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Laboratory parameters in prognostic sickle cell disease cohort studies in children: a systematic review and lessons for an appropriate strategy for analysis

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Research article

Keywords: Systematic review - Longitudinal data - Cohort - Sickle cell disease - Children

Posted Date: December 6th, 2019

DOI: https://doi.org/10.21203/rs.2.18322/v1

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Abstract

BACKGROUND: There is a variability of the results in the published prognostic sickle cell disease (SCD) studies. The aim of this article is to assess and demonstrate how the methods used for analysis of laboratory parameters impact the prognostic study results in children with sickle cell disease.

METHODS: Firstly, a systematic review of published studies was conducted to identify the methods used in statistical analyses on laboratory parameters. Secondly, four different identified analysis were successively applied on laboratory data collected prospectively in our cohort of newborns with SCD in order to evaluate the association between laboratory parameters and cerebral vasculopathy, depending on the analysis strategy.

RESULTS: Thirty-one studies were included in the systematic review in which hemoglobin and reticulocyte counts were the most frequently collected parameters. Through this review, we noted four different statistical strategies. In almost one half of the cohort studies, laboratory parameter was collected only one time per patient. When several values were available, only one statistical analysis considered the time variation. When we applied these four different statistical strategies to our cohort of SCD children, we highlighted that the conclusion on prognostic value of parameters (either as protective or as risk factor) depends on the analysis strategies applied.

CONCLUSION: The laboratory parameter prognostic value depends on the analysis strategy chosen for its analysis. It raises fundamental questions about methodological approaches that concern clinician researchers. Identifying prognostic factors is currently essential for better understanding of SCD. Taking into account longitudinal data and standardization of analyses in prognostic cohort studies should allow drawing valuable and reproducible conclusions.

Background

Physicians and researchers have sought to identify factors to explain the phenotypic variability of sickle cell disease (SCD) and to predict the clinical course in patients: although all patients with SCD share a specific genotype mutation, clinical variability in the pattern and severity of disease manifestations is broad and unpredictable. Over the last decades, more than fifty prognostic studies have been published, especially in children, seeking to understand the factors associated with increased morbidity and mortality. Several studies have identified prognostic variables [1] potentially associated with increased disease severity, including fetal hemoglobin level [2][3], leukocyte count [4][5], reticulocyte count [6] or hemoglobin level [7] [8].

However, there is a variability of the results in the published studies. For example, fetal hemoglobin was shown to be protective against cerebral vasculopathy (CV) in one study [9] while others failed to demonstrate this [6] [7]. The difference in the results might be due to differences in the methods used to consider these laboratory parameters.

Indeed, blood levels of these parameters change over time, particularly in children. First, baseline levels vary physiologically depending on age. For example, the rate of fetal hemoglobin usually decreases by 60% at 3 months of age down to 25% at one year of age [9]. In addition, blood levels may vary as a result of the disease course, the occurrence of complications or during some treatments.
The purpose of our study was to assess how the laboratory parameters are collected and analysed in epidemiological observational studies in order to determine whether the methods employed had an impact on the conclusions drawn with regard to an association between laboratory parameters and clinical SCD events in the paediatric context.
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Methods

The first step was to conduct a systematic literature review to collect how the laboratory parameters were measured and analysed in published prognostic studies in SCD. The second step was to perform various statistical analyses on the same data set in order to assess the impact of the methodological strategy on the results for a given cohort and therefore, potentially, on the overall conclusions of the studies.

Systematic review

The review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement [10] (See Appendix 1).

Inclusion criteria

We included the observational studies conducted in children from 0 to 18 years of age and which evaluated the association between prognostic SCD laboratory variables, i.e. hemoglobin and/or fetal hemoglobin and/or reticulocyte count, and a clinical event linked to SCD. Selected studies were those published in English in one of the following categories, i.e., paediatric or hematology or general journals, and in one of the four leading journals in terms of impact factor according to Journal Citation Reports, 2016. The studies were extracted from the electronic database PubMed using the timeframe from January 2007 to July 2017. We did not include congress reports or short communications, commentaries or letters to editors as the methods are usually poorly reported in such articles. Controlled trials were not included, as the goals are different, and the analysis usually follows a different logic than that of observational studies. The electronic search strategy is described in Appendix 2.

Study selection

Two researchers (EL, JS) independently screened the records retrieved and selected all studies that fulfilled the eligibility criteria. Eligible studies were downloaded to the reference management database Zotero. The researchers (EL, JS) independently screened the titles and abstracts to identify relevant studies. Full texts were read when abstracts met inclusion criteria or when abstracts were not sufficiently clear to determine eligibility. Full-text screening was completed independently. All disagreements were discussed and resolved by consensus.

