Nonlinear treatment effects in IPD-MA: an introduction to modelling absolute risk differences using splines

Michail Belias , Maroeska M. Rovers , Jeroen Hoogland, Johannes B. Reitsma, Thomas P.A. Debray, Joanna IntHout

Health Evidence, Radboud university medical center, Mailbox 133, P.O. Box 9101, Nijmegen 6500 HB, The Netherlands

Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, PO Box 85500, 3508 GA Utrecht, The Netherlands

Cochrane Netherlands, University Medical Center Utrecht, Utrecht University, PO Box 85500, Utrecht, 3508 GA, The Netherlands

Corresponding author

# Introduction

One of the main goals of an individual participant data (IPD) meta-analysis (MA) is to investigate whether treatment effect differences are present, and how they are associated with patient characteristics [1]. Investigating a treatment effect conditional on a continuous variable (e.g. BMI or age) may be challenging, since often the association between the outcome and the continuous variable is not a-priori known.

A naïve but often used approach is to ignore the functional form of the outcome-covariable association by either categorisation of the continuous variables or by assuming a linear effect. Categorisation leads to loss of information, and may result in model misspecification, reduced power, inflation of the type I error rates, and biased results [2–5]. Linear modelling may be problematic, as it ignores potential non-linearities and may thus also lead to biased results.

Modelling treatment effect differences whilst accounting for non-linear functional shapes may provide the opportunity to accurately make inferences whether a patient should be treated or not. An approach to account for non-linearities is to first estimate the functional shape of the associations and then investigate potential treatment effect differences [6]. So far, a variety of methods that account for non-linear functional shapes have been proposed [7–16]. In this manuscript we consider the use of splines to account for potential non-linearities since splines allow to capture both non-linear main effects and non-linear treatment-covariable interaction effects, without the need to pre-specify their functional form, thus avoiding mis-specification. Furthermore, it is possible to choose between fully parametric, semi-parametric or even non-parametric approaches, and to allow for penalisation and clustering. Thus, splines are very flexible to address a great variety of fitting problems. The most relevant types of splines in order to investigate possible differences in treatment effect for clinical decision making are based on piece-wise polynomials. Four commonly used spline approaches are restricted splines [17], B-splines [18, 19], Smoothing splines [20] and P-splines[21].

Splines are being used in single studies, both in intervention and prediction studies. However, the use of splines in IPD-MA is less common. In IPD-MA, splines may be modelled in either one or two stages. In a one-stage approach, a generalised additive mixed effects model (GAMM) [22] is applied. GAMMs fit a generalised additive model using covariables with or without spline transformation, while adjusting for within-study clustering of the participants. In two-stage IPD-MA, at the first stage an appropriate statistical model is fitted per study including spline transformations. Subsequently we can either extract study specific estimated regression lines with their standard errors, or the coefficients of the estimated functions along with their variance-covariance matrix. At the second stage, we may either pool the extracted regression curves through pointwise meta-analysis [14] or pool the extracted coefficients through multivariate meta-analysis [23].

One reason why splines are not often used in IPD-MA may be that the available guidance is limited. White et al. [24] did compare pointwise meta-analysis and multivariate meta-analysis techniques in presence of non-linear associations, but used fractional polynomials instead of splines. Gasparrini et al. [23] described the use of B-splines in combination with multivariate meta-analysis. They mention that multivariate meta-analysis may be combined with other approaches to account for non-linearities but don’t provide details. Riley et al. [25] described multivariate meta-analysis and one-stage mixed effects modelling. However, most of the examples were limited to either linear associations or a combination of restricted cubic splines and multivariate meta-analysis.

The estimand we focus on is the absolute risk difference between interventions conditional on a continuous covariable, as we consider this measure the most relevant for clinical decision making. Our goal therefore is to explain and illustrate how to model conditional absolute treatment effects using the aforementioned spline approaches in scenarios with multiple studies, using artificial data-sets for illustration. We describe the various spline approaches mentioned above and their application in IPD-MA using pointwise meta-analysis [14], multivariate meta-analysis [23], and GAMMs [22], and we provide the corresponding R-code. We also describe the results of the aforementioned spline and pooling methods using an empirical individual participant data-set, investigating the effect of antibiotics in children with acute otitis media (AOM) [26].

# Illustrative examples

In order to illustrate the aforementioned spline and IPD-MA approaches we generated artificial data to mimic a previously reported nonlinear association between BMI and mortality [27, 28]. We consider the case where the outcome is binary, but note that splines may be used to other types of outcomes such as continuous and time-to-event outcomes. For the control group we generated a J-shaped association showing increased mortality for underweight and overweight participants, while obese participants show a BMI-dependent exponentially increasing mortality risk. For the experimental group we assume a levelled J-shaped association, where the underweight and overweight participants have approximately the same risk as the participants with normal weight without treatment, while the obese participants show again a BMI-dependent exponential increase in their risk, but less steep. The functional shape for the associations in the control and experimental group is quadratic and quartic respectively, see Figure 1.

Figure 1. approximately here

To illustrate the performance of splines in IPD-MA we generated three distinct IPD-MA scenarios, each consisting of 5 RCTs with 500 participants per study. In the first scenario, which we refer to as the heterogeneous data-set with equal BMI ranges, the association between BMI and mortality is different across studies, see Figure 2, but the distribution and ranges of BMI are the same. In the second scenario, which we refer to as non-heterogeneous data-set with different BMI ranges, the parameter values of the association for both the treated and control group are identical across all studies, but the ranges of available BMIs vary across studies (see Figure 3). In the third scenario, to which we refer as the combined data-set with different BMI ranges and with between study differences in the mortality risks, both the ranges of BMI and the association of BMI with the mortality risk vary across studies, see Figure 4. Exact equations are given in the Appendix.

Figure 2. approximately here

Figure 3. approximately here

Figure 4. approximately here

# Treatment effect (measure) modification

“Treatment effect modification”, also called “treatment effect measure modification” [29, 30] is the phenomenon where the effect of a treatment varies across the levels or strata of a certain variable. We prefer the term “treatment effect-measure modification” since effect modification may be present for one measure (e.g. risk difference) but not for another (e.g. odds ratio, risk ratio) [29–33]. The scale in which the results are presented is therefore a vital first decision.

A commonly applied approach to investigate treatment effect measure modification is to model the interaction of a potential effect modifier with the treatment. In case of non-linear associations, a spline transformed version of the continuous modifier can be used. Therefore, we model the association between the modifier and the outcome by including a spline transformed version of the modifier, both as main effect and in interaction with the treatment. In case of a binary outcome like mortality, a logit link function can be used in the model. In order to calculate the absolute risk difference between the treatment arms, we back-transform the predicted outcome per treatment arm with the inverse logit function. To calculate the confidence interval of the absolute risk difference we use the proposal of Newcombe [34].

