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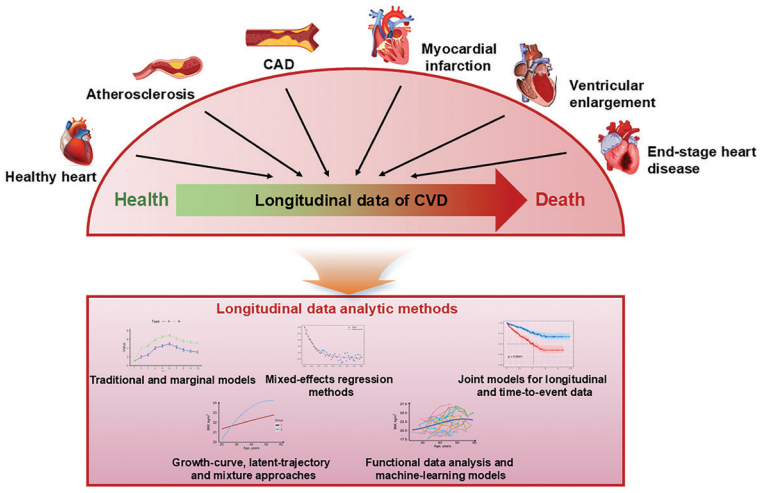
REVIEW

Statistical Methods for Longitudinal Cardiovascular Disease Research Design: A Narrative Review

Graphical Abstract



Highlights

- Longitudinal studies of cardiovascular disease present analytic challenges: repeated measurements, irregular follow-up, missing data, and nonlinear trajectories.
- Five main method classes were reviewed: marginal, mixed-effects, joint, trajectory and mixture, and functional or machine learning approaches.
- For each class, we discuss assumptions, strengths, limitations, sensitivity analyses, and cardiovascular applications.
- Practical guidance covers method selection aligned with questions and data characteristics, validation, reporting, and missing data handling.
- No single method fits all; future needs include developing accessible hybrid models, integrating sparse/dense data, and improving reporting.

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In brief

This narrative review categorizes longitudinal cardiovascular study methods into five classes (marginal, mixed-effects, joint, trajectory/mixture, and functional/ML approaches). It provides practical guidance on selection, validation, missing data handling, and transparent reporting. No single method fits all; the analytic strategy must align with the clinical question and data characteristics. Future developments should focus on accessible hybrid models and improved reporting standards.

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REVIEW

Statistical Methods for Longitudinal Cardiovascular Disease Research Design: A Narrative Review

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Abstract

Cardiovascular disease develops through gradual accumulation of risk factors and progressive vascular damage. Longitudinal studies are well suited to determine when and how these changes occur, but they introduce several analytic challenges, including repeated measurements on the same individuals, irregular or sparse follow-up schedules, missing data, and non-linear trajectories. We conducted a narrative, application-focused review categorizing methods into five major classes: traditional or marginal models, mixed-effect models, joint models, trajectory and mixture models, and functional or machine-learning approaches. For each class, we provide intuitive descriptions, typical cardiovascular applications, and a balanced discussion of assumptions, strengths, limitations, and recommended sensitivity analyses. We emphasize practical guidance for method selection, model validation, and transparent reporting. In summary, no single method addresses every research goal. The analytic strategy should fit both the clinical question and data characteristics, with clear definition of objectives, careful assessment of assumptions, appropriate handling of missing data, and validation on independent samples whenever possible. Future methodological development should focus on making hybrid models more accessible, improving integration of sparse and dense data sources, and advancing reporting standards for longitudinal cardiovascular research.

Keywords: Cardiovascular disease; Longitudinal design; Traditional marginal models; Mixed-effects regression; Joint models; Trajectory analytic methods; Functional and machine-learning approaches

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Introduction

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide: it accounted for nearly 19.2 million deaths in 2023 and represents a substantial proportion of global disability-adjusted life years, particularly in low- and middle-income countries [1]. The etiology of major CVD, including ischemic heart disease, stroke, and heart failure, is multifactorial and develops over decades [2]. This progression reflects the cumulative effects of biological, behavioral, and social exposures; therefore, repeated measurement is essential to understand when and how risk accumulates.

Longitudinal study designs are particularly valuable in cardiovascular research because they allow investigators to track changes within individuals over time. This design enables temporal relationships between risk factors and outcomes to be established, and it supports the analysis of exposure patterns and time-varying effects that cannot be captured in cross-sectional studies [3–5]. When combined with modern analytical methods that handle time-varying confounding, well-executed longitudinal studies can strengthen causal inference and provide more robust evidence linking dynamic risk factors to cardiovascular outcomes [6]. In such settings, time-varying confounders may be influenced by prior exposure and may simultaneously affect subsequent exposure and outcomes, thereby creating feedback mechanisms. These dynamic relationships cannot be adequately addressed by using standard regression models and, if not properly handled, they may yield biased estimates when causal interpretation is intended.

Despite their advantages, longitudinal cardiovascular data pose several analytical challenges. Repeated measurements within the same individual are inherently correlated and therefore violate the assumption of independence. This intra-individual correlation reflects a fundamental statistical feature of longitudinal data. In contrast, time-varying confounding is a source of bias that arises when a covariate is influenced by prior exposure, and subsequently affects future exposure and outcomes. In addition, follow-up schedules are often irregular, and participants may miss visits or withdraw from studies, thus further complicating analysis and potentially introducing missing data issues. Data may also include a combination of continuous variables, counts, and time-to-event outcomes [7–12]. Furthermore, modern cardiovascular research increasingly incorporates high-dimensional and multimodal information, such as repeated imaging, multi-omics biomarkers, and continuous physiological signals from wearable devices. Addressing this complexity requires statistical methods capable of accounting for intra-individual correlation, informative dropout, non-normal data distributions, and dynamic patterns of change. Approaches including mixed-effects and marginal models, joint longitudinal–survival methods, functional data analysis, and contemporary machine-learning techniques have been developed to meet these demands.

Given the clinical importance of CVD and the increasing complexity of cardiovascular data, a clear need exists for practical and accessible guidance on choosing and applying longitudinal analytic methods. This review aims to meet that need by (i) describing the main categories of longitudinal approaches in plain language; (ii) comparing their assumptions, strengths, and limitations for frequently encountered cardiovascular outcomes; and (iii) illustrating method selection with examples from epidemiologic and clinical research. Our goal is to support clinicians and investigators in conducting rigorous, reproducible analyses that generate meaningful insights into cardiovascular health.

Longitudinal Data Analytic Methods

In cardiovascular research, many key indicators, such as risk factors, biomarkers, and clinical outcomes, are repeatedly measured in the same individuals. These longitudinal measurements can help reveal how cardiovascular health changes over time. However, their analysis requires specialized statistical methods, each of which has distinct assumptions, strengths, and appropriate applications. This review summarizes the main families of longitudinal analytic methods used in cardiovascular research. Traditional marginal models, including repeated-measures analysis of variance (RM-ANOVA) and generalized estimating equations (GEEs), are frequently applied to estimate population-level associations. However,

causal interpretation of these estimates requires careful consideration of potential biases, including time-varying confounding, participant selection, and measurement error. In particular, when time-varying confounders are influenced by prior exposure, standard regression approaches may yield biased estimates unless appropriate causal inference methods are used. Mixed-effects regression models extend traditional marginal models by capturing variation within individuals over time, and therefore are suitable for assessing evolving risk profiles and associations with time-varying exposures. Joint longitudinal–time-to-event models link repeated biomarker measurements with survival outcomes. These models account for informative dropout (for example, when patients die or are lost to follow-up because of worsening disease), and they enable dynamic risk prediction. Growth-curve, latent-trajectory, and mixture models identify subgroups of individuals sharing distinct temporal patterns. These methods are valuable for uncovering variation in disease progression and patient subtypes. Finally, functional data analysis and machine-learning methods are designed to process dense or high-frequency data, such as continuous blood pressure (BP) or wearable sensor signals, to extract meaningful features and improve prediction.

Traditional or Marginal Models

Repeated-Measures Analysis of Variance

Analysis of variance, originally developed by R. A. Fisher in the early 20th century, provided a foundation for many modern statistical methods. Its extension to repeated-measures designs, frequently known as RM-ANOVA, emerged in the mid-20th century and was among the earliest tools for analyzing longitudinal data [12–14]. RM-ANOVA is appropriate when the same individuals are assessed at several fixed time points under controlled conditions, such as in clinical trials or laboratory studies. Conceptually, RM-ANOVA decomposes the total variability of the outcome into three components: (i) between-subject variation, reflecting stable differences among individuals; (ii) within-subject (time-related) variation, representing systematic changes over repeated measurements; and (iii) residual error, capturing unexplained variability. By formally comparing these variance components, the method tests whether mean outcome values significantly differ across time points or between treatment groups.

RM-ANOVA performs optimally in balanced study designs, such as those in which all participants are assessed at the same time points, the data are approximately normally distributed, and missing observations are minimal [15]. When the sphericity assumption is mildly violated, corrections such as the Greenhouse–Geisser or Huynh–Feldt adjustments can be applied to modify the degrees of freedom and decrease type I error. Alternatively, a multivariate analysis of variance approach may be used, which does not require sphericity but typically requires larger sample sizes. However, these adjustments address only specific assumption violations but do not resolve more substantial challenges, such as irregular follow-up intervals, time-varying covariates, or non-random missingness. Because these issues are common in real-world cardiovascular research, RM-ANOVA remains most appropriate for balanced experimental designs with fixed measurement schedules rather than long-term observational studies with variable follow-up and participant dropout.