Data extraction

The researchers (EL, JS) independently extracted data from each relevant study using a standardized computerized data collection form developed for the study. Disagreements were resolved by discussion and consensus.

General study information was extracted: authors, article title, publication year, journal, country, setting, number of patients included, study design and SCD clinical events studied.

Additional items that could influence the results of statistical analyses on laboratory variables were extracted, i.e. patients' ages at study inclusion and SCD genotypes.

To assess the heterogeneity of measurements and methods of analysis, we recorded the methods used to collect data, i.e. at a single timepoint in the study, (either at study inclusion or last known value), or repeatedly throughout the study, as well as the statistical methods, i.e. continuous or categorical variable when one value per patient was available; mean, median or modelisation when several values per patient were used. We focused on cohort studies because cross-sectional or case-control studies are based on a snapshot, therefore, only one measurement is generally expected.

When information was not available, investigators were contacted by the researchers to request additional information.

Illustration of the used method impact

To illustrate the impact of methods by which prognostic variables are taken into account in analyses, we used a SCD cohort dataset. The studied outcome is the cerebral vasculopathy (CV), an important complication of sickle cell disease in children

Study Population

We used laboratory data set collected prospectively from the National Paediatric Sickle Cell Reference Centre at the Robert Debre University Hospital in Paris, France, as previously described [9]. In this cohort, laboratory data and concurrent clinical status were recorded prospectively at 3, 6 and 12 months of age and yearly thereafter. The cohort of 375 patients included 363 patients with homozygous HbS and 12 patients with heterozygous sickle cell/beta°-thalassaemia. Median follow-up was 6.8 years (2677 patient-years). Each laboratory variable was measured a median of 6 times (min:1; Q1: 4, Q3: 9, max: 16).

Statistical Methodology

Laboratory parameters were assessed as risk factors for CV using a Cox survival multivariable model. Time-to-CV was calculated from the date of birth to the date of diagnosis of CV. Patients who remained CV-free at their last follow-up before 31 December 2010 were right-censored at this date. Patients not seen during the 12 months prior to 31 December 2010 were considered lost to follow-up and censored at their last follow-up. We used the seven available laboratory variables evaluated as risk factors in this

cohort study: reticulocyte count, platelet count, neutrophil count, mean corpuscular volume, leukocyte count, hemoglobin level and fetal hemoglobin level.
To take into account laboratory parameters in this model, we conducted our analyses according to the results of the systematic review and observed the variability of the results concerning associations between the prognostic laboratory variables and CV.

So we conducted four analyses where laboratory variables were included either as (1) one value per patient at inclusion in the study (from 1 to 12 months of age); or (2) last known value, whatever the age at the last follow-up; or (3) mean of all non-censored values per patient; or (4) modelisation of all non-censored values in a two-step approach as previously described [11] [12] [13] [14].

For each analysis, we included all laboratory variables in the final multivariable model, firstly without selection (to interpret the variability of the results without any selection) and secondarily with stepwise selection classically applied in multivariate analysis. Use of a stepwise selection procedure allows for selection of statistically significant variables only at the last step of the procedure. The alpha level was set at 0.05 for all analyses. The results are presented as the hazard ratio (HR) with 95% confidence interval (95% CI). It was performed with SAS 9.4 (Cary, NC, USA).

Results

Systematic review

Literature Search

The electronic search strategy retrieved a total of 571 references, from which 65 were selected based on the titles and abstracts. Thirty-one studies met the inclusion criteria and were included in the review. The flowchart is presented in Fig. 1.

Study Characteristics

Characteristics of the studies included are shown in table 1. More than three out of four studies were published in hematological journals (24/31, 77%). United States (18/31, 58%) and France (7/31, 23%) were the largest providers of articles. More than one out of two studies was a cohort study (16/31). Age at inclusion was mentioned in all studies; the dispersion of age at inclusion spanned as much as 17 years.

Neurological complications, i.e. CV, stroke, silent infarct or headaches, were the most frequently evaluated clinical events (16/31, 52%). Description of included studies is provided in appendix 3.

Measurement and statistical analyses of laboratory parameter in cohort studies

The characteristics of the prognostic laboratory parameters used are presented in Table 2. The mean of patient follow-up can reach 11.4 years. Each laboratory parameter was collected several times per patient in barely two-thirds of the studies and was