# Spline approaches in a single study

In a setting where the association between an outcome and a continuous variable X is nonlinear, one of the options for curve fitting is to use splines. Splines represent a continuous variable as a linear additive combination of (often) local parts which each have a simple mathematical form and are known as basis functions. Numerous basis functions have been developed involving various mathematical forms, such as polynomials, radials and Fourier series. However, for our goal basis functions based on piecewise polynomials are most relevant. As the term piecewise implies, the range of X is divided into intervals, using cut-offs called knots. Within each interval a d-degree polynomial of X is used to model the association between the outcome Y and X. These polynomials are connected across adjacent intervals. This way, instead of estimating a global non-linear association over the full range of data, we estimate the linear association between the outcome and a local transformation of X. Third degree polynomials are the simplest basis functions with good smoothness properties. Therefore, they are commonly applied in splines, but polynomials of higher or lower degree can also be used. In practice, smoothing with polynomials of a higher than third degree will usually give similar results to the third degree [35].

Two important choices have to be made, in addition to the degree of the basis functions: (1) the number and the position of the knots, and (2) whether a penalty should be applied. Splines calculated with the use of knots and without penalties are often called regression splines. The most commonly used regression splines are restricted or natural splines [36] and B-splines [18, 37]. Splines where a penalty is applied are called penalised splines. The most commonly used penalised splines are P-splines [21] and Smoothing splines [20]. A short summary of these four types of splines is presented below. Details are presented in the Appendix. Figure 5 shows how the aforementioned spline methods are associated with each other.

Figure 5. approximately here

## Regression splines

In order to understand the rationale to use splines, we start with a short description of other approaches to model nonlinear associations. Generalised linear models (GLMs) are often used to model the association between an independent variable X and an outcome Y. In case of non-linear associations, a transformed version of X can be used in a GLM instead of X, for example a third degree polynomial. Yet, global functions, over the full range of X, may have poor fit near the boundaries of X due to the instability of the estimated polynomial in this area. To avoid this, polynomials fitted on different intervals of X, also called piecewise polynomials, may be preferred to global functions. However, piecewise polynomials when fitted in two consecutive intervals will show different predicted values at the adjacent boundaries of the intervals (*i.e.* at the knots), thus their functional shape will be discontinuous. A solution could be to fit a higher-degree global polynomial, and model the deviations from this globally defined shape within truncated parts of X. A disadvantage of these truncated power series is that they can still show erratic behaviour near the boundaries of X.

## **Natural or restricted splines**

A solution to this erratic behaviour is to fit a linear (first-degree) global polynomial over the full range of X and model the deviations from it. Note that also splines need to be continuous over the knots. Therefore, specific truncated transformations of X are used, which we present in the Appendix. These splines are often called natural or restricted (polynomial) splines. The number and location of the knots may be based on clinical knowledge or on descriptive statistics. For instance, Harrell suggests the use of quantiles and advocates that four knots in most cases are adequate [17, 38], see Table 1. Depending on the available sample size and required complexity of the functional shape we may use a different number of knots.

In our single study example, we used restricted cubic spline transformations of X both as main effects and as interactions with the treatment. Following Harrell’s suggestion, we placed 5 knots at values corresponding to 5%, 27.5%, 50%, 72.5% and 95% quantiles of X. In Figure 6(a), we present the predicted mortality risks per treatment arm, conditional on BMI, along with the 95% confidence intervals. Subsequently, we calculated the effect of the treatment conditional on BMI, by calculating the conditional risk for the control minus the conditional risk for the treated, see Figure 7(a). To calculate the absolute risk difference’s 95% confidence intervals, we followed the proposal of Newcombe, see section 3. Note that in our artificial data the boundaries and distribution of BMI-values for the treated and control group are the same. In practice, this may not be true and knots may be placed at different positions for the main effects and the interaction terms.

## **B-splines**

B-splines are another commonly applied regression spline approach. They are based on a parametrisation of polynomial splines. Their basis functions are based on step functions called zero-degree basis functions equal to 1 within an interval and 0 otherwise. First degree basis functions are calculated from the zero-degree basis functions, and so on. More details are given in the Appendix.

In Figure 6(b) we show the results of the B-splines approach for the simulated single study data. In order for B-splines and restricted cubic splines to be comparable in terms of the degrees of freedom, we used second degree B-spline transformations of X both for the main effects and for the interactions with the treatment. We used 4 equidistant knots; 2 inner knots at BMI values 25.65 and 32.84 plus 2 at the boundaries 18.5 and 40. Subsequently, we calculated the effect of the treatment conditional on BMI, see Figure 7(b), similar as for the restricted cubic splines.

## **Properties of regression splines**

The main advantages of regression splines are their simplicity and the fact that they can be represented by a formula. As a consequence, the estimated regression coefficients can be reported and used in further analysis, e.g. meta-analysis. Also, both restricted splines and B-splines are straightforward extensions of GLMs, with low computational cost.

B-splines of any degree are calculated based on zero-degree basis functions defined within each interval. This provides great local support and numerical stability [35]. Restricted splines fit a “basic” linear model and estimate deviations from it, which provides limited local support, since some basis functions are defined over the whole range of the variable, while others are not. Restricted splines with κ knots require degrees of freedom, while dth degree B-splines require degrees of freedom [35]. Furthermore, the model fit of regression splines (e.g. as quantified using the log-likelihood) depends on the number and position of the knots, thus careful modelling is required to avoid overfitting. In some occasions clinical knowledge on the expected curvature or descriptive statistics may be used to define the knots, but in others it is unclear how many knots should be used and where they should be placed. Commonly used criteria such as Akaike’s information criterion (AIC) can be used for a data-based choice of the number and position of the knots Yet, to avoid this selection process, penalised splines have been proposed, which we describe in section 4.2.

## Penalised splines

The two commonly applied penalised splines that we discuss, P-splines and Smoothing splines, increase the number of knots to a large set (usually, 10-40) or even to be equal to the number of observations. This way they circumvent the problem of choosing the number and positions of the knots. Since estimating one parameter for each observation would clearly lead to a perfect fit and thus generate functional shapes with extreme variability, penalised splines introduce in their optimisation functions a penalty term () multiplied by a non-negative , often called a tuning parameter. As the term “tuning” implies, changing the value of changes the magnitude of the penalisation.

Penalised splines may circumvent the problem of knot selection, but at a cost. By using a penalty in their optimisation function, they introduce bias in their estimate in order to obtain a more stable solution. Furthermore, in both P-splines and Smoothing splines the tuning parameter must be specified. Too high or too low values of may lead to over- or undersmoothing respectively. Several approaches have been proposed in order to determine the “optimal” , such as Akaike’s information criterion AIC [39], “leave one out” generalised cross-validation (GCV) [40] or mixed-effects modelling [22]. These processes are automated in most of the statistical packages. Briefly, when using the AIC, a series of models fitted with different values are compared and the one with the lowest AIC is selected. “Leave one out” GCV is an iterative process, the algorithm goes as follows: 1) one observation is omitted 2) a model is fitted 3) using the model a prediction of the omitted value is generated and 4) the distance between the observed and predicted value is calculated. This procedure is repeated for each observation and for a series of values. The that minimizes the sum of the squared distances, i.e. the GCV score, is selected. In Bayesian/mixed effects modelling approaches the penalty term is estimated in a similar way as a random effects parameter [22]. More details are shown in the Appendix.