Despite its limitations, RM-ANOVA remains useful in well-controlled experimental or intervention studies using balanced designs and fixed measurement schedules. For example, in a randomized trial assessing the effect of a legume-enriched diet on lipid profiles, glycemic indices, and BP among participants with a family history of diabetes, 26 individuals were randomly assigned to either a legume-enriched diet (n = 13) or a habitual diet (n = 13) for 6 weeks. Because the groups had equal sample sizes and identical follow-up schedules, RM-ANOVA was appropriate for comparing changes within and between groups. Consuming four servings of legumes per week was not found to significantly affect glycemic or lipid profiles but was associated with a modest, borderline significant decrease in both systolic and diastolic BP [16].

Generalized Estimating Equations

GEEs, first introduced by Liang and Zeger and later refined by Prentice, were developed to analyze correlated data from longitudinal or clustered studies at

the population level [17–19]. A GEE estimates the average effect of predictors across all participants rather than modeling individual-specific trajectories. Therefore, instead of evaluating how an exposure influences a particular person's outcome over time, GEE methods assess how the mean outcome changes in response to a treatment or exposure in the overall study population.

GEEs correct for the correlation among repeated measurements within the same individual, thereby preventing the biased results that might occur if the observations were incorrectly treated as independent. However, importantly, adjusting for within-subject correlation alone does not address bias due to time-varying confounding, particularly when confounders are influenced by prior exposure. GEEs are highly flexible and can be applied to a wide range of outcome types, including continuous, binary, and count data; therefore, they are well suited to clinical and public health research. A key advantage of GEEs is that the estimates have clear population-level interpretations, such as quantifying the mean decrease in BP associated with a given intervention. GEEs also perform reliably when data are missing completely at random (MCAR) [20]. However, when data are missing at random (MAR) or missing not at random, the resulting estimates may be inefficient or biased.

GEEs are widely used in cardiovascular research, particularly in randomized trials and studies with repeated measurements over time. For example, the China Rural Hypertension Control Project, analyzed by Wang and colleagues, evaluated whether intensive BP management might decrease cardiovascular events in patients with isolated systolic hypertension. Villages were randomly assigned in a 1:1 ratio to either an intervention or usual care. In the intervention group, trained non-physician community health providers implemented comprehensive BP management targeting a BP <130/80 mmHg, whereas the usual-care group continued with standard treatment. Participants were assessed every 6 months over 36 months. Because the study used cluster randomization at the village level, the data exhibited within-cluster correlation. Therefore, a GEE was used to estimate treatment effects. After 36 months, significantly lower systolic BP (by 21.5 mmHg; 95% CI: 20.6–22.3; $P < 0.001$) and diastolic BP (by 7.6 mmHg; 95% CI: 7.0–8.1; $P < 0.001$) were observed in the intervention group than the control group, thus demonstrating the effectiveness of the intensive management strategy [21].

Mixed-Effects Regression Methods

Linear Mixed-Effects Model

The linear mixed-effects model (LMM), also known as a random-effects model, is widely used for analyzing continuous repeated measures. First formalized for longitudinal data by Laird and Ware, LMM extends traditional linear regression by accounting for correlations among repeated observations within the same subject, and allowing baseline values and rates of change to vary across participants [22]. This flexibility makes LMM well suited to examining both population-level trends and individual trajectories over time.

Conceptually, an LMM separates two sources of information:

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{\gamma} + \boldsymbol{\varepsilon},$$

where $\boldsymbol{y}$ is an outcome vector, $\mathbf{X}$ is the design matrix of observed variables, $\boldsymbol{\beta}$ is the vector of fixed-effects parameters (population-average effects), $\mathbf{Z}$ is the design matrix of observed variables, $\boldsymbol{\gamma}$ is the vector of random-effects parameters (subject-specific deviations), and $\boldsymbol{\varepsilon} \sim N(0, 1)$. Fixed effects $\boldsymbol{\beta}$ represents the overall population trend (e.g., the average rate of BP increase with age), and random effects $\boldsymbol{\gamma}$ captures how each subject's trajectory deviates from that average (e.g., one patient's BP rises faster than another's). By including both, the model can estimate how exposures influence outcomes while also tracking subject-specific trajectories.

LMM is a flexible and practical approach for analyzing longitudinal data. It can accommodate unbalanced designs, irregular follow-up schedules, and missing data under a MAR mechanism. However, LMM accuracy depends on correctly specifying how individual differences are modeled and the assumed outcome distribution, because misspecification can lead to biased estimates.

In cardiovascular research, LMM is frequently used to evaluate the effects of interventions on outcomes and to examine the relationships between risk factors and clinical outcomes in repeated-measures

settings. For example, Li and colleagues have investigated the association between hypertension and semen quality among 1,381 young men from the general population [23]. Semen samples were collected in a private setting at baseline and across four follow-up intervals: 1–15 days, 16–31 days, 32–63 days, and 64–90 days. Because of missing measurements during follow-up, LMM was used instead of RM-ANOVA to estimate the associations between hypertension and repeated sperm quality parameters. Hypertension, particularly stage 2 hypertension, was associated with reduced sperm morphology.

A key limitation of LMM is its assumption that outcomes are continuous, and residual errors and random effects follow approximately normal distributions. When outcomes are binary (e.g., presence or absence of arrhythmia) or counts (e.g., number of hospitalizations), a more flexible approach, such as the generalized linear mixed model (GLMM), is needed. Developed by Breslow and Clayton, GLMM extends the mixed-effects framework to accommodate non-normal outcomes [24]. Like LMM, it separates population-level effects from individual-level variation, but introduces link functions, such as the logit for binary outcomes or the log for count data, to model different types of outcomes. This flexibility enables GLMM to handle the continuous, binary, categorical, and count data frequently observed in cardiovascular research [25]. When the outcome is continuous, the GLMM reduces to the LMM; therefore, the LMM is a special case of the GLMM.

GLMM is widely used in cardiovascular research to model event counts, binary outcomes, and other non-normal longitudinal data. For example, Bergeman et al. have conducted a multicenter study in patients with catecholaminergic polymorphic ventricular tachycardia to evaluate whether adding flecainide to beta-blocker therapy might decrease arrhythmic events. Because the outcome of interest, the number of arrhythmic episodes, was a count variable, the investigators applied a GLMM with a negative binomial distribution and patient-level random effects. This approach accounted for individual variability and demonstrated that flecainide was associated with a significant decrease in arrhythmic events across the cohort [26].

Nonlinear Mixed-Effects Model

Certain cardiovascular processes exhibit nonlinear rather than linear trajectories. For example, BP fluctuates throughout the day, and recovery of left ventricular function after an intervention often follows a curved trend rather than a steady increase or decrease. The nonlinear mixed-effects model (NLMEM), specifically designed for these situations, allows researchers to fit biologically plausible curves that better capture the underlying dynamics. Detailed methods have been described by Davidian and Giltinan, and practical guidance has been provided by Pinheiro and Bates [27, 28].

NLMEM extends the linear mixed-effects framework by modeling outcomes as a combination of population-level parameters and individual deviations, while using nonlinear functions, such as logistic growth or exponential decay, to represent patterns of recovery, growth, or cyclic fluctuations. Compared with GLMM, NLMEM requires greater computational resources and is more sensitive to the initial assumptions during model estimation [29].

In cardiovascular research, NLMEM is particularly well suited to modeling physiological processes with nonlinear trajectories. For example, Van Rijn-Bikker and colleagues have applied NLMEM to characterize 24-hour systolic BP (SBP) patterns by estimating parameters that directly reflect daytime and nighttime SBP levels. Because BP exhibits a nonlinear diurnal pattern, NLMEM provided an appropriate framework to describe 24-hour SBP patterns. This approach captured the curved pattern of SBP changes and quantified both typical population values and interindividual differences [30]. This example illustrates how NLMEM can describe both overall population trends and important individual-level variability in complex cardiovascular processes.

Joint Models for Longitudinal and Time-to-Event Data

In cardiovascular research, investigators are often interested not only in how a marker changes over time but also in how these changes affect the risk of a clinical event. For example, researchers may seek to understand how temporal changes in BP influence the risk of myocardial infarction.

However, separate analyses of longitudinal and time-to-event data present important methodological challenges. First, repeated biomarker measurements are subject to measurement error. Conventional time-dependent Cox models typically treat observed biomarker values as error-free, thus potentially attenuating effect estimates and bias inference regarding the true association between the biomarker and clinical risk. Second, dropout is frequently associated with the outcome process. Patients with rapidly worsening biomarker profiles may experience earlier cardiovascular events and consequently discontinue follow-up. In such settings, the probability of missing data depends on the underlying unobserved biomarker trajectory, thus leading to informative dropout and violation of the missing-at-random assumption.

Joint models have been developed to address these challenges within a unified framework that simultaneously characterizes biomarker evolution and event risk. By modeling the longitudinal and survival processes together, joint models appropriately account for measurement error and outcome-related dropout, thereby decreasing bias and increasing estimation efficiency.

