Evaluate Cutpoints: Adaptable continuous data distribution system for determining survival in Kaplan-Meier estimator

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Background and Objective: Growing evidence of transcriptional and metabolomic differentiation induced many studies which analyze such differentiation in context of outcome of disease progression, treatment or influence of many different factors affecting cellular and tissue metabolism. Particularly, cancer researchers are looking for new biomarkers that can serve as a diagnostic/prognostic factor and its further corresponding relationship regarding clinical effects. As a result of the increasing interest in use of dichotomization of continuous variables involving clinical or epidemiological data (gene expression, biomarkers, biochemical parameters, etc.) there is a large demand for cutoff point determination tools with simultaneous lack of software offering stratification of patients based on continuous and binary variables. Therefore, we developed “Evaluate Cutpoints” application offering wide set of statistical and graphical methods for cutoff point optimization enabling stratification of population into two or three groups.

Methods: Application is based on R language including algorithms of packages such as survival, survMisc, OptimalCutpoints, maxstat, ROCR, ggplot2, GGally and plotly offering Kaplan-Meier plots and ROC curves with cutoff point determination.

Results: All capabilities of Evaluate Cutpoints were illustrated with example analysis of estrogen, progesterone and human epidermal growth factor 2 receptors in breast cancer cohort. Through ROC curve the cutoff points were established for expression of ESR1, PGR and ERBB2 in correlation with their immunohistochemical status (cutoff: 1301.253, 243.35, 11,434.438, respectively; sensitivity: 94%, 85%, 64%, respectively; specificity: 93%, 86%, 91%, respectively). Through disease-free survival analysis we divided patients into two and three groups regarding expression of ESR1, PGR and ERBB2. Example algorithm cutp showed that lower expression of ESR1 and ERBB2 was more favorable (HR = 2.07, p = 0.0412; HR = 2.79, p = 0.0777, respectively), whereas heightened PGR expression was correlated with better prognosis (HR = 0.192, p = 0.0115).

Conclusions: This work presents application Evaluate Cutpoints that is freely available to download at http://wbnipk.umed.lodz.pl/Evaluate-Cutpoints/. Currently, many softwares are used to split continuous variables such as Cutoff Finder and X-Tile, which offer distinct algorithms. Unlike them, Evaluate Cutpoints allows not only dichotomization of populations into groups according to continuous variables and binary variables, but also stratification into three groups as well as manual selection of cutoff point thus preventing potential loss of information.

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1. Introduction

In the advent of high-throughput technologies such as next generation sequencing, mass spectrometry and metabolomics along with quantitative analysis of data, more and more biomedicai research is focused on identification of associations of potential biomarkers with outcome that may serve as prognostic factor; furthermore assessing its relationship with health effect. As a result of increasing interest in the use of dichotomization of continuous variables involving clinical or epidemiological data (gene expressions, biomarkers, biochemical parameters, etc.) there is a large demand for cutoff point determination tools. The “optimal” cutoff point being defined as that threshold value of the continuous covariate
distribution, which best separates low and high risk patients with respect to some outcome [1,2]. In general, there are several simple approaches that can possibly determine the cutoff point (i.e. using the mean, median or quantile distribution). However, due to the fact that many factors can affect the pathogenic process of cancer and the effect of treatment cannot be unequivocally confirmed, due to the risk of recurrence, it is necessary to use the appropriate statistical methods. One of the best known methods accounting for the uncertainties associated with clinically relevant endpoints is survival analysis. Survival analysis is a set of statistical methods used to determine the time elapsed for any event to occur; in biomedical research for instance it can be death or relapse. At the other hand choosing the best cutoff point is often a compromise between the highest sensitivity (the smallest percentage of false negative results) and the highest specificity (the smallest percentage of false positive results) which for measurable data is graphically represented in the form of ROC curve [3,4]. Often the categorization of only two groups results in loss of information, therefore in some cases it is necessary to distinguish the third, middle group. There are several possibilities for use continuous variable with survival analysis and Kaplan-Meier plot. The simplest approach is to divide continuous predictor into groups based on quantile groups an example is PROGgene web application [5]. Unfortunately, such non-systematic determination of cut-off point lacks reproducibility and different studies are difficult to compare. The statistical-mathematical methods of a cut-off point determination for continuous predictor are implemented in several algorithms like OptimalCutpoints [6], maxstat [7] but those programs are not user friendly and require minimal programming knowledge. Cutoff Finder is a one of the best web based and user friendly applications that one may use with continuous variable predictor [8]. Another one is X-tile a standalone software [9]. Both programs are widely used to assess the association between marker of gene expression and survival, and automatically select the optimum cut point in continuous variable like gene expression or CpG methylation studies [10–12]. Here we present Evaluate Cutoffpoint software enabling estimation of one or two cutoffs, by using different kinds of statistical methods, and graphical visualization of results. Our software suite has several advantages over algorithms mentioned above and the most important is a wider selection of statistical algorithms for cut-off point determination. Application has been tested using clinical and gene expression data of Breast Invasive Carcinoma (BC) cohort from The Cancer Genome Atlas (TCGA). We investigated whether the expression together with immunohistochemically determined status of ESR1, PGR and ERBB2 have significant impact on disease free survival in breast cancer patients.