## **P-splines**

A specific type of penalised splines, P-splines, proposed by Eilers and Marx [21], is a penalised version of B-splines, using a specific penalty term based on the sum of p-order differences between the coefficients of two consecutive intervals . The first order differences are defined as follows: , but Eilers and Marx propose the use of second order differences, which are the first order differences of the first order differences. Note that the degree of the underlying B-splines may be different from the order of the differences. A common combination is that of a third-degree B-spline with a second order difference. Using a penalty based on a zero-degree order difference results in the ridge penalty [41].

P-splines are based on equidistant knots. It is possible to use a knot sequence that is not evenly spaced; but in this case, weights need to be introduced [22, 35]. As P-splines with non-equidistant knots are rarely used in practice we don’t consider them in this article. In our single study example, we used P-spline transformations of X for both main effect and interaction terms. We used an arbitrary number of 17 equidistant knots; 15 inner knots plus the boundaries, while the parameter was selected through a ‘leave one out’ GCV process as described above. In Figure 6(c), we present the resulting mortality risks per treatment arm conditional on BMI, along with the 95% confidence intervals. Subsequently, the effect of the treatment conditional on BMI, calculated as the difference between the two curves in Figure 6(c), is presented in Figure 7(c).

## **Smoothing splines**

Smoothing splinesare another member of the family of penalised spline methods. Similar to P-splines the idea is to increase the number of knots, but this time to be equal or approximately equal to the number of observations. O’ Sullivan [41] suggested that a penalty based on Reinsch’s integral of the second derivative of , where is a cubic spline, multiplied by a tuning parameter, has good smoothing properties. This results in the following penalty term for Smoothing splines: .

In our single study example, we use Smoothing spline transformations of X for both the main effect and the interaction term, while the parameter is selected through a ‘leave one out’ GCV process as described above. In Figure 6(d), we present the resulting mortality risks per treatment arm conditional on BMI, along with the 95% confidence intervals. The effect of the treatment conditional on BMI is presented in Figure 7(d).

Figure 6. approximately here

Figure 7. approximately here

## **Properties of penalised splines**

Penalised splines are penalised extensions of the regression splines based on many knots. For instance, P-splines are B-splines with an order difference penalty applied on the coefficients, while Smoothing splines are cubic splines with a derivative based penalty. The penalisation contributes to the selection between a complex and a simple model in a similar way as in other well-known penalised GLM approaches, for instance LASSO, ridge or elastic-net. Penalised splines reflect ones belief that the predicted regression lines are more likely to be smooth than not. Therefore, their main advantage is that they are more likely to show smoother functional shapes as compared to unpenalised splines. Another great advantage is that they circumvent the need to specify the positions and the number of knots, which in most cases are not known beforehand and may need to be estimated.

Penalisation also affects the inference, due to the bias-variance trade-off. For instance, the coefficient estimates are subject to a smoothing bias, therefore their interpretation may be problematic. Note that this issue does not necessarily apply to the predicted outcomes. A related issue is that the degrees of freedom have to be modified to account for the penalisation. Wood [42] suggests the use of effective degrees of freedom of a model. Effective degrees of freedom are calculated using the Welch‑Satterthwaite approximation formula and can be used to compare models fitted with different types of splines. Note that in this context the effective degrees of freedom may be decimal and not integers [43].

# Individual participant data meta-analysis using splines

In the previous sections we focused on estimating nonlinear main effects and interactions with the treatment in a single study. As trials are rarely powered to investigate effect modifiers, exploring non-linear effects in a single study may often be even more problematic or yield very wide confidence intervals. Depending on the underlying curvature, splines need a high amount of data and therefore their use is more feasible in the context of an IPD-MA, where they enable the statistical modelling of complex relationships such as non-linear associations [44]. They can be applied in a two-stage or one-stage meta-analysis approach. We apply the methods on three IPD-MA scenarios of 5 studies each. In the first scenario, the regression lines are heterogeneous whilst the BMI ranges are the same across studies. In the second scenario, the regression lines are homogeneous but the BMI ranges are different across studies. In the third scenario, both the regression lines and the BMI ranges are different across studies.

## Two-stage pointwise meta-analysis

In pointwise meta-analysis a separate meta-analysis is conducted per distinct value (point) of X, using the outcomes and standard errors as estimated per study. In the first stage of two-stage pointwise meta-analysis, as proposed by Royston and Sauerbrei [14], we may fit an appropriate model and estimate the predicted outcome per study, optionally controlling for individual‐level confounders. Note that instead of using fractional polynomials as in Royston and Sauerbrei, we may use any of the spline approaches described in section 4. At this point we should decide, e.g. by plotting the results, whether it is sensible to pool the predicted outcomes across studies. At the second stage, for each distinct value of X a meta-analysis is performed on the resulting predicted values and standard errors, using either a fixed or random effects approach [14]. Given a continuous variable X the algorithm proceeds as follows:

**Stage 1**

1. Select a spline approach and fit per study an appropriate model including interaction between X and the treatment. Since in pointwise meta-analysis we are pooling the predicted outcomes we can apply any good fitting model per study. As a consequence, different modelling techniques may be applied across studies, including linear models, fractional polynomials, and splines of different degrees and with different knot specifications. During this stage we may use criteria to the find the best fitting model per study, e.g. Aikaike information criterion, GCV or likelihood ratio tests (possibly with a nominal significance level larger than 0.05 as proposed by Sauerbrei and Royston [14].
2. Using the models from step 1, estimate regression lines and for the treated and control group in study *j* respectively, along with their standard errors and 95% confidence intervals. In order to smooth the pooled regression lines from stage 2, we can extrapolate the regression lines to cover the full domain of X. Automatically, the standard errors of the predicted outcomes in the extrapolated regions will be increasing along with the extent of extrapolation, and ensure small weights for the extrapolated outcomes in the meta-analysis.
3. Depending on the outcome we wish to show and depending on the scale on which we wish to make inferences, we may choose to use a link function :
   * 1. If, in stage 2, we aim to show the predicted outcome per treatment arm and conditional on X, we calculate the predicted outcome per treatment arm, and
     2. If, in stage 2, we aim to show the effect of the treatment conditional on X, we first calculate per study the absolute treatment effect or the relative treatment effect and calculate the corresponding confidence interval, see section 3. Note that if the goal of our meta-analysis is to make inferences on the treatment effect, this approach is preferable to step 3.1, to avoid amalgamating the within and between study heterogeneity [45, 46].

**Stage 2**

1. For each value within the boundaries of X we perform either a fixed or random effects meta-analysis to get the pooled outcome of choice as a function of X along with its pointwise 95% confidence interval. Note that if the available data across the studies vary over different regions of X, pooling of the predicted outcomes may produce unsmooth results, see Figures 8 and 9, especially in the second and third scenario.

We applied pointwise meta-analysis using all aforementioned spline approaches in all 3 IPD-MA scenarios. First, we estimated per study and treatment arm the mortality risk conditional on BMI (step 3.1), and we estimated per study the risk difference and the confidence interval (step 3.2). In the second stage we pooled both the regression lines per treatment arm and their risk difference, using random effects meta-analyses with REML estimators for τ2. The pooled mortality risks per treatment arm are presented in Figure 8, and the pooled treatment effects conditional on BMI in Figure 9. For the spline approaches, we positioned knots per study as follows: for the restricted cubic splines, we placed 4 knots, following Harrell’s suggestion to use the 5%, 35%, 65% and 95% quantiles of BMI, for B-splines 3 equidistant knots (1 inner knot plus the boundaries per study), and for P-splines 17 equidistant knots (15 inner knots plus the boundaries per study). For the penalised splines (P-splines and Smoothing splines) the tuning parameter λ was selected through a ‘leave one out’ GCV process.