Key early formulations included Wulfsohn and Tsiatis (1997), and comprehensive treatments have been described by Rizopoulos [30, 31]. A typical shared-random-effects joint model combines two submodels: a longitudinal model for the latent marker trajectory $m_i(t)$, and a survival model for the event hazard $h_i(t)$.

$$\begin{cases} \text{Longitudinal}: y_i(t) = m_i(t) + \varepsilon_i(t), m_i(t) = \mathbf{X}_i\ (t)^T \beta + \mathbf{Z}_i(t)^T \boldsymbol{b}_i \\ \text{Survival:} \qquad\qquad h_i(t \mid b_i) = h_0(t) exp\left(\gamma^T \boldsymbol{\omega}_i + \alpha \mathcal{A}\left\{m_i(t)\right\}\right) \end{cases}$$

where $y_i(t)$ denotes the observed longitudinal measurement for subject i at time t; $m_i(t)$ is the latent marker trajectory; $\mathbf{X}_i(t)$ denotes the design vector for fixed effects, representing population-level covariates and time functions; β is the corresponding regression coefficient; $\mathbf{Z}_i(t)$ denotes the design vector for random effects; $\boldsymbol{b}_i$ represents subject-specific random effects, capturing individual deviations from the population-average trajectory, $\varepsilon_i\ (t) \sim N(0, \sigma^2)$, which is independent of $\boldsymbol{b}_i$; $h_i(t)$ is the hazard function for subject i at time t; $h_0(t)$ is an unspecified baseline hazard function; γ is the regression coefficients for baseline covariates; ω_i is vector of baseline covariates; $\mathcal{A}\left\{m_i(t)\right\}$ denotes the association function linking the longitudinal process to survival risk; and α is the association parameter quantifying the strength of the biomarker–event relationship.

A typical shared-random-effects joint model consists of two interconnected components: a longitudinal submodel for the latent marker trajectory and a survival submodel for the event hazard. The longitudinal submodel characterizes the trajectory of the repeated marker over time, whereas the survival submodel relates that trajectory to the instantaneous risk of the clinical event. These submodels are linked through shared subject-specific random effects, thus allowing individual deviations in a patient's biomarker profile to directly influence their event risk.

A critical consideration in joint modeling is how the longitudinal marker is associated with event risk. Three common strategies are typically used. First, the current value approach links the hazard at a given time to the patient's biomarker level at that time. For example, elevated troponin may indicate elevated short-term risk of heart failure hospitalization. This approach is intuitive and robust when the marker directly influences immediate risk. Second, the slope approach links risk to the rate of change in the biomarker rather than its absolute level. Moreover, it can capture dynamic processes, such as a rapid rise in B-type natriuretic peptide signaling impending decompensation. Accurate slope estimation requires frequent or reliable measurements. Third, the cumulative exposure approach, often quantified as the area under the curve (AUC), links risk to the overall burden of exposure over time. For example, elevated BP for decades may increase cardiovascular risk even if an individual's current measurement is normal. This approach reflects long-term accumulation but requires comprehensive longitudinal data.

In practice, selecting the appropriate joint-model link depends on three key factors: the biological question, the data structure, and model complexity. The biological question determines whether interest lies in the biomarker's current level, its rate of change, or cumulative exposure. Sparse or irregular measurements may limit reliable estimation of slopes or AUC. Adding multiple link functions can provide richer insights but increases computational

and estimation challenges. Researchers typically evaluate candidate models by using fit statistics, predictive accuracy, and clinical interpretability. Detailed guidance for choosing the appropriate association is summarized in Table 1.

When properly specified, joint models provide more reliable and clinically meaningful estimates than separate analyses. Their principal strength lies in linking a patient's evolving biomarker trajectory with subsequent event risk within a coherent probabilistic framework, thereby reflecting the dynamic nature of cardiovascular disease progression.

To better appreciate this advantage, we compare joint models with frequently used alternative approaches. In cardiovascular research, associations between repeated biomarkers and time-to-event outcomes can also be analyzed with alternative approaches, most frequently time-dependent Cox regression models or two-stage methods based on parameters derived from longitudinal models. In a time-dependent Cox model, observed biomarker values are included as external time-varying covariates in the hazard function. Although straightforward to implement, this approach treats observed measurements as error-free and does not explicitly account for within-subject measurement error or informative dropout. Consequently, regression dilution bias may occur when biomarkers are measured with variability or irregular timing [32–34].

Another common strategy is a two-stage approach, in which a linear mixed-effects model is first fitted to estimate subject-specific parameters (such as baseline level or slope), and these estimated quantities are subsequently entered into a Cox regression model. However, this method does not fully propagate uncertainty from the first-stage estimation into the survival model, and it may potentially lead to biased standard errors and attenuated effect estimates [31, 35].

In contrast, joint longitudinal–survival models simultaneously estimate the longitudinal trajectory and the survival process within a unified framework. By linking the two submodels through shared random effects or association structures (e.g., current value, slope, or cumulative exposure), joint models explicitly account for measurement error, informative dropout, and dynamic risk prediction [36]. This integrated estimation improves inferential validity when biomarker evolution and event risk are intrinsically related. Therefore, whereas time-dependent Cox and two-stage methods may be adequate when measurement error is negligible and dropout is noninformative, joint models are generally preferable when repeated biomarker processes and survival outcomes are strongly interdependent.

An example illustrating cumulative exposure modeling comes from research on low-density lipoprotein cholesterol (LDL-C). Cumulative exposure to LDL-C, quantified as the long-term AUC, has

Table 1 Summary of Preferred Links in CVD Studies.

Association functions	When preferred	Data requirements	Typical CVD examples	Notes/limitations
Current value	Instantaneous marker level is hypothesized to drive short-term risk	Repeated measures (irregular)	NT-proBNP level → HF hospitalization; current BP → stroke	Robust with sparse data; ignoring dynamic or cumulative effects
Current slope	Rapid change (deterioration/improvement) triggers events	Requiring 5–10 observations per participant or strong parametric/smoothing assumptions	Rapid BNP rise → imminent decompensation; sharp BP increases → acute events	Rate of change potentially unstable with sparse data; sensitive to smoothing
AUC/cumulative exposure	Risk increases with accumulated burden over time	Long-term follow-up with repeated measures	Cumulative systolic BP → CHD/stroke; cumulative LDL → atherosclerosis	AUC estimation sensitive to sparse or irregular measurements

Note: AUC, area under the curve; BNP, brain natriuretic peptide; BP, blood pressure; CHD, coronary heart disease; CVD, cardiovascular disease; HF, heart failure; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro brain natriuretic peptide.

been shown to predict cardiovascular events more effectively than single-time-point measurements, thus supporting the accumulation model of disease development. Using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study, Domanski and colleagues have evaluated the association between the area under the LDL-C–age curve and incident CVD events [37]. A mixed-effects model incorporating cubic splines was applied to estimate subject-specific LDL-C trajectories between the ages of 18 and 40 years. The area under the LDL-C–age curve over this period [AUC(18–40 y)] was calculated to represent cumulative LDL-C exposure and expressed in mg/dL × years. Subsequently, multivariable Cox proportional hazards regression models were further used to assess the relationship of cumulative LDL-C exposure to CVD events after the age of 40 years. The findings demonstrated that greater cumulative LDL-C exposure during early adulthood was significantly associated with a higher risk of subsequent CVD events ($P < 0.001$).

Growth-Curve, Latent-Trajectory, and Mixture Approaches

This group of methods is designed to examine changes in individuals over time and how these patterns relate to cardiovascular outcomes. Latent growth models summarize each subject's trajectory by using a small set of key parameters, such as baseline level and rate of change. In contrast, mixture and trajectory models identify subgroups of individuals who follow distinct patterns, for example, those with persistently low versus steadily increasing BP. Collectively, these approaches can reveal whether cardiovascular risk develops gradually, accelerates during specific life stages, or varies across hidden subpopulations. Table 2 summarizes their main features, data requirements, and typical applications.

Latent Growth Curve Model

The latent growth curve model (LGCM), introduced by Meredith and Tisak (1990), provides a framework for summarizing individual trajectories by using structural equation modeling [38]. Instead of analyzing each repeated measurement separately, LGCM models change over time by specifying latent variables called growth factors, typically including an intercept, representing baseline level, and a slope, representing rate of change. These growth factors capture the average trajectory across individuals and can subsequently be associated with later cardiovascular outcomes [39].

LGCM is based on an assumption that all individuals originate from a single population and share a common trajectory shape, while still allowing for individual deviations through random effects. This approach performs best when measurement times are similar across participants, and the data approximate a normal distribution. LGCM offers a concise summary of both average and individual patterns of change, but requires specialized software and can be difficult to apply when follow-up schedules are highly irregular.

In cardiovascular research, LGCM is frequently used to examine how risk factors change over time and how these trajectories relate to subsequent outcomes. For example, Pai and colleagues have applied LGCM to study changes in activities of daily living (ADL) at 1, 3, 6, and 12 months after stroke [40]. The authors selected LGCM because their primary goal was to model the average recovery trajectory rather than to identify distinct subgroups. Using this framework, they further examined associations between these trajectories and the National Institutes of Health Stroke Scale (NIHSS) score within 24 hours after stroke onset. Lower NIHSS scores were associated with higher initial ADL levels and predicted a faster decline over time. This finding underscores the importance of including early NIHSS scores as predictors in stroke rehabilitation planning to support ADL recovery.

