$. We also compared CV to independent within-study validation using our simulation model. For the independent within-study validation, we iteratively pair two datasets generated using identical regression coefficients and gene expression distributions. Subsequently, we train a model on the first dataset and evaluate it on the second one. As can be seen in Supplementary Figure S5, CV values, as expected, are slightly smaller than for the independent within-study validations.

### 4 DISCUSSION AND CONCLUSION

In applying genomics to clinical problems, it is rarely safe to assume that the studies in a research environment faithfully represent what will be encountered in clinical application, across a variety of populations and medical environments. From this standpoint, study heterogeneity can be a strength, as it allows to quantify the degree of generalizability of results, and to investigate the sources of the heterogeneity. This aspect has long been recognized in meta-analysis of clinical trials (Moher and Olkin, 1995). Therefore, we expect that an increased focus on quantifying cross-study performance of prediction algorithms will contribute to the successful implementation of the personalized medicine paradigm.
matrix, such as between studies where all or no patients received hormonal treatment. We note that incorporation of clinical prognostic factors into genomic prognostic models could likely produce gains in CSV accuracy, and that such multi-factor prognostic models could also be assessed by the proposed matrix of CSV statistics.

In practice it is neither possible nor desirable to eliminate all sources of heterogeneity between studies and between patient populations. The adoption of ‘leave-one-in’ CSV, in settings where at least two comparable independent datasets are available, can provide more realistic expectations of future prediction model performance, identify outlying studies or clusters of studies, and help to develop ‘generalist’ prediction algorithms which will hopefully be less prone to fit to dataset-specific characteristics. Further work is needed to formalize the identification of clusters of comparable studies, to develop databases for large-scale cross-study assessment of prediction algorithms, and to develop better ‘generalist’ prediction algorithms. Appropriate curated genomic data resources are available in Bioconductor (Gentleman et al., 2004) through the curatedCRCData, curatedBladderData and curatedOvarianData (Ganzfried et al., 2013) packages, and in other common cancer types through InSilicoDB (Taminau et al., 2011). In realms where such curated resources are available, CSV is in practice no more difficult or CPU-consuming than cross-validation, and should become an equally standard tool for assessment of prediction models and algorithms.

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