Figure 8. approximately here

Figure 9. approximately here

## Two-stage multivariate meta-analysis

Instead of using pointwise meta-analysis per distinct value of X, the functional shapes can also be pooled using multivariate meta-analysis. This approach, as proposed by Gasparrini et al. [23], pools the set of regression coefficients estimated in the first stage, accounting for their within- and (if applicable) between-study correlation, using a fixed or random effects multivariate meta-analysis approach. The coefficients of the penalised splines are biased due to penalisation and pooling them may be problematic and show biased results. However, we may use unpenalised spline approaches such as described in section 4.1. Note that in order to pool the results of the first stage, each study should provide the same set of coefficients, estimated in the same domain of X. Therefore, in order to apply multivariate meta-analysis, the basis functions for the splines in the individual studies should be of the same degree, and also defined on the same intervals across studies, using the same knot positions. In case of different ranges of X across studies, the use of common positions for the knots may leave some coefficients inestimable in some studies and meta-analysing them may cause complications [23]. A solution is to conduct data augmentation as a preliminary step. Data augmentation as described by White et al. [47] and Riley et al. [25] refers to the generation of pseudo data beyond the per study boundaries of X, with minimal weight and arbitrary outcome. Note that in multivariate meta-analysis careful specification of the knots is required as convergence issues may occur during the second stage.

The multivariate meta-analysis algorithm proceeds as follows:

**Stage 1**

1. As a preliminary step choose the knots corresponding to the optimal locations across the studies along with the degree of the unpenalised spline method we wish to apply.
2. Per study j fit a model including interaction between X and the treatment with the chosen specifications of step 1.
3. With Q the total number of coefficients and q [1, 2, …, Q], extract per study the estimated coefficients along with their variance-covariance matrix.

**Stage 2**

1. Use either fixed or random effects multivariate meta-analysis to estimate the pooled
2. To calculate the predicted outcome given X and treatment T multiply the pooled estimates with the design (or model) matrix containing the values of X along with their spline transformed values.
3. To estimate the treatment effect conditional on X, subtract the pooled-per-treatment arm outcomes and calculate the confidence interval as described in section 3.

We applied multivariate meta-analysis in combination with regression splines in all three scenarios. To do so we performed data augmentation as a preliminary step [25, 48] in the second and third scenario. This way all studies had curves estimated over the full range of BMI. In stage 1, per study we fitted restricted cubic spline and B-spline transformations of BMI both as main effects and as interactions with the treatment. For the restricted cubic spline transformations, we used 5 knots, following Harrell’s suggestion to use the 5%, 27.5%, 50%, 72.5% and 95% quantiles of BMI, for B-splines 4 equidistant knots (2 inner knots plus the boundaries per study). Note that we positioned the knots over the full domain of BMI. Subsequently, we pooled the estimated coefficients using a random-effects meta-analysis with the REML estimation method. We calculated regression lines per treatment arm by multiplying the design (or model) matrix with the pooled coefficients. Absolute risk differences were calculated by subtracting the pooled mortality risks of the treated minus the control, while for the confidence intervals we used the proposal of Newcombe [34]. The pooled mortality risks per treatment arm are presented in Figure 10, and the pooled treatment effects conditional on BMI in Figure 11. Note that in the second and third scenario multivariate meta-analysis showed high sensitivity to the model specifications.

Figure 10. approximately here

Figure 11. approximately here

## One-stage generalised additive mixed effects model

Instead of using a two-stage meta-analysis, we may also conduct the analysis in one stage, using a mixed effect model with splines, i.e. a generalised additive mixed effect model (GAMM). Hereby, we may include spline transformations of X as main effects and as interactions with the treatment as described in section 3. Note that spline transformations of X are the sum of several basis functions, as described in section 4. Researchers may choose for the effects of each basis function to be either fixed (common), random or stratified [49]. The fixed (common) effect assumption is that the effect of the basis function is identical across all studies. The random effects assumption is that the effect of the basis function comes from a distribution of effects, while the stratified effects assumption is that for each study the effect of the basis function may be different and is estimated per study. The fixed (common) effect may be modelled straightforward by including the basis function as they are. Stratified effects can be modelled by including an interaction of the basis function with the (categorical) clustering variable (e.g. study). Random effects can be modelled by penalising the interaction of the basis function with the clustering variable, as Wood [22, 50, 51] and Kimeldorf and Wahba [52] have shown.

Depending on the estimand of choice and the assumptions researchers wish to make they may use any combination of the above assumptions for their model. Note that interaction terms included in one-stage mixed effect models may be prone to ecological bias and amalgamate the within and across study effects [45, 53–55]. To avoid this, two methods have been proposed. One approach is to stratify by study all or some of the main effects including at least the treatment effect [25]. Another approach is to center the covariate X about its study‐specific mean creating a new variable j. Subsequently, include Z,j, and the interaction of Z with the treatment in the one-stage model [25, 56].

In our 3 scenarios, we used the 4 aforementioned spline transformations both as main effect and in interaction with the treatment. We used a random intercept and random slope for BMI in combination with a fixed spline part. For the restricted cubic splines, we used 5 knots (the 5%, 27.5%, 50%, 72.5% and 95% quantiles of BMI), for B-splines 4 equidistant knots (2 inner knots plus the boundaries), and for P-splines we used 17 equidistant inner knots (15 inner knots plus the boundaries). Note that we positioned the knots over the full domain of BMI and that no data-augmentation nor extrapolation was needed.

Figure 12. approximately here

Figure 13. approximately here

## Properties of the pooling methods

We illustrated the association between BMI and mortality risk in 3 scenarios using 4 spline methods and 2 two-stage approaches (pointwise and multivariate meta-analysis) and 1 one-stage approach (GAMM). Table 2 summarises the properties of the aforementioned approaches. An advantage that both two-stage approaches share is that during their first stage they provide a better insight in the underlying associations per study. Furthermore, in two-stage methods we may use heterogeneity measures such as Cochran’s Q statistic, τ2, and prediction intervals per value of X to assess whether it is sensible to pool the associations. Therefore, it is always informative to investigate the results per study similar to the first stage in two-stage methods.  **Pointwise meta-analysis: robust and flexible but non-smoothness may occur** The main advantages of pointwise meta-analysis are its flexibility, robustness and ease of use. In pointwise meta-analysis we are allowed to fit different models across the studies, as we are pooling the predicted outcomes rather than the coefficients. For instance, in one study we may apply a restricted cubic spline transformation of X, in another a second degree B-spline and in another we may choose to not transform X. Also, we are allowed to vary the number and position of knots per study. Therefore, we may choose the predicted outcomes from the best fitting model per study. Since performing a meta-analysis for each value of X offers great local support pointwise meta-analysis is more robust to model misspecification than multivariate meta-analysis and GAMMs, where we pool coefficients within prespecified subintervals of X. When the ranges of X are different across studies, pointwise meta-analysis may still use the whole domain of X even without data augmentation. The main disadvantage of pointwise meta-analysis is that when the ranges of X are not the same across studies the pooled curve may be unsmooth. Also, since we are performing a meta-analysis for each value of X pointwise meta-analysis may be more computationally intensive than multivariate meta-analysis and GAMMs.