Latent Class Growth Model

The latent class growth model (LCGM), also known as the group-based trajectory model, was introduced by Nagin (1999) and later expanded in fields such as criminology and epidemiology. LCGM is a finite mixture modeling approach designed to identify groups of individuals who share similar patterns of change over time [41, 42]. The model is based on an assumption that the population consists of several distinct trajectory groups, each defined by a specific functional form of change, such as linear

Table 2 Detailed Summaries of Growth-curve, Latent-trajectory, and Mixture Approaches.

Method	Data format requirements	Latent classes (grouping)	Random effects included	Recommended use
LGCM	Wide-format data typically required; multilevel modeling can use long-format; measurement time points should be aligned	No	Yes (random intercept/slope)	If the research question assumes a single developmental trajectory shared by all participants, LGCM is the most appropriate choice.
LCGM	Wide-format data; measurement time points should be aligned	Yes	No (no random effects within group)	If the goal is to explore or describe broad subgroups of change patterns, and the data are regular, LCGM is suitable.
LGMM	Wide-format (e.g., Mplus, SEM framework) or long-format (e.g., mixed models in R) data, depending on software; measurement time points often aligned	Yes	Yes (random intercept/slope within group)	If investigators expect multiple latent trajectory subgroups with meaningful within-subgroup variability and regularly spaced data, LGMM offers an appropriate balance between flexibility and interpretability.
LCMM	Long-format data required; accommodates irregular measurement time points	Yes	Yes (random intercept/slope within group)	LCMM is the preferred choice if investigators expect multiple latent trajectory subgroups with meaningful within-subgroup variability, particularly for irregular data, nonlinear time trends, or multiple outcome types.

Note: LCGM, latent class growth model; LCMM, latent class mixed model; LGCM, latent growth curve model; LGMM, latent growth mixture model; SEM, structural equation model.

or quadratic. Unlike LGCM, LCGM does not allow for within-group variability, and all members of a trajectory group are assumed to follow the same pattern. This simplifying assumption enhances computational efficiency and interpretability, and is particularly useful for exploratory analyses aimed at identifying a small number of typical patterns. However, because the absence of within-group variation can oversimplify reality, trajectory groups should be interpreted as descriptive rather than definitive biological categories. LCGM estimation is straightforward with dedicated software (e.g., PROC TRAJ in SAS), and model selection typically combines statistical criteria with clinical plausibility. This method is particularly suitable for describing a limited number of developmental patterns and examining their associations with subsequent outcomes, such as long-term treatment adherence or risk-factor evolution.

In cardiometabolic research, LCGM has been applied to study longitudinal patterns of BP and lipids. For example, Liu and colleagues have used data from the China Health and Nutrition Survey to investigate the relationship between SBP trajectories and incident diabetes risk [43]. The authors assumed that the population could be divided into distinct SBP trajectory groups, and subsequently applied LCGM to identify them. Four trajectories were found: normotensive-stable, prehypertension-stable, stage I hypertension-increasing, and stage II hypertension-increasing. Participants in the rising hypertension groups had substantially higher risks of developing diabetes than those who remained normotensive, thus highlighting the value of trajectory-based risk profiling for identifying high-risk individuals.

Latent Growth Mixture Model

The latent growth mixture model (LGMM), also called the growth mixture model, integrates the strengths of LGCM and LCGM. Developed by Muthén and colleagues in the late 1990s and building on Nagin's work on group-based trajectories, LGMM allows for multiple latent trajectory classes, similarly to LCGM, but also accommodates within-group variability via random effects [41, 44, 45]. Therefore, distinct subgroup trajectories and individual deviations within those subgroups can be

simultaneously modeled. LGMM identifies latent subgroups, such as "stable-low," "increasing," or "high-declining," each of which has a characteristic trajectory. This method is particularly useful when risk factor patterns vary across individuals rather than following a single trend. A key limitation is that LGMM generally requires measurement times to be regularly aligned across participants, thus limiting flexibility for irregular or unbalanced longitudinal designs.

LGMM has been widely applied to cardiovascular risk factors, including BP and lipid trajectories. For example, Tielemans and colleagues have analyzed data from two prospective cohorts of middle-aged men to characterize 10-year BP trajectories and their association with cardiovascular mortality [46]. By assuming multiple latent trajectory groups and allowing for variability within each group, the authors used LGMM but not LCGM to identify distinct BP trajectories. Subsequently, a Cox proportional hazards model was applied to assess the relationship between these trajectories and cardiovascular mortality. Certain elevated trajectories were associated with a two- to four-fold elevated risk of cardiovascular death, thereby demonstrating that both absolute BP and the pattern of change over time are strong predictors of cardiovascular outcomes.

Latent Class Mixed Model

The latent class mixed model (LCMM), also known as the latent class growth mixed model, the most flexible and general approach within this family, integrates the strengths of mixture modeling with the flexibility of mixed-effects models. LCMM extends LGMM by incorporating group-specific fixed effects, flexible random-effects structures, and a variety of time functions, including linear, polynomial, spline, or nonlinear forms [47]. Unlike LGMM, LCMM can accommodate irregularly spaced measurements and multiple outcome types, such as continuous, binary, and count data. Because of this flexibility, LCMM can be considered a unifying framework: LGCM, LCGM, and LGMM are all special or restricted cases of LCMM, differing only in the assumptions that they impose. However, this flexibility comes with increased computational demands and requires careful attention to model selection, convergence, and interpretability.

LCMM has been applied in cardiovascular research to identify subgroups of individuals with distinct BMI or BP trajectories, and to examine their associations with hypertension and other cardiovascular outcomes. For example, Fan and colleagues have used LCMM to characterize longitudinal BMI trajectories during young adulthood (20–40 years of age) in a Chinese cohort, in which BMI was measured 4 to 11 times between 2004 and 2015 [48]. Because the data contained sparse and irregular measurements, and the investigators assumed several distinct BMI trajectory groups while allowing within-group variability, LCMM, but not LCGM or LGMM, was considered the most appropriate method for this analysis. Using this approach, the investigators identified four distinct BMI trajectory patterns: low-stable, moderate-increasing, high-increasing, and sharp-increasing. Cox regression analysis demonstrated that these distinct BMI trajectories significantly influenced the risk of developing hypertension, and the period between 20 and 30 years of age emerged as a critical window for incident hypertension. This study illustrates how LCMM can capture diverse developmental patterns and their long-term cardiovascular implications.

Functional and Machine-Learning Approaches

With the increasing use of wearable devices, continuous monitoring technologies, and large-scale cohort studies, cardiovascular research now frequently generates high-frequency, long-term, and complex longitudinal data. Because traditional statistical methods often struggle to capture the detailed temporal dynamics and nonlinear patterns present in such datasets, more advanced analytic approaches are needed.

Among advanced approaches, functional data analysis (FDA) offers a flexible framework by treating each individual's longitudinal measurements as smooth curves, thereby enabling direct characterization and comparison of key temporal features. Moreover, machine-learning and deep-learning sequence models can automatically capture complex temporal patterns from large high-dimensional datasets, and therefore are

particularly valuable for risk prediction and pattern recognition.

Each approach offers distinct advantages: FDA emphasizes interpretability and explicit modeling of temporal smooth curves, whereas deep learning methods excel in predictive performance and automated feature extraction. Regardless of the method chosen, aligning the analytic strategy with the underlying data structure and the specific research objectives is essential, while ensuring rigorous validation and transparent reporting to produce reliable and clinically meaningful insights.

Functional Data Analysis

FDA treats each individual's repeated measurements as a continuous and smooth curve rather than isolated time points, according to a framework established in foundational monographs by Ramsay and Silverman (1997, 2005) [49, 50]. This approach is particularly well suited to dense, high-frequency data, such as 24-hour ambulatory BP monitoring, continuous electrocardiography, or accelerometer-based activity records [51]. Although FDA can also be applied to sparse or irregular data, it performs best when measurements are frequent and regularly spaced. By modeling smooth curves, FDA enables the investigation of full-day temporal patterns, identification of salient features, and extraction of summary measures that would be missed by simple visit-to-visit comparisons [52]. However, because results may be sensitive to choices in smoothing and curve alignment, methodological transparency is critical.

In cardiovascular research, FDA has been used to analyze entire temporal profiles rather than relying on single-time-point averages, thereby capturing the full shape of risk or behavioral trajectories. For example, Wrobel and colleagues have applied functional regression to 24-hour accelerometry-derived activity curves from nearly 90,000 adults in the UK Biobank, despite the sparse and irregular nature of accelerometry data [53]. Daily physical activity patterns were found to substantially change with age and to differ between men and women. These findings demonstrate how FDA can reveal nuanced temporal patterns that may inform prevention strategies.

Machine-Learning Sequence Models

Machine-learning methods provide a powerful approach for analyzing longitudinal data, particularly when sequences are long, nonlinear, and high-dimensional. Two frequently used architectures are long short-term memory networks [54] and attention-based models such as the Transformer [55]. Long short-term memory networks capture long-range temporal dependencies by using a memory mechanism, whereas Transformers leverage self-attention to identify relationships between any pair of time points and can be trained in parallel on very large datasets.