2. Materials and methods

Evaluate Cutoffpoints is an application developed using the R language [13]. Shiny framework and R packages (R version 3.4.1): survival, survMisc, OptimalCutpoints [6], maxstat [7], ROCR, ggplot2, GGally and plotly. It consists of two main layers – the first one generates HTML dynamically, the second separates the real-time data analysis. Software is available through the web interface.

2.1. Workflow

The input dataset can be any tab-separated file. Observations should be placed in rows, columns should represent variables. The next step is to choose the number of resulting groups (two or three). The user then defines variables needed to perform the analysis: biomarker, survival time and outcome. When the user wants to split the cohort into two groups, they also determine whether the cutoff value should be estimated manually or based on the significance of correlation with the binary outcome or survival. The user also specifies the statistical method that will be used to calculate the cutoff point. When the user chooses to divide the population into three groups, they also indicate if the cutoff point should be estimated using the hierarchical clustering method, or whether they will set the cutoff points manually. The query is then processed by the server and the results include estimated cutoff points and graphical visualization.

2.2. Methods for cutoff point value determination

Compartmentation of the population into two groups can be performed with four statistical methods based on significance of correlation with binary outcome or survival time. The user can also determine the cutoff point value manually.

Methods based on significance of correlation with binary outcome: Youden index and minimization of the distance between PROC plot and point (0.1). Application uses the package Optimal-Cutpoints to generate ROC plots and estimate: cutoff points, positive predictive value (PPV), negative predictive value (NPV), sensitivity (Se), specificity (Sp), positive diagnostic likelihood ratio (LR+), negative diagnostic likelihood ratio (LR_), false positives (FP), false negatives (FN).

Methods based on significance of correlation with survival time: maximally selected rank statistics and Cox proportional hazard model. Calculation of maximally selected rank statistics and estimation of cutoff value (the most significant split based on the standarized log-rank test) is performed with the use of maxstat package. As for the second method, application uses coxph function from the survival package to fit Cox proportional hazard model to the binary (outcome) and continuous (survival time and biomarker value) covariates. Cutoffpoint is then computed with the cutp function (survMisc package).

Adaptive (manual) selection of the cutoff value: the user can select a cutoff value based on a scalable, interactive heatmap (generated with the use of plotly package) that illustrates all cutoff points (biomarker values arranged from lowest to highest). The intensity of the color (from blue to yellow) of each field presents the probability value, calculated using the coxph function from the survival package. Selection of a field on the heatmap results in generation of Kaplan-Meier plot and a table with estimated hazard ratio, confidence interval and p-value. Cutoffpoint adaptability increases the value of the algorithm, since the statistically significant cutoff value may not be optimal biologically or medically. In such cases, despite lower statistical significance, we may receive better information from a medical point of view.

Classification of the population into three groups based on survival time and binary outcome can be executed in the application with the hierarchical clustering method (function tier from the Rorl package). Firstly, the algorithm splits the cohort into two groups by estimation of the optimal cutoff with the highest log-ranking statistics. The procedure is then repeated in the resulting groups to obtain two supplementary cutoff values. Second optimal cutoff is the one with larger test statistics. Application omits all rows (observations) with NA values. User can also manually select two cutpoints from the sliders to observe the changes in the Kaplan-Meier plot and pairwise comparison of hazard ratio, confidence intervals and p-values between the resulting groups.