**Multivariate meta-analysis: efficient if specified “correctly”, but lacks robustness and flexibility**

White et al. show that the main advantage of multivariate meta-analysis is, if the fitted curves are correctly specified, that multivariate meta-analysis appears to be more efficient with narrower confidence intervals than pointwise meta-analysis [48]. However, in splines this argument may not be relevant. In practice, specifying correctly a non-linear association is challenging. For instance, in our illustrative examples we generated quadratic and quartic associations for the control and treated group, respectively. However, during the analysis we used splines to model these associations and the resulting curves were not a perfect representation of the prespecified curves; therefore our model may be considered mis-specified. Only in the first scenario, the multivariate meta-analysis had approximately the same efficiency as pointwise meta-analysis and GAMMs. A major limitation of multivariate meta-analysis is that it lacks robustness to changes in the model. For instance, in the second and third scenario multivariate meta-analysis showed very different results when combined with RCS or B-splines, see Figures 10 and 11. Furthermore, it is less flexible compared to pointwise meta-analysis, since the models fitted per study should have the same parametrisation e.g. same type of spline, same number and positions of knots and the same range of X [23]. This restriction may be problematic in cases where a subset of studies included in the meta-analysis has a limited number of participants. In that case modelling the association between the outcome and the spline transformations of X may fail to converge, and only multivariate meta-analysis based on simple linear models may be possible. Finally, since multivariate meta-analysis pools the coefficients estimated during the first stage it may not be compatible with approaches where penalisation to those coefficients is applied.

**GAMM can handle different study domains and sample sizes, whilst producing smooth pooled regression curves, but careful modelling is required**

The main advantage of GAMMs is that they can handle differences in the distributions of X across studies, include all studies regardless of the number of observations, and result in smooth pooled curves and confidence intervals. The main disadvantage of GAMMs is that we may lose the insight in the underlying associations per study offered during the first stage of the two-stage methods. Furthermore, GAMMs require careful modelling, especially when aggregation (ecological) bias might be present, as discussed by Riley et al., Hua et al., Belias et al. [25, 46, 56].

# Software

All analyses were performed with the statistical software R version 3.6.0. For data manipulation we used the **tidyverse** [57] package, for the splines and GAMMs we used the **mgcv** [50] package, including its predict function for the confidence intervals, and for pointwise and multivariate meta-analysis the **meta** [58] and **mvmeta** [23] packages respectively. It is also possible to estimate splines in other software such as Stata or SAS. However, since R is freely available for every researcher, we provide the scripts to apply splines in multiple studies scenarios only in R, using the third illustrative scenario as an example.

# Empirical example

To illustrate the use of splines combined with the aforementioned pooling methods in a real example we consider a previously published IPD-MA investigating the effect of antibiotics in children with acute otitis media [26]. Rovers et al. collected IPD from six randomised clinical trials with a total of 1643 children, aged from 0 to 12 years old. The primary outcome was fever and/or ear-pain 3-7 days (yes/no) after antibiotics or placebo treatment. Hereby, we investigate the effect of antibiotics across the values of age, in children with unilateral or bilateral acute otitis media (AOM).

## Methods

From a total of 6 studies, we used 5 studies and omitted one study with 315 participants from all subsequent analyses as the information for unilateral or bilateral AOM was not reported. We used data of children till 9 years old, as AOM above 9 years is seldom and we had a limited number of children over that age (only 15). From the remaining 5 studies, one study (Appelman et al.) had a limited number of events (children with fever/ear pain) and for some age-bilateral AOM combinations no events at all. Therefore, we followed different strategies across the pooling methods for this study.

**Pointwise meta-analysis**

For pointwise meta-analysis, for the Appelman study we fitted a logistic regression model including the main effects of bilateral AOM, treatment and age and their two-by-two interactions, without any spline transformation for age. For the remaining studies we fitted per study a logistic regression model including the main effects of bilateral AOM, treatment, and age transformed by each of the aforementioned spline approaches, and we included the interactions of the spline transformed age with bilateral AOM (yes/no) and treatment (both two-way). For restricted cubic splines we followed Harrell’s suggestion and used per study 3 knots at the 10%, 50%, and 90% quantiles of age; for B-splines we used second degree basis functions and per study 3 equidistant knots (1 inner knot plus the boundaries per study), while for P-splines we used third degree basis functions and per study 17 equidistant knots (15 inner knots plus the boundaries per study). For the penalised splines (P-splines and Smoothing splines) the tuning parameter λ was selected through a ‘leave one out’ GCV process. Subsequently, we extracted the predicted outcomes for fever/ear pain in logit scale and pooled them using a random-effects meta-analysis approach with REML τ2 estimator. To show the pooled risk conditional on children’s age, bilateral AOM and treatment group, we back-transformed the pooled curves into risks curves. To show the treatment effect conditional on children’s age and bilateral AOM, we first back-transformed per study the predicted fever/ear pain risk. To estimate the risk difference between the treated and control along with their confidence intervals, we followed the proposal of Newcombe [34], see Section 3. Finally, we pooled the risk differences using a random-effects meta-analysis approach with REML τ2 estimator.

**Multivariate meta-analysis**

For multivariate meta-analysis, we omitted the Appelman study mentioned above. Therefore, multivariate meta-analysis was based on the 4 remaining studies with a sufficient number of observations to fit splines. Also, since in multivariate meta-analysis the ranges of age across the studies need to be the same, we performed data-augmentation as a preliminary step. In the first stage of the multivariate meta-analysis, we fitted a logistic regression model including the main effects of treatment, bilateral AOM and spline transformed age, and the two-way interactions of spline transformed age with treatment and bilateral AOM. Since in multivariate meta-analysis the positions of knots need to be the same across the studies, for restricted cubic splines we used 3 knots at 10%, 50%, 90% quantiles of age calculated on the 4 studies combined; for B-splines we used second degree basis functions and 3 equidistant knots (1 inner knot at 2.5 age plus 2 at the boundaries of age). Subsequently, we extracted the regression coefficients and their variance-covariance matrix and pooled them using a random-effects meta-analysis approach with REML estimator for τ2. Finally, to show the risk of developing fever/ear pain conditional on age, treatment and bilateral AOM, we multiplied the pooled coefficients with the corresponding design matrix and back-transformed the pooled outcomes using the inverse logit function. To calculate the absolute risk differences and their confidence intervals, we followed the proposal of Newcombe [34], see section 3.

**Generalised additive mixed effects models**

For GAMMs, we included all 5 studies. We fitted a logistic regression model including the main effects of treatment, bilateral AOM and spline transformed age and the two-way interactions of spline transformed age with treatment and bilateral AOM. We used similar definitions for knot positioning and degrees of splines as in pointwise and multivariate meta-analysis, but using the whole data-set without data-augmentation nor extrapolation. We followed Wood’s proposal and included random-effects for the intercept and for the slope of age additively to account for the within study clustering of participants [42].