These models are particularly effective for continuous physiological signals, such as wearable sensor data, electrocardiographic recordings, medical imaging sequences, or intensive-care vital signs [56]. They can be used to predict future observations, generate risk scores, and classify clinical outcomes. Consequently, these models are oriented primarily toward prediction and should not be interpreted as causal models without additional methodological assumptions and careful study design. However, deep-learning approaches require large training datasets, substantial computational resources, and careful adjustment of model settings. A major limitation is interpretability: unlike traditional statistical models, deep networks often operate as "black boxes." Therefore, rigorous validation, calibration, and explainability are required to ensure clinical applicability.

In cardiovascular research, machine-learning sequence models are particularly valuable for prediction. For example, cardiac surgery-associated acute kidney injury is a common postoperative complication that contributes to both morbidity and mortality. Traditional risk-prediction models may struggle to capture complex nonlinear associations and fully integrate intraoperative and early postoperative variables. Machine-learning approaches address this gap by modeling high-dimensional temporal patterns. For example, Zheng has applied a machine-learning model by using comprehensive perioperative data to predict cardiac surgery-associated acute kidney injury. A gradient boosting decision tree achieved the highest performance (AUC = 0.87), thus demonstrating its utility for postoperative risk estimation. A recent review by Zhou and

colleagues has further emphasized that machine-learning methods can generate real-world evidence to support clinical decision-making and complement traditional statistical approaches.

Comparison of Methods

This section summarizes the major analytic approaches for longitudinal data, and compares their data requirements, interpretability, and suitability for cardiovascular research. Collectively, these methods offer a flexible and comprehensive toolkit for addressing a wide range of clinical and epidemiologic questions. Table 3 provides an overview of key methodological features and applications in cardiovascular studies, and Table 4 summarizes frequently used software implementations for longitudinal analyses.

Marginal models, such as RM-ANOVA and GEE, are well suited to estimating population-level effects when measurements are collected at regular time points [57]. They are computationally efficient and straightforward to interpret, and therefore are particularly useful in short-term trials or studies with balanced follow-up. However, they are less well suited to individual-specific inference, and they may yield biased estimates when missingness is not completely random.

Linear and generalized linear mixed-effects models offer enhanced flexibility when follow-up is irregular, or outcomes are of different types [27, 58, 59]. Because these models account for inter-individual variability and can accommodate realistic missing-data mechanisms, they are well suited to long-term observational studies. However, their validity depends on correctly specifying the random-effects structure and verifying model assumptions. NLMEM further extends this framework to nonlinear physiological processes, such as diurnal BP rhythms or post-intervention recovery curves. When the underlying biological process is appropriately modeled, these models yield parameters with direct clinical relevance, although with high computational cost, and enhanced sensitivity to starting values and model specification.

Joint longitudinal–time-to-event models address the related challenge of linking dynamic biomarker trajectories to clinical outcomes. These models estimate how an individual's evolving biomarker profile affects event risk while appropriately handling measurement error and informative dropout [31, 60]. Their performance depends on accurate selection of the biomarker–risk association structure, guided by both biological knowledge and data density.

Trajectory and mixture models identify latent subgroups with distinct longitudinal patterns. These approaches are valuable for identifying meaningful differences in risk-factor development or disease progression across individuals [38, 41, 42, 61–63]. Nevertheless, class enumeration and interpretation are sensitive to modeling choices, and rigorous validation is needed to avoid overinterpretation.

Finally, FDA and machine-learning sequence models are particularly advantageous for dense, high-frequency data, such as continuous electrocardiography or 24-hour BP monitoring. FDA characterizes entire trajectories as smooth functions, whereas machine-learning approaches automatically extract complex temporal features for prediction [49, 50, 52]. FDA and machine-learning approaches require substantial datasets and careful validation, and machine-learning approaches particularly require attention to transparency and explainability for clinical applicability.

Model Diagnostics and Goodness-of-Fit Evaluation

Appropriate model selection in longitudinal cardiovascular research should be accompanied by method-specific diagnostic evaluation to ensure valid inference and adequate model fit. Because different analytic frameworks rely on distinct assumptions, diagnostic strategies must be tailored accordingly.

For RM-ANOVA, model adequacy depends on normality, homogeneity of variance, and the sphericity assumption, which can be assessed by using residual diagnostics and Mauchly's test, with Greenhouse–Geisser or Huynh–Feldt corrections applied when needed [64, 65]. In GEE, which is based on quasi-likelihood estimation, model comparison typically relies on the quasi-likelihood under the independence model criterion, together with evaluation of residual patterns, working correlation structures, and overdispersion for count outcomes [66, 67].

Table 3 Comparison of Major Longitudinal Analytic Methods Frequently Used in Cardiovascular Research.

Analytical method	Key research question	Methodological role	Interpretation of key results	Data structure	Assumptions	Strengths	Limitations
RM-ANOVA	Do mean outcomes differ across fixed time points or treatment groups under controlled conditions?	Decomposes total variance into inter-individual, intra-individual, and residual components; tests mean differences in balanced repeated designs	Estimated mean differences reflect population-average change under strict design assumptions	Balanced, fixed-visit trials	Sphericity; balanced and fixed measurement schedule; normally distributed residuals; minimal missing data	Simple, interpretable	Sensitive to violations of sphericity; limited handling of missing data and irregular follow-up, and time-varying covariates
GEE	What is the average effect of an exposure on repeated outcomes at the population level?	Estimates population-average effects while accounting for intra-individual correlation via working correlation structures	Regression coefficients represent average change in outcome associated with predictors	Longitudinal or clustered data	Correct marginal mean specification; working correlation structure; MCAR	Direct population-averaged effects; robust to working-correlation misspecification; computationally efficient	No individual-specific predictions; sensitive to missingness mechanisms and informative dropout
Mixed-effects models	How do individual trajectories evolve over time, and how do predictors influence individual-specific change?	Models fixed and random effects; accommodates unbalanced data and irregular follow-up	Fixed effects indicate population trends; random effects quantify inter-individual variability	Irregular repeated continuous outcomes	Random effects normally distributed; MAR	Handles unbalanced data, random slopes; interpretable population and participant effects	Sensitive to random-effects misspecification; conditional normal distribution
Joint longitudinal–survival models	How are evolving biomarkers associated with time-to-event risk?	Simultaneously models longitudinal trajectory and hazard process; accounts for measurement error and informative dropout	Association parameters quantify the effect of current value, slope, or cumulative exposure on event risk	Longitudinal biomarkers + time-to-event data	Correct longitudinal submodel; correct association function	Accounts for measurement error and informative dropout; coherent hazard estimates	Complex specification; computationally intensive; sensitive to misspecification of association function
Latent growth/ mixture/ trajectory models	Are subgroups distinct, with heterogeneous longitudinal patterns?	Identifies latent classes according to trajectory shape; may include within-class variability, depending on specification	Class-specific trajectories describe subgroup patterns; posterior probabilities indicate classification uncertainty	Trajectory subgrouping over time	Finite class structure; class identifiability	Identifies distinct trajectory subgroups	Class number subjective; classes may be statistical artifacts
FDA	What features characterize densely sampled longitudinal signals?	Represents trajectories as smooth functions; extracts functional components via basis expansion or FPCA	Functional coefficients summarize dominant temporal patterns across individuals	Dense, continuous curves (24-h ABPM, wearable streams)	Smoothness and basis representation; dense sampling preferred	Uses whole-curve information; functional PCA for dimension reduction	Sensitive to choices for smoothing and curve alignment; complex model and poor interpretability

Table 3 (Continued)

Analytical method	Key research question	Methodological role	Interpretation of key results	Data structure	Assumptions	Strengths	Limitations
Machine-learning sequence models	Can complex longitudinal or multimodal data improve prediction?	Learns nonlinear temporal patterns from high-dimensional sequences; optimized for predictive accuracy	Performance metrics evaluate predictive utility rather than causal effects	High-frequency multimodal time series	Large labeled datasets; careful preprocessing	Powerful nonlinear modeling; handles high-dimensional inputs and long-range dependencies	Data-hungry; black-box; computationally intensive; limited causal interpretability

Note: ABPM, ambulatory blood pressure monitoring; AF, atrial fibrillation; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; ECG, electrocardiogram; EHR, electronic health record; FDA, functional data analysis; FPCA, functional principal component analysis; GEE, generalized estimating equation; GLMM, generalized linear mixed model; HF, heart failure; LDL, low-density lipoprotein; LMM; linear mixed-effects model; LVEF, left ventricular ejection fraction; MAR; missing at random; MCAR, missing completely at random; NLMEM, nonlinear mixed-effect model; NT-proBNP, N-terminal pro brain natriuretic peptide; PCA, principal component analysis; RM-ANOVA, repeated-measures analysis of variance; SBP, systolic blood pressure.

For mixed-effects models, including linear and generalized forms, residual-versus-fitted plots, quantile–quantile plots, and assessment of random-effects distributions are frequently used. Marginal and conditional R^2 statistics quantify explained variance, whereas information criteria such as the Akaike information criterion and Bayesian information criterion guide model comparison [27, 67]. Overdispersion diagnostics and link-function adequacy are particularly relevant for generalized linear mixed models. Nonlinear mixed-effects models additionally require evaluation of convergence, parameter identifiability, and visual predictive checks [29].