One should bear in mind that slight differences in results given by employed methods, when they are indeed comparable, are natural. Firstly, theory behind an R function might use slightly different mathematics (as in the case of cutp and maxstat.test [14]), secondly - there are potential differences in implementation of algorithms performing the calculations, as well as in algorithms themselves. Moreover, various methods can be expected to give consistent results provided that cutoffs are defined unambiguously for the data at hand. If there is an intermediate region
in the range of the interesting covariate that does not clearly differentiate between survival curves, one can in principle end up with significantly non-equal cutpoints from the former.

2.3. Data visualization and analysis

For each cutoff point estimation method, the application generates the histogram, Kaplan-Meier plot and calculates hazard ratio, confidence intervals and p-values (pairwise in case of division into three groups). Kaplan-Meier plot is generated using a combination of `survival`, `ggplot2` and `plotly` packages. It is scalable and interactive (when the user hovers over the plot, survival probability and time is shown). Hazard ratio, confidence intervals and p-values are estimated with the use of `coxph` function from `survival` package.

3. Results

Once the input data has been prepared, we illustrate the functionality of Evaluate Cutpoints application by the analysis of estrogen (ESR1), progesterone (PGR) and human epidermal growth factor 2 (ERBB2) receptors in BC tissue. BC is heterogeneous disease, therefore the evaluation of its molecular subtype is essential step in clinical management.

We used publicly available gene expression (RNAseqV2, RSEM normalized) and clinical data of BC cohort extracted from TCGA (data status of Jan 28th, 2016). We defined cutoff points for all three receptors using all algorithms available in Evaluate Cutpoints application and all resulting figures were automatically generated.

Among the methods which we used for cutoff point determination was the one based on the measurement of specificity and sensitivity. We focused on two methods established based on ROC curve (Youden Index and minimization of the distance between ROC plot and point (0,1)). The methods optimize the cutoff point using correlation between gene expression measurements as biomarker and outcome information which in our case was immunohistochemically determined receptors status. The cutoff point for ESR1 was determined as 1301.253 with sensitivity greater than 94% and specificity greater than 93% and was exactly the same for both methods (Fig. 1). For PGR the optimal cutoff point was also the same for both methods and was determined as 243.35

![Graphs](image_url)

**Fig. 1. Cutoff point determination using ROC curve.** A) and B) Histogram of ESR1 gene expression in 1093 breast cancer patients from TCGA data classifying them into two groups according to cutoff point determined by Youden index and ROC plot (0,1), respectively; C) ROC curve as a result of Youden index method with optimal cutoff point for ESR1 and the quality of the prediction assessed by the AUC; D) ROC curve as a result of method of minimizing distance to the point (0,1) with optimal cutoff point for ESR1 and the quality of the prediction assessed by the AUC.
with sensitivity equal 85% and specificity greater than 86%. In the case of ERBB2 the results were different depending on the method. Youden Index indicated cutoff point as 11,434.438 with sensitivity greater than 64% and specificity greater than 91% while according to the method minimizing the distance, the cutoff point was 10,753.2 with sensitivity greater than 65% and specificity greater than 88%. In addition, the area under the curve (AUC) was equal 0.96, 0.922 and 0.813 for ESR1, PGR and ERBB2, respectively, indicating high quality of the test prediction (Table 1). Figures for PGR and ERBB2 may be found in Supplementary information (Supplementary Figures S1-S8).

Cutpoint, optimal cutoff point; se, sensitivity; sp, specificity; PPV, positive predictive value; NPV, negative predictive value; DLR+, value set for positive diagnostic likelihood ratio, DLR−, value set for negative diagnostic likelihood ratio; FP, false positives; FN, false negatives; AUC, area under curve.