## Results

Figures 14, 16 and 18 show the pooled regression curves of pointwise meta-analysis, multivariate meta-analysis and GAMMs, conditional on age and bilaterality of AOM. Figures 15, 17, and 19 show the absolute risk difference between the treated and control group (the treatment effect) conditional on age and bilaterality of AOM. Since this is an empirical example, the underlying true associations are not known and we cannot draw firm conclusions with respect to the appropriateness of the different approaches. However, we show the pooled curves and compare them with regard to their smoothness and width of confidence intervals, and report convergence issues if any.

As we were investigating interactions between treatment, bilaterality of AOM and age, in some studies the combinations of these variables created groups of patients with a limited number of events at certain age ranges. Consequently, pointwise meta-analysis resulted in wide confidence intervals in some sub-domains of age. Furthermore, as in the artificial data-sets, the predicted pooled regression lines were not always smooth due to differences in the age ranges across the studies.

Multivariate meta-analysis resulted in smooth pooled regression curves for both the restricted cubic splines and the B splines approaches, see Figures 16 and 17. GAMMs combined with penalised splines resulted in smooth pooled regression lines and confidence intervals, see Figure 18 and Figure 19, while GAMMs combined with regression splines resulted in less smooth regression lines and wider confidence intervals in sub-domains of age.

Figure 14. approximately here

Figure 15. approximately here

Figure 16. approximately here

Figure 17. approximately here

Figure 18. approximately here

Figure 19. approximately here

# Discussion

Our results, in which we illustrated four spline-based approaches (restricted splines, B-splines, P-splines and Smoothing splines), and three pooling methods (pointwise meta-analysis, multivariate meta-analysis and GAMMs) on three scenarios with artificial data, showed that all approaches performed equally well in modelling the underlying true association analysis in case of a heterogeneous data-set with similar ranges of the effect modifier. In the two scenarios with different ranges, pointwise meta-analysis resulted in non-smooth pooled regression lines and wide confidence intervals in some sub-domains of BMI. Multivariate meta-analysis resulted in smooth pooled curves, wide confidence intervals and showed high sensitivity in model specification. GAMMs resulted in both smooth pooled regression lines and confidence intervals, and robustness in model changes. When applying the splines and pooling methods on an empirical example investigating the association between age and the effect of antibiotics in children from 0-9 years with unilateral or bilateral otitis media, we found that GAMMs, especially when combined with penalised splines, resulted in smooth pooled regression lines and reasonable confidence intervals for the whole range of the potential treatment effect measure modifier (age), while pointwise meta-analysis resulted in non-smooth and very wide confidence intervals, and multivariate meta-analysis was limited to a subset of studies.

The major strength of our manuscript is that as far as we are aware, we are the first to provide an introduction on how to apply a variety of spline methods in both single and multiple studies, in order to investigate treatment effect differences when non-linearities are present. In our illustrative examples we introduced three features into our generating mechanisms. First, the association of mortality risk with BMI was simple and realistic as it was based on previously published papers [27, 28]. Second, we generated IPD-MA scenarios suitable to pool as the between-study heterogeneity of the regression curves was limited to I2 less than 40%. Finally, in the second and third scenarios we generated per study different boundaries for BMI, in order to illustrate the performance of the pooling methods in scenarios where the regression curves have limited overlap.

Some potential limitations should also be mentioned. First, we did not illustrate the performance of the aforementioned approaches in a scenario with homogeneous associations and similar ranges of the effect modifier across studies. We considered that this scenario is rarely present in practice and that all approaches would produce similar results. We chose the settings as they generated realistic data, appropriate for pooling, and suitable for the purpose of an introductory paper in IPD-MA, as illustration for the stronger and weaker points of the approaches. Second, we did not illustrate the performance of the pooling methods in scenarios with ecological bias. Modelling choices that avoid ecological bias in presence of non-linear associations still require further research and were thus outside the scope of this article. Last, corresponding to our main aim to provide an introduction to splines, we limited our study to spline-based approaches whereas other techniques might also be able to deal with non-linear associations, e.g. tree-based approaches [7–11], meta-stepp [12, 13], locally (weighted) estimated scatter-plot smoothing (lo(w)ess), or fractional polynomials [14, 15, 36].

Other researchers have also drawn the attention to the importance of modelling non-linear associations in IPD-MA [25, 48]. These studies focused on estimating relative treatment effect functions whereas we focused on estimating the absolute risk differences. Our examples and results show that accounting for nonlinearities is also of great importance if the aim is to investigate treatment effect differences on the absolute scale. Therefore, we believe that this introduction on how to apply splines in IPD-MA will aid researchers to consider non-linear relations with a potential effect modifier. Doing so may provide better insight in the underlying associations and contribute to more evidence-based conclusions and thus better clinical decision making.

In conclusion, taking into account non-linear associations whilst combining multiple studies needs careful modelling. Across 3 common IPD-MA scenarios and one empirical example we showed that pointwise meta-analysis is robust to model changes and flexible, but non-smoothness may occur, multivariate meta-analysis may be efficient if specified “correctly”, but lacks robustness and flexibility, and GAMM can handle different study domains and sample sizes, whilst producing smooth pooled regression curves, but careful modelling is required. Splines provide a helpful tool to capture nonlinear treatment effect differences in IPD-MA.

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|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Number of knots | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th |
| 3 | 10% | 50% | 90% |  |  |  |  |
| 4 | 5% | 35% | 65% | 95% |  |  |  |
| 5 | 5% | 27.5% | 50% | 72.5% | 95% |  |  |
| 6 | 5% | 23% | 41% | 59% | 77% | 95% |  |
| 7 | 2.5% | 18.33% | 34.17% | 50% | 65.83% | 81.67% | 97.5% |

Table 1: Number of knots and their quantile location based on Harrell’s proposal for the restricted cubic splines approach.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristic | Pointwise meta-analysis | | Multivariate meta-analysis | | GAMM | |
| Base for pooling | Uses the predicted outcome  per value of X | | Uses coefficients of fitted curves | | Uses a common curve with random effects to account for across studies differences | |
| Allows study specific fitting strategies (different model specifications) with regard to the effect modifier | Yes | | No | | No | |
| Difficulty to perform | Easy | | Difficult | | Intermediate | |
| Performance | Same distribution of X across studies: wider confidence intervals than multivariate-meta-analysis and GAMM | Different distributions of X across studies:  more flexible than multivariate meta-analysis | Same distribution of X across studies:  narrower  confidence intervals than pointwise meta-analysis  and GAMM | Different distributions of X across studies:  lacks robustness | Same distribution of X across studies: wider confidence intervals than multivariate meta-analysis | Different distributions of X across studies: wider confidence intervals than multivariate meta-analysis |
| Main advantage | Flexible | | Efficient | | Smooth | |
| Main disadvantage | May show discontinuities in the pooled curves | | Lack of robustness, very sensitive to modelling choices  Needs all parameters to be the same across studies.  Data augmentation might be needed as a preliminary step. | | Could lead researcher to lose insight into the underlying associations per study, if done without the first stage of the two-stage methods | |
| Can be performed using all types of splines | Yes.  Plus: different models may be applied across studies | | No  Not suitable in combination with penalised splines | | Yes | |

**Table 2. Comparison of the pooling methods**

# Highlights

## What is already known

* Analysing associations between outcomes and continuous patient characteristics in IPD-MA may be challenging when non-linear associations are present.
* Splines offer great flexibility but are rarely used.