In joint longitudinal–survival models, diagnostics should address both submodels, including residual assessment for the longitudinal component, proportional hazards evaluation for the survival component, and predictive performance metrics such as time-dependent discrimination and prediction error, supported by likelihood-based criteria [36, 68]. Latent trajectory and mixture models rely on the Akaike information criterion, Bayesian information criterion, entropy, and posterior class probabilities to determine optimal class structure, and clinical interpretability remains essential [69]. In FDA, cross-validation and explained functional variance guide smoothing and model adequacy [52]. For machine-learning approaches, evaluation emphasizes cross-validation, discrimination and calibration metrics, prediction error measures, and assessment of overfitting [70, 71].

Discussion

This review highlights both the opportunities and limitations of contemporary longitudinal analytic methods in cardiovascular research. Each class of methods poses unique challenges yet also provides distinct advantages when applied in appropriate settings. A clear understanding of these trade-offs is critical for selecting analytic strategies that yield valid, interpretable, and clinically meaningful results.

Methodological Challenges

Multiple recurring challenges influence the validity and interpretability of longitudinal analyses in

Table 4 Summary of Software Implementation for Longitudinal Methods in Cardiovascular Research.

Statistical methods	Common software	Example implementation command
Repeated-measures ANOVA	SAS, SPSS, R	SAS: PROC GLM; REPEATED time; SPSS: Analyze → General Linear Model → Repeated Measures R: aov()
Generalized estimating equations	SAS, STATA, R	SAS: PROC GENMOD; REPEATED SUBJECT=ID; STATA: xtgee R: geepack::geeglm()
Linear mixed-effects model	SAS, STATA, R	SAS: PROC MIXED; STATA: mixed R: lme4::lmer()
Generalized linear mixed model	SAS, STATA, R	SAS: PROC GLIMMIX; STATA: melogit R: lme4::glmer()
Nonlinear mixed-effects model	SAS, STATA, R	SAS: PROC NLMIXED; STATA: menl R: nlme::nlme(), saemix package
Joint models for longitudinal and time-to-event data	R, STATA	R: JM, JMbayes2 packages STATA: stjm
Latent growth curve model	SAS, R	SAS: PROC MIXED/GLIMMIX or PROC CALIS (also PROC NLMIXED possible) R packages: nlme, lme4 (multilevel models), lavaan (SEM)
Latent class growth model	SAS, R	SAS: PROC TRAJ R: gbmt, lcmm (no random effects mode)
Latent growth mixture model	R	lcmm::hlme, flexmix, depmixS4
Latent class mixed model	R	lcmm::hlme, lcmm()
Functional data analysis	R	refund, fda, mgcv packages
Machine learning methods	R, Python	R: randomForest, xgboost Python: scikit-learn, tensorflow

cardiovascular research. First, missing data often occur because patients may become more ill or die, thus leading to incomplete biomarker measurements. If these mechanisms are ignored, the results may be biased; therefore, methods such as joint models must be carefully specified and accompanied by sensitivity analyses [59]. Second, measurement error and time-varying covariates are pervasive, because biomarkers are often imprecise and influenced by prior treatment or changing health status. Mixed-effects and joint models are particularly useful for addressing measurement error and confounding [31, 58]. Third, irregular or sparse sampling frequently limits method applicability. Mixed-effects and joint models accommodate such data structures, whereas approaches assuming dense and regular measurements, such as RM-ANOVA, may be inefficient or inappropriate in these settings [27, 49, 50]. Fourth, the identification of variability and trajectory patterns represents both an opportunity and a challenge. Mixture and group-based trajectory models can reveal clinically meaningful subgroups, such as distinct midlife BP patterns. However, subgroup identification is inherently sensitive to model assumptions. Rigorous validation and transparent reporting of uncertainty in subgroup membership are therefore essential [41, 44, 63]. Finally, model specification and selection can substantially affect conclusions. Decisions such as the form of the biomarker–event association in joint models (e.g., current value, slope, or cumulative exposure) or the random-effects structure in mixed models can alter both interpretation and effect estimates. Graphical diagnostics, comparison of plausible models, and sensitivity analyses are critical to ensure credible and reproducible inference [27, 59].

Practical Selection Guidance

Given these challenges, method selection should align with the primary objective of the study, whether descriptive, predictive, or causal. Although many longitudinal models (e.g., RM-ANOVA, GEE, and mixed-effects models) are frequently used to estimate associations, causal interpretation requires additional assumptions and careful handling of potential biases, including time-varying confounding, selection processes, and measurement error. In the analyses of longitudinal cardiovascular data, investigators should first define the primary estimand, specifying whether the goal is to estimate a population-average effect, characterize individual-specific trajectories, evaluate a dynamic biomarker–event association, or identify latent subgroups. This clarification guides both model choice and result interpretation. In addition to population-average effects, individual-specific trajectories, dynamic biomarker–event associations, and latent subgroup identification, some studies may aim primarily to develop accurate prediction models for future outcomes. Figure 1 shows the decision framework for selecting longitudinal analytic methods in cardiovascular research.

When continuous repeated outcomes are collected with irregular follow-up, mixed-effects models are preferred, because they incorporate individual-specific variation, accommodate unequal numbers of observations, and appropriately address missing-at-random mechanisms [27]. When investigators focus on the relationship between repeated biomarker measurements and clinical events with time-to-event data, joint models are recommended. The choice of association function, such as current value, slope, or cumulative exposure, should be guided by biological plausibility and the frequency of longitudinal measurements [72, 73]. Trajectory or mixture models should be used with pre-specified validation strategies, including sensitivity analyses and assessments of subgroup number and variance structure, to ensure robust and interpretable subgroup identification [41, 61, 63]. FDA is most suitable for dense, high-frequency monitoring and can be combined with mixed-effects or joint modeling to enhance interpretability while retaining complete temporal information [49, 50]. Across all these approaches, missing data assumptions must be explicitly reported, justified, and evaluated through sensitivity analyses to ensure transparent and reliable inference [59].

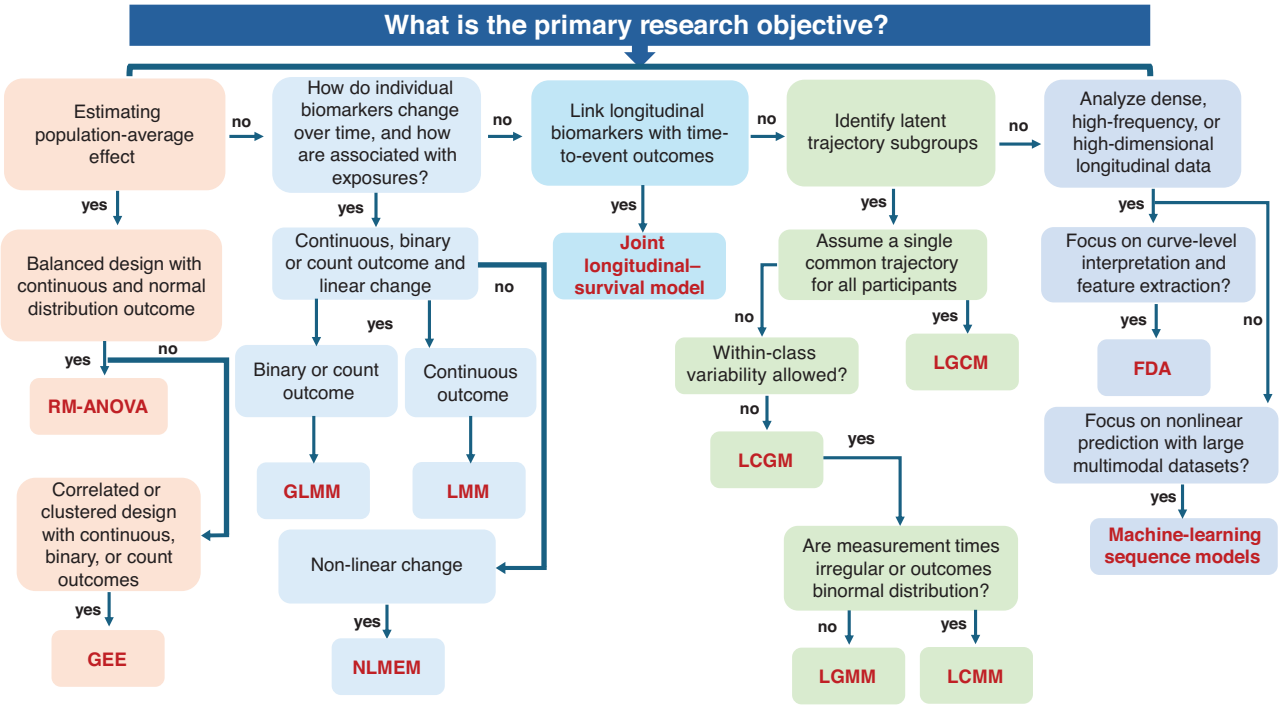

Figure 1 Decision Framework for Selecting Longitudinal Analytic Methods in Cardiovascular Research.