Disease-free survival (DFS) analysis showed that regardless of algorithm used, lowered ESR1 and ERBB2 expression was related with better recurrence prognosis (cutfp: HR = 2.07, p = 0.0412 (Fig. 2); maxstat: HR = 2.39, p = 0.0423 (Fig. 3); adaptive: HR = 2.42, p = 0.0396 (Fig. 4) and cutp: HR = 2.79, p = 0.0777; maxstat: HR = 2.78, p = 0.0791; adaptive: HR = 2.79, p = 0.0777, respectively), whereas higher expression of PGR was more favorable (cutfp: HR = 0.192, p = 0.0115; maxstat: HR = 0.222, p = 0.0246; adaptive: HR = 0.108, p < 0.01 (Table 2). Figures for PGR and ERBB2 may be found in Supplementary information (Supplementary Figures S9-S12; S13-S18; S19-S24).

Next, we stratified the population of patients into three groups with simultaneous consideration of the survival variables using Rorl package. For ESR1 the first optimal cutoff was determined as 134.9501 and the second one as 1932. For PGR first cutoff point was 4.0357 and second cutoff point was 6667.0359, and for ERBB2 first cutoff point was determined as 3743.1147 and second cutoff point as 10,819.5356 (Table 3). Kaplan-Meier plots were generated and showed the differences in DFS between patients with low, medium and high expression of ESR1, PGR and ERBB2. In case of ESR1 and ERBB2 low expression of receptors was correlated with the most favorable prognosis, while low expression of PGR was associated with the worst prognosis (statistically significant are only: ESR1: HR = 0.167, p = 0.0446 for low vs high; PGR: HR = 0.786+0.09, p = 0.00301 for low vs high and HR = 9.13, p = 0.00865 for medium vs high) (Table 4). Additionally, we could also chose Adapt option

### Table 1

Statistics for cutoff point optimization using methods based on ROC curve.

<table>
<thead>
<tr>
<th></th>
<th>cutpoint</th>
<th>se</th>
<th>sp</th>
<th>PPV</th>
<th>NPV</th>
<th>DLR+</th>
<th>DLR−</th>
<th>FP</th>
<th>FN</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>1301.253</td>
<td>0.947</td>
<td>0.932</td>
<td>0.979</td>
<td>0.837</td>
<td>14.022</td>
<td>0.057</td>
<td>16</td>
<td>43</td>
<td>0.96</td>
</tr>
<tr>
<td>PGR</td>
<td>243.35</td>
<td>0.85</td>
<td>0.868</td>
<td>0.929</td>
<td>0.739</td>
<td>6.457</td>
<td>0.173</td>
<td>45</td>
<td>105</td>
<td>0.922</td>
</tr>
<tr>
<td>ERBB2</td>
<td>11,434.438</td>
<td>0.646</td>
<td>0.912</td>
<td>0.684</td>
<td>0.898</td>
<td>7.387</td>
<td>0.388</td>
<td>49</td>
<td>58</td>
<td>0.813</td>
</tr>
</tbody>
</table>

#### Fig. 2. Disease-free survival analysis according to expression of ESR1 performed with cutp algorithm. A) Kaplan-Meier plot according to expression cutoff of ESR1 calculated. B) Histogram of ESR1 gene expression in 1093 breast cancer patients from TCGA data classifying them into two groups according to cutoff point determined by cutp survival algorithm.

#### Fig. 3. Disease-free survival analysis according to expression of ESR1 performed with maxstat algorithm. A) Plot of standardized log-rank statistics of ESR1 expression. B) Histogram of ESR1 gene expression in 1093 breast cancer patients from TCGA data classifying them into two groups according to cutoff point determined by maxstat survival algorithm. C) Kaplan-Meier plot according to expression cutoff of ESR1.
Table 2
Statistics of DFS analysis according to three algorithms.

<table>
<thead>
<tr>
<th>gene</th>
<th>cutpoint</th>
<th>No. of patients in group &lt; cutpoint</th>
<th>No. of patients in group cutpoint &gt;</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>3890</td>
<td>328</td>
<td>711</td>
<td>2.07</td>
<td>1.01–4.42</td>
<td>0.0412</td>
</tr>
<tr>
<td>PGR</td>
<td>5542</td>
<td>896</td>
<td>197</td>
<td>0.192</td>
<td>0.0459–0.798</td>
<td>0.0115</td>
</tr>
<tr>
<td>ERBB2</td>
<td>3743</td>
<td>181</td>
<td>912</td>
<td>2.79</td>
<td>0.851–9.13</td>
<td>0.0777</td>
</tr>
<tr>
<td>maxstat</td>
<td></td>
<td></td>
<td></td>
<td>2.39</td>
<td>1–5.69</td>
<td>0.0423</td>
</tr>
<tr>
<td>ESR1</td>
<td>1836</td>
<td>298</td>
<td>795</td>
<td>0.222</td>
<td>0.0512–0.93</td>
<td>0.0246</td>
</tr>
<tr>
<td>PGR</td>
<td>6538</td>
<td>928</td>
<td>165</td>
<td>0.78</td>
<td>0.848–9.09</td>
<td>0.0791</td>
</tr>
<tr>
<td>ERBB2</td>
<td>3714</td>
<td>180</td>
<td>913</td>
<td>0.108</td>
<td>0.0148–0.794</td>
<td>0.00816</td>
</tr>
<tr>
<td>adaptive</td>
<td></td>
<td></td>
<td></td>
<td>2.42</td>
<td>1.02–5.75</td>
<td>0.0396</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence intervals.

Fig. 4. Disease-free survival analysis according to expression of ESR1 performed with adaptive algorithm. A) Kaplan-Meier plot according to expression cutpoint of ESR1. B) Histogram of ESR1 gene expression in 1093 breast cancer patients from TCGA data classifying them into two groups according to cutoff point determined by adaptive algorithm. C) Heatmap showing p-values of distinct cutoff points for each from ESR1 expression value, which are represented by differential intensity of the colors.

for manual selection of optimal cutoff points classifying patients into three groups (Fig. 5B and D). Figures and statistic tables for PGR and ERBB2 may be found in Supplementary information (Supplementary Figures S25–S28).

As an Evaluate Cutoffs validation method we used Cutoff Finder tool. From example data set GSE2034 downloaded from Cutoff Finder website we chose ESR_20522_at as a biomarker. For cutoff point determination based on ROC curve, immunohistologically determined estrogen receptor status (ER_HIC) was employed as an outcome variable. Both Cutoff Finder and Evaluate Cutoffs reported nearly identical results. Cutoff point and AUC obtained from Cutoff Finder were 10.11 and 0.94 respectively (Supplementary Figures S39–S32), and from Evaluate Cutoff 10.128 and 0.939, both ROC(0,1) and Youden methods (Supplementary Figures S33–S36). All methods showed also the same sensitivity and specificity values, 91.9% and 85.7%, respectively. Of the example data set we could not perform comparative DFS analysis due to lack of appropriate data; instead distant metastasis-free survival (DMFS) was examined with dmfstime as time variable, dmf_event as outcome variable and ESR1 expression (ESR1 205225_at) as biomarker. Both tools showed exactly the same results (cutpoint: 12.52, HR = 1.41 (95% CI: 0.96–2.06), p = 0.079) (Supplementary Table S1, Supplementary Figures S37–S40). Adaptive selection of cutpoint showed range of 11.8–12.8 with 12.5–12.6 being the most statistically significant cutoff points (Supplementary Figures S41–S42).

4. Discussion

In our work, we present the freely available web application Evaluate Cutoffs (http://wnbikp.umed.lodz.pl/ Evaluate-Cutoffs/), which allows estimation of one or two cutoff points using various statistical algorithms and graphical visualization of the results. The program is straightforward to use: the user uploads the molecular data and select the method for cutoff point determination. As a results user get estimated optimal cutoff points, Kaplan-Meier plots and other overview plots as well as interactive heat map, which allows manual selection of the optimal cutoff point.

Many programs and applications that are used to split continuous data exist with the example of X-tile and Cutoff Finder [8,9]. They differ from each other with the statistical algorithms offered, the way the data are presented, the number of groups resulting from the division and ease of use. We believe that Evaluate Cutoffs combines the advantages and eliminates the deficiencies of prior created software. It allows split of studied population into groups according to continuous variables (time, biomarker value) and binary variables (observation result). Major advantage of
Table 3
Cutoff point optimization using Rolr package and number of patients in each from three groups (low, medium, high).

<table>
<thead>
<tr>
<th>GENE</th>
<th>CUTPOINT1</th>
<th>CUTPOINT2</th>
<th>LOW</th>
<th>MEDIUM</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>134.9501</td>
<td>1932.0478</td>
<td>155 (14.2%)</td>
<td>145 (13.3%)</td>
<td>793 (72.6%)</td>
</tr>
<tr>
<td>PCGR</td>
<td>4.0357</td>
<td>6667.0359</td>
<td>17 (1.56%)</td>
<td>915 (83.7%)</td>
<td>161 (14.7%)</td>
</tr>
<tr>
<td>ERBB2</td>
<td>3743.1147</td>
<td>10,819.5356</td>
<td>181 (16.6%)</td>
<td>640 (58.6%)</td>
<td>272 (24.9%)</td>
</tr>
</tbody>
</table>

Evaluate Cutpoints is availability of algorithm for stratification not only into two but also into three groups, unlike the Cutoff Finder, which offers dichotomization only. Such approach seems to be the most natural, however could cause loss of information. Additionally, the user can manually adapt the cutoff point and observe how the survival probability and biological properties fit together. Moreover, the software generates interactive heat map, in which the color is changing according to p-value for each possible cutoff point. Cutoff Finder is not characterized by the flexibility of choice and in consequence it is not possible to identify other potential cutoff points with high statistical significance. Furthermore, Evaluate Cutpoints estimates cutoff point using five different statistical methods and calculates hazard ratio, which is not available in X-tile software. We have tested our software in comparison with Cutoff Finder using the same data and when the same statistical algorithms were used, results are identical (supplementary data).

In general, Evaluate Cutpoints as well as Cutoff Finder and X-Tile were developed to analyze data from growing high-throughput gene expression experiments. However, such algorithms may be used with another genomic continuous variable like recently reported differential methylation of CpG islands in clear cell renal cell carcinoma [11]. There are several important concerns pointing that discretization of continuous variables generates discontinuous model of response that is not truly relevant for a data like gene expression analysis.

Fig. 5. Disease-free survival analysis according to expression of ESR1 performed with Rolr and adaptive algorithms. A) Kaplan-Meier plot according to expression cutpoint of ESR1 by Rolr algorithm. B) Kaplan-Meier plot according to expression cutpoint of ESR1 by adaptive algorithm. C and D) Histogram of ESR1 gene expression in 1093 breast cancer patients from TCGA data classifying them into two groups according to cutoff point determined by Rolr and adaptive algorithm, respectively.
expression. On another hand, most researchers and clinicians agree to use immunohistochemical staining for survival analysis though its categorized test data represents continuous protein concentration. Another concern is that majority of microarray based gene expression experiments are hardly comparable and using those for survival analysis has a very limited clinical usage. This is also true, but we should point that those are rather preliminary results directing follow-up analyses and not a true clinical marker selection. Most important is that growing evidence of a standardized RNAseq data like TCGA database are very useful for comparable survival analysis. cbioPortal on-line tool are quite useful for TCGA database analysis including survival analysis based on gene expression variables [15,16]. However, cbioPortal survival analysis is based on cutoff point chosen arbitrary by researcher that requires numerous tests. Alternatively, as showed in our analysis, TCGA gene expression data can be used for survival analysis using our Evaluate Cutpoints or another algorithm discussed above.

Evaluate Cutpoints was developed to facilitate the cutoff point estimation and optimization from continuous data. The software was created for application in biomedical research, but it could be also utilized in other areas where the division of continuous data is necessary. The program can also be further extended with other methods for estimating cutoff point and their visualization.

Acknowledgments

All co-authors testify that our article entitled Evaluate Cutpoints: adaptable continuous data distribution system for determining survival in Kaplan-Meier estimator submitted to Computer Methods and Programs in Biomedicine has not been published in whole or in part elsewhere is not currently being considered for publication in another journal and all authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

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**Conflict of interest**

All co-authors declare no conflict of interests.

**Supplementary materials**

Supplementary material associated with this article can be found in the online version, at doi: 10.1016/j.cmpb.2019.05.023.

**References**