## What is new?

* We provide an introduction and guidance on how to model non-linear treatment effects using restricted splines, B-splines, P-splines and Smoothing splines and three IPD-MA methods to pool the results of multiple studies: pointwise meta-analysis, multivariate meta-analysis, and generalised additive mixed effects models (GAMMs).
* We illustrate the performance of the splines and pooling methods on three common IPD-MA scenarios and provide R code.
* We illustrate their performance of the splines and pooling methods on real acute otitis media data

## Potential impact for other fields

* Splines provide a helpful tool to capture nonlinear treatment effects in IPD-MA.
* Modelling nonlinear associations may provide personalised treatment effects with more accuracy, thus aiding to more precise clinical decision making.

# Appendix

For readability we adopt the following notation throughout the manuscript:

* The trials as j = 1,2, …, n
* Trial participants as i = 1,2, …,,
* Continuous effect modifier: X
* Binary treatment indicator: T with value 0 for the control group and 1 for the experimental group.
* The true association of X with the outcome: f(X)
* Smoothed estimated function:
* the boundaries of X and the boundaries of X per trial j
* The number of (inner) knots:
* w є [1,…, κ +1] the intervals defined by the knots
* g: a link function and its inverse function
* B(X;d) a basis function of dth degree

## Polynomial regression and truncated power series

In general, to model the association between an independent variable X and an outcome Y, generalised linear models (GLMs) are used. In case of non-linear associations, transformed versions of X can be used instead of X. For instance, the statistical model for a GLM with link function g and with a d-degree polynomial of X is:

However, a global function over the full range of X may have poor fit near the boundaries due to instability of the estimated polynomial in this area. To avoid these issues, piecewise polynomials may be preferred to global functions. The model for a d-degree polynomial for interval *w*, between knot *tw* and *tw+1*, would be:

These piece-wise polynomials, when fitted in two consecutive intervals, will show different predicted values at the boundaries of the intervals (*i.e.* at the knots), thus their functional shape will be discontinuous. For this reason, we may use restrictions to “connect” interval-specific polynomials. One convenient solution is to fit a global polynomial, and model the deviations from this globally defined shape within truncated parts of X. Thereto, each basis function is a polynomial with one term. Given a non-decreasing sequence of knots a truncated power series basis is defined by the following basis functions:

and the statistical model for the association between X and Y is:

The + subscript denotes that for a given z

The first term in equation (4) generates the global polynomial, often called the “basic” polynomial, whereas the second term, often called the “secondary” polynomial, is modelling the deviations from it. The resulting splines, using truncated power series basis functions, are often called polynomial ‘regression’ splines [59]. The term truncated reflects the fact that the intervals for the power series in the secondary polynomial are shortened to produce estimates only in sub-domains of X. A disadvantage of truncated power series is that they can still show erratic behaviour near the ranges of X.

# Regression splines

## Natural or restricted splines

A solution to this erratic behavior near the boundaries is to restrict the truncated power series to be linear near the boundaries of X [17]. These splines are often called natural or restricted (polynomial) splines. Given a non-decreasing sequence of knots the statistical model is given as:

where

and for *w* є [2, κ-1]

Harrell shows that restricted cubic splines can also be written as truncated power series with a linear “basic” polynomial, by dividing the basis functions by [36]. Therefore, an equivalent statistical model to (5) may be written as follows:

The number and location of the knots may be based on clinical knowledge or on descriptive statistics. For instance, Harrell suggests the use of quantiles and advocates that four knots in most cases are adequate [17, 38], see Table 1. Depending on the available sample size and required complexity of the functional shape we may use a different number of knots.

In Figure A1(a) we show the basis functions - scaled by for the restricted cubic splines approach with 5 knots placed at the 5%, 27.5%,50%, 72.5%, 95% quantiles. In our single study example, we used restricted cubic spline transformations of X both as main effects and as interactions with the treatment. Following Harrell’s suggestion, we placed 5 knots at values corresponding to 5%, 27.5%, 50%, 72.5% and 95% quantiles of X.

.

## B-splines

B-splines are another commonly applied spline approach. They are based on a parametrisation of polynomial cubic splines. Given a non-decreasing knot sequence and X є , the dth degree B-splines basis functions are calculated by the following algorithm proposed by De Boor [19].

First, d additional knots are generated before and d additional knots after . These are often called outer knots and their choice is arbitrary. We can set them to be equidistant or even equal to the boundary values and of X. A new knot sequence is generated, where:

are the left outer knots or endpoints,

,

, , …, the inner knots

,

the right outer knots

Within each interval *w* a zero-degree B-spline is calculated. Zero-degree B-splines are step functions equal to 1 within an interval and 0 otherwise.

All succeeding basis functions, with degree >1, are calculated using the following formula:

where [1, 2, …, d]. For example, the first degree is calculated using the zero degree B-splines, and the second degree from and so on, using formula 7.

Three variations of B-splines based on the inner knot positionings have been proposed. B-splines with a uniform knot vector use equidistant knots and are the most typically applied B-splines [35]. B-splines with an open uniform knot vector also use equidistant knots but they allow analysis of closed curves. Non-uniform B-splines use non-equidistant knots, placed at positions of the researcher’s choice. To our topic, uniform and non-uniform B-splines are the most relevant. Non-uniform B-splines may reflect the a-priori knowledge of a researcher over the underlying complexity of the functional form and/or distribution of the continuous variable.

In Figure A1(b) we show the basis functions of a 2nd degree B-spline with 4 equidistant knots; 2 inner knots plus the boundaries [α, β], placed at values α=0, 0.33, 0.66 and 1=β.