Beyond classical regression-based approaches, increasingly complex data structures have also motivated the use of advanced machine-learning techniques [74, 75]. Although methods such as deep-learning sequence models offer powerful tools for analyzing high-frequency and high-dimensional longitudinal data, they impose substantial computational demands. Training deep neural networks often requires large sample sizes, high-performance computing infrastructure, and extensive hyperparameter tuning [74, 75]. Memory requirements can be considerable in processing continuous sensor streams or imaging data. Beyond hardware constraints, software implementation presents challenges. Although open-source platforms such as R and Python provide accessible machine-learning libraries, the development, validation, and reproducibility of complex models require technical expertise in programming and model optimization [76, 77]. Researchers must also address issues of overfitting, model calibration, and external validation [71, 76, 78]. Therefore, when selecting analytic methods, investigators should align the choice of method with the study objective: machine-learning sequence models are most appropriate for prediction-focused tasks, whereas regression-based or joint-modeling approaches are generally more suitable for etiologic or causal inquiries. Additionally, researchers should carefully consider the trade-offs among model complexity, interpretability, computational feasibility, and available infrastructure.

Regardless of model complexity, rigorous diagnostics and reproducibility remain fundamental. Across all analyses, investigators should evaluate model fit (e.g., residual patterns and random-effects structures), conduct proportional-hazards assessments where relevant, and provide code or sufficiently detailed procedures to support replication. For prediction-focused applications, model performance should be assessed by using appropriate accuracy and calibration metrics, ideally including external validation. For more complex or "black-box" techniques, explainability tools are necessary to clarify model behavior and support clinical trust.

Several methodological priorities warrant future attention. First, more scalable and user-friendly versions of joint models are needed to handle high-dimensional longitudinal biomarkers. Second, methods that integrate causal inference into mixed-effects and joint modeling frameworks are required to properly analyze time-varying exposures. Third, unified analytic pipelines should be developed to combine sparse clinical visits with dense data streams from wearable devices. Finally, reporting standards tailored to longitudinal and dynamic prediction studies, such as extensions of STROBE or TRIPOD, should be expanded. Progress in these areas would strengthen transparency, reproducibility, and clinical translation.

These methodological developments are closely associated with the increasing interdisciplinarity of modern cardiovascular research. Contemporary studies often rely on collaboration across medicine, epidemiology, statistics, bioinformatics, and computer science [79]. The increasing availability of high-dimensional omics data, including genomics, transcriptomics, proteomics, and metabolomics, requires analytical strategies capable of integrating complex biomarker trajectories over time [80, 81]. Bioinformatics approaches, such as pathway enrichment analysis, network modeling, and multi-omics integration frameworks, can be combined with longitudinal mixed-effects or joint models to better characterize dynamic biological processes underlying cardiovascular disease progression [82].

In addition, systems biology methods enable the modeling of interactions among biomarkers across time, whereas machine-learning techniques facilitate the integration of multimodal data sources, including imaging, electronic health records, and wearable-device signals [75, 83]. Such interdisciplinary integration not only enhances predictive performance but also improves mechanistic understanding. Future methodological advances are likely to emerge from collaborative efforts that bridge clinical insight with advanced computational modeling.

Finally, longitudinal complexity in cardiovascular research does not arise solely from repeatedly measured biomarkers. Instead, clinical outcomes themselves are often dynamic, involving recurrent events, disease progression, and transitions among clinical states, such as moving from stable coronary disease to myocardial infarction, heart failure hospitalization, or death. In such settings, standard longitudinal regression approaches may be insufficient, and more specialized statistical

methods are required. When outcomes are recurrent or represent transitions across clinical states, multi-state models, recurrent event models (e.g., Andersen–Gill or frailty-based approaches), and illness–death models may be appropriate [84–86]. When both longitudinal biomarkers and event outcomes evolve over time, joint longitudinal–event models can provide a coherent framework. For complex disease progression processes, state-space models or hidden Markov models may also be considered. By incorporating these state-transition approaches, longitudinal cardiovascular analysis can better capture the dynamic nature of clinical outcomes beyond continuous biomarker trajectories.

Conclusions

Classical marginal and mixed-effects models, joint longitudinal–survival methods, latent-trajectory approaches, and functional data analysis together provide a comprehensive toolkit for longitudinal cardiovascular research. Method selection should be guided by a clear alignment between the analytic approach, the research objective, and the structure of the data. Equally important are transparent diagnostic evaluations, sensitivity analyses, and rigorous reporting to ensure robustness, interpretability, and reproducibility. When applied appropriately, these methods can transform complex longitudinal data into actionable insights on disease progression, prognosis, and prevention, thereby supporting more effective cardiovascular research and improving patient care.

Data Availability Statement

Not applicable.

Author Contributions

Y.C. drafted the initial manuscript and contributed to the conceptualization and design of the study. Y.Y., Y.W., and C.L. performed the literature review and data curation. X.W. made substantial contributions to manuscript revision and critically improved the intellectual content. T.X. and Y.R. contributed to method development and interpretation of the analytical framework. G.F. supervised the study and provided critical revisions. T.Z. contributed to study design, overall supervision, and final approval of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. Global Burden of Cardiovascular Diseases and Risks 2023 Collaborators. Global, regional, and national burden of cardiovascular diseases and risk factors in 204 countries and territories, 1990-2023. J Am Coll Cardiol 2025;86(22):2167–243.
2. Mensah GA, Fuster V, Murray CJL, Roth GA; Global Burden of Cardiovascular Diseases and Risks Collaborators. Global burden of cardiovascular diseases and risks, 1990-2022. J Am Coll Cardiol 2023;82(25):2350–473.
3. Meng Y, Sharman JE, Koskinen JS, Juonala M, Viikari JSA, Buscot MJ, et al. Blood pressure at different life stages over the early life course and intima-media thickness. JAMA Pediatr 2024;178(2):133–41.
4. Shang X, Zhang X, Huang Y, Zhu Z, Zhang X, Liu S, et al. Temporal trajectories of important diseases in the life course and premature mortality in the UK Biobank. BMC Med 2022;20(1):185.
5. Flores M, Wolfe BL. The influence of early-life health conditions on life course health. Demography 2023;60(2):431–59.
6. VanderWeele TJ, Jackson JW, Li S. Causal inference and longitudinal data: a case study of religion and mental health. Soc Psychiatry Psychiatr Epidemiol 2016;51(11):1457–66.

7. Jones L, Barnett A, Vagenas D. Common misconceptions held by health researchers when interpreting linear regression assumptions, a cross-sectional study. PLoS One 2025;20(6):e0299617.
8. Rabe-Hesketh S, Skrondal A. Ignoring non-ignorable missingness. Psychometrika 2023;88(1):31–50.
9. Yang X, Li J, Shoptaw S. Imputation-based strategies for clinical trial longitudinal data with non-ignorable missing values. Stat Med 2008;27(15):2826–49.
10. Pullenayegum EM, Lim LS. Longitudinal data subject to irregular observation: a review of methods with a focus on visit processes, assumptions, and study design. Stat Methods Med Res 2016;25(6):2992–3014.
11. Chen Y, Ning J, Cai C. Regression analysis of longitudinal data with irregular and informative observation times. Biostatistics 2015;16(4): 727–39.
12. Schober P, Vetter TR. Repeated measures designs and analysis of longitudinal data: if at first you do not succeed-try, try again. Anesth Analg 2018;127(2):569–75.
13. Carr GJ, Chi EM. Analysis of variance for repeated measures. Data: a generalized estimating equations approach. Stat Med 1992;11(8):1033–40.
14. Keselman HJ, Algina J, Kowalchuk RK. The analysis of repeated measures designs: a review. Br J Math Stat Psychol 2001;54(Pt 1):1–20.
15. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. Cardiovasc Res 1994;28(3):303–11.
16. Saraf-Bank S, Esmaillzadeh A, Faghihimani E, Azadbakht L. Effects of legume-enriched diet on cardiometabolic risk factors among individuals at risk for diabetes: a crossover study. J Am Coll Nutr 2016;35(1):31–40.
17. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988;44(4):1049–60.
18. Chang YC. Residuals analysis of the generalized linear models for longitudinal data. Stat Med 2000;19(10):1277–93.
19. Prentice RL. Correlated binary regression with covariates specific to each binary observation. Biometrics 1988;44(4):1033–48.
20. Lipsitz SR, Fitzmaurice GM, Orav EJ, Laird NM. Performance of generalized estimating equations in practical situations. Biometrics 1994;50(1):270–8.
21. Wang C, Liu S, Miao W, Ye N, Xie Z, Qiao L, et al. Intensive blood pressure control in isolated systolic hypertension: a post hoc analysis of a cluster randomized trial. Lancet Reg Health West Pac 2024;48:101127.
22. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38(4):963–74.
23. Li G, Xu S, Pan S, Shao H, Gaskins AJ, Zhang Y, et al. Hypertension and semen quality among 1381 young men: a cohort study with repeated measurements. Innov Med 2024;2(4):100099.
24. Breslow NE, Clayton DG. Approximate inference in generalized linear mixed models. J Am Stat Assoc 1993;88(421): 9–25.
25. Tuerlinckx F, Rijmen F, Verbeke G, De Boeck P. Statistical inference in generalized linear mixed models: a review. Br J Math Stat Psychol 2006;59(Pt 2):225–55.
26. Bergeman AT, Lieve KVV, Kallas D, Bos JM, Rosés I, Noguer F, et al. Flecainide is associated with a lower incidence of arrhythmic events in a large cohort of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation 2023;148(25):2029–37.
27. Pinheiro JC, Bates DM. Mixed-effects models in S and S-PLUS. New York: Springer; 2000.
28. Davidian M, Giltinan DM. Some general estimation methods for nonlinear mixed-effects models. J Biopharm Stat 1993;3(1):23–55.
29. Lindstrom ML, Bates DM. Nonlinear mixed effects models for repeated measures data. Biometrics 1990;46(3):673–87.
30. van Rijn-Bikker PC, Snelder N, Ackaert O, van Hest RM, Ploeger BA, van Montfrans GA, et al. Nonlinear mixed effects modeling of the diurnal blood pressure profile in a multiracial population. Am J Hypertens 2013;26(9):1103–13.
31. Wulfsohn MS, Tsiatis AA. A joint model for survival and longitudinal data measured with error. Biometrics 1997;53(1):330–9.
32. Ibrahim JG, Chu H, Chen LM. Basic concepts and methods for joint models of longitudinal and survival data. J Clin Oncol 2010;28(16):2796–801.
33. Fisher LD, Lin DY. Time-dependent covariates in the Cox proportional-hazards regression model. Annu Rev Public Health 1999;20:145–57.
34. Putter H, van Houwelingen HC. Understanding landmarking and its relation with time-dependent Cox regression. Stat Biosci 2017;9(2): 489–503.
35. Henderson R, Diggle P, Dobson A. Joint modelling of longitudinal measurements and event time data. Biostatistics 2000;1(4):465–80.
36. Rizopoulos D. Dynamic predictions and prospective accuracy in joint models for longitudinal and time-to-event data. Biometrics 2011; 67(3):819–29.
37. Domanski MJ, Tian X, Wu CO, Reis JP, Dey AK, Gu Y, et al. Time course of LDL cholesterol exposure and cardiovascular disease event risk. J Am Coll Cardiol 2020;76(13):1507–16.
38. Meredith W, Tisak J. Latent curve analysis. Psychometrika 1990;55: 107–22.
39. Felt JM, Depaoli S, Tiemensma J. Latent growth curve models for biomarkers of the stress response. Front Neurosci 2017;11:315.
40. Pai HC, Lai MY, Chen AC, Lin PS. Change in activities of daily living in the year following a stroke: a latent growth curve analysis. Nurs Res 2018;67(4):286–93.
41. Nagin DS. Analyzing developmental trajectories: a semiparametric, group-based approach. Psychol Methods 1999;4(2):139–57.
42. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. Annu Rev Clin Psychol 2010;6:109–38.
43. Liu Y, Chen X, Li C, Fan B, Lv J, Qu Y, et al. Life-course blood pressure trajectories and incident diabetes: a longitudinal cohort in a Chinese

population. Front Endocrinol (Lausanne) 2022;13:1035890.
44. Muthen B, Shedden K. Finite mixture modeling with mixture outcomes using the EM algorithm. Biometrics 1999;55(2):463–9.
45. Muthen B, Muthén LK. Integrating person-centered and variable-centered analyses: growth mixture modeling with latent trajectory classes. Alcohol Clin Exp Res 2000;24(6):882–91.
46. Tielemans SM, Geleijnse JM, Menotti A, Boshuizen HC, Soedamah-Muthu SS, Jacobs DR Jr, et al. Ten-year blood pressure trajectories, cardiovascular mortality, and life years lost in 2 extinction cohorts: the Minnesota Business and Professional Men Study and the Zutphen Study. J Am Heart Assoc 2015;4(3):e001378.
47. Proust-Lima C, Phillipps V, Liquet B. Estimation of extended mixed models using latent classes and latent processes: the R Package lcmm. J Stat Softw 2017;78(2):1–56.
48. Fan B, Yang Y, Dayimu A, Zhou G, Liu Y, Li S, et al. Body mass index trajectories during young adulthood and incident hypertension: a longitudinal cohort in Chinese population. J Am Heart Assoc 2019;8(8):e011937.
49. Ramsay JO, Silverman BW. Principal components analysis for functional data. In: Functional data analysis. Springer series in statistics. New York: Springer; 1997.
50. Ramsay JO, Silverman BW. Functional data analysis. 2nd ed. New York: Springer; 2005.
51. Gertheiss J, Rugamer D, Liew BXW, Greven S. Functional data analysis: an introduction and recent developments. Biom J 2024;66(7):e202300363.
52. Guo W. Functional data analysis in longitudinal settings using smoothing splines. Stat Methods Med Res 2004;13(1):49–62.
53. Wrobel J, Muschelli J, Leroux A. Diurnal physical activity patterns across ages in a large UK based cohort: the UK Biobank Study. Sensors (Basel) 2021;21(4):1545.
54. Hochreiter S, Schmidhuber J. Long short-term memory. Neural Comput 1997;9(8):1735–80.
55. Vaswani A, Shazeer N, Parmar N, Uszkoreit J, Jones L, Gomez AN, et al. Attention is all you need. Adv Neural Inf Process Syst 2017;30:5998–6008.
56. Dai J, Xu H, Chen T, Huang T, Liang W, et al. Artificial intelligence for medicine 2025: navigating the endless frontier. Innov Med 2025;3(1):100120.
57. Diggle P, Heagerty P, Liang K, Zeger S. Analysis of longitudinal data. 2nd ed: Oxford University Press; 2002.
58. Serroyen J, Molenberghs G, Verbeke G, Davidian M. Non-linear models for longitudinal data. Am Stat 2009;63(4):378–88.
59. Rizopoulos D. Joint models for longitudinal and time-to-event data: with applications in R. Boca Raton, FL: Chapman and Hall/CRC Press; 2012.
60. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis. 2nd ed. Hoboken, New Jersey: John Wiley & Sons, Inc; 2011.
61. Miettunen J, Nordstrom T, Kaakinen M, Ahmed AO. Latent variable mixture modeling in psychiatric research – a review and application. Psychol Med 2016;46(3):457–67.
62. Slaughter JC, Herring AH, Thorp JM. A Bayesian latent variable mixture model for longitudinal fetal growth. Biometrics 2009;65(4):1233–42.
63. Dhana K, van Rosmalen J, Vistisen D, Ikram MA, Hofman A, Franco OH, et al. Trajectories of body mass index before the diagnosis of cardiovascular disease: a latent class trajectory analysis. Eur J Epidemiol 2016;31(6):583–92.
64. Girden ER. ANOVA: repeated measures. Newbury Park, CA: Sage; 1992.
65. Field A. Discovering statistics using IBM SPSS statistics: and sex and drugs and rock "N" roll. 4th ed. Los Angeles, London, New Delhi: Sage; 2013.
66. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Stat Med 1997;16(20):2349–80.
67. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics 2001;57(1):120–5.
68. Proust-Lima C, Séne M, Taylor JM, Jacqmin-Gadda H. Joint latent class models for longitudinal and time-to-event data: a review. Stat Methods Med Res 2014;23(1):74–90.
69. Kim SY. Determining the number of latent classes in single- and multiphase growth mixture models. Struct Equ Modeling 2014;21(2):263–79.
70. Harrell FE. Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis. 2nd ed. Cham: Springer; 2015.
71. Steyerberg EW. Clinical prediction models: a practical approach to development, validation, and updating. 2nd ed. Cham: Springer; 2019.
72. Oulhaj A, Aziz F, Suliman A, Iqbal N, Coleman RL, Holman RR, et al. Joint longitudinal and time-to-event modelling compared with standard Cox modelling in patients with type 2 diabetes with and without established cardiovascular disease: an analysis of the EXSCEL trial. Diabetes Obes Metab 2023;25(5):1261–70.
73. Yang L, Yu M, Gao S. Prediction of coronary artery disease risk based on multiple longitudinal biomarkers. Stat Med 2016;35(8):1299–314.
74. Esteva A, Robicquet A, Ramsundar B, Kuleshov V, DePristo M, Chou K, et al. A guide to deep learning in healthcare. Nat Med 2019;25(1):24–9.
75. Rajkomar A, Oren E, Chen K, Dai AM, Hajaj N, Hardt M, et al. Scalable and accurate deep learning with electronic health records. NPJ Digit Med 2018;1:18.
76. Hastie T, Tibshirani R, Friedman J. The elements of statistical learning: data mining, inference, and prediction. 2nd ed. New York, NY: Springer; 2009.
77. Beam AL, Kohane IS. Big data and machine learning in health care. JAMA 2018;319(13):1317–8.
78. Van Calster B, McLernon DJ, van Smeden M, Wynants L, Steyerberg

EW. Calibration: the Achilles heel of predictive analytics. BMC Med 2019;17:230.
79. Johnson KW, Shameer K, Glicksberg BS, Readhead B, Sengupta PP, Bjorkegren JLM, et al. Enabling precision cardiology through multiscale biology and systems medicine. JACC Basic Transl Sci 2017;2(3):311–27.
80. Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, et al. Genomic risk prediction of coronary artery disease in 480,000 adults: implications for primary prevention. J Am Coll Cardiol 2018;72(16):1883–93.
81. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol 2017;18(1):83.
82. Barabasi AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet 2011;12(1):56–68.
83. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nat Med 2019;25(1):44–56.
84. Andersen PK, Keiding N. Multi-state models for event history analysis. Stat Methods Med Res 2002;11(2):91–115.
85. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. Stat Med 2007;26(1):2389–430.
86. Hougaard P. Multi-state models: a review. Lifetime Data Anal 1999;5(3):239–64.