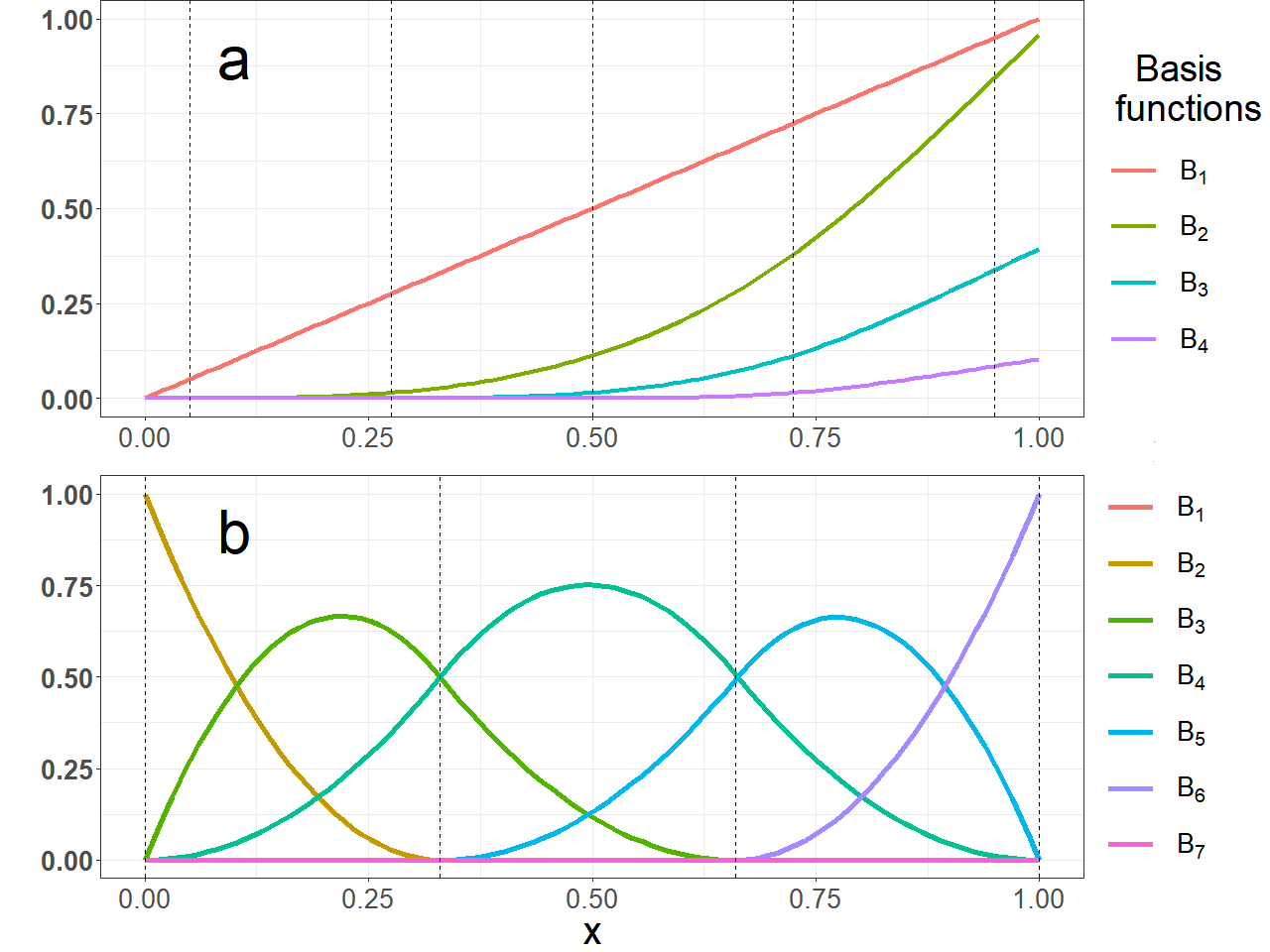


Figure A1. approximately here

# Penalised splines

The two commonly applied penalised splines that we discuss, P-splines and Smoothing splines, increase the number of knots to a large set (usually, 10-40) or even to be equal to the number of observations. This way they circumvent the problem of choosing the number and positions of the knots. Since estimating one parameter for each observation would clearly lead to a perfect fit and thus generate functional shapes with extreme variability, penalised splines introduce in their optimisation functions a penalty term () multiplied by a non-negative , often called a tuning parameter. As the term “tuning” implies, changing the value of changes the magnitude of the penalisation.

In GLMs the estimation of the regression coefficients is accomplished through optimisation of functions of . For Gaussian outcomes the least squares optimisation is estimating the that minimise the squared distance of the predicted and the observed values of the outcome, while for outcomes belonging to the exponential family (Gaussian, Binary, Poisson etc) we estimate the maximising the likelihood function of . Adding a penalty term () results in the following optimisation equations:

* Least squares approach
* Maximum Likelihood approach

Penalised splines circumvent the problem of knot selection, but at a cost. By using a penalty in their optimisation function, they introduce bias in their estimate in order to obtain a more stable solution. Further, in both P-splines and Smoothing splines the tuning parameter must be specified. Too high or too low values of may lead to over- or undersmoothing respectively. Several approaches have been proposed in order to determine the “optimal” , such as Akaike’s information criterion AIC [39], “leave one out” generalised cross-validation (GCV) [40] or mixed-effects modelling [22]. These processes are automated in most of the statistical packages. Briefly, when using the AIC, a series of models fitted with different values is compared and the one with the lowest AIC is selected. “Leave one out” GCV is an iterative process, the algorithm goes as follows: 1) one observation is omitted 2) a model is fitted 3) using the model a prediction of the omitted value is generated and 4) the distance between the observed and predicted value is calculated. This procedure is repeated for each observation and for a series of values. The that minimizes the GCV minimizes the sum of the squared distances, i.e. the GCV score, is selected. In Bayesian/mixed effects modelling approach the penalty term is estimated in a similar way as random effects parameters.

## P-splines

A specific type of penalised splines, P-splines, proposed by Eilers and Marx [21], is a penalised version of B-splines, using a specific penalty term based on the sum of p-order differences between the coefficients of two consecutive intervals . The first order differences are defined as follows: , but Eilers and Marx propose the use of second order differences, which are the first order differences of the first order differences

.

Note that the degree of the underlying B-splines may be different from the order of the differences. A common combination is that of a third-degree B-spline with a second order difference. Using a penalty based on a zero-degree order difference results in the ridge penalty [41]. Note that in some occasions penalised splines and penalised B-splines are misinterpreted as P-splines, but not all penalised B-splines or penalised splines are P-splines. For instance, ordinary B-splines may be fitted using a Smoothing splines approach, but this does not make them P-splines, unless they are penalised using the approach suggested by Eilers and Marx.

P-splines are based on equidistant knots. It is possible to use a knot sequence that is not evenly spaced; but in this case, weights need to be introduced [22, 35]. As P-splines with non-equidistant knots are rarely used in practice we don’t consider them in this article.

## Smoothing splines

Smoothing splinesare another member of the family of penalised spline methods. Similar to P-splines the idea is to increase the number of knots, but this time to be equal or approximately equal to the number of observations. O’ Sullivan [41] suggested that a penalty based on Reinsch’s integral of the second derivative of , where is a cubic spline, multiplied by a tuning parameter, has good smoothing properties. This results in the following penalty term for Smoothing splines: .

## Single study artificial data-set simulation functions

The risk of mortality per participant in the single study data-set was generated using the following formulas:

* For the control group

* For the treated group

Equivalently equations (1) and (2) can also be combined into a single equation:

where would be the association of BMI with mortality risk for the control and the additive effect of the treatment.

## Multiple studies artificial data-set simulation functions

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | True underlying function forms | | BMI Ranges | | | | |
| Scenarios | Control | Treated | 1st Study | 2nd  Study | 3rd  Study | 4th  Study | 5th Study |
| Heteroge-neous data-set with equal BMI ranges |  |  | [18.5,40] | [18.5,40] | [18.5,40] | [18.5,40] | [18.5,40] |
| Non-hetero-geneous data-set with different BMI ranges |  |  | [18.5,27] | [21.2,30.2] | [24.5,33.5] | [27.8,36.7] | [31.40] |
| Combined data-set with different BMI ranges and between study differences in the mortality risks |  |  | [18.5,27] | [21.2,30.2] | [24.5,33.5] | [27.8,36.7] | [31.40] |

The risk of mortality per participant and per study j in the three multiple studies scenarios was generated using the following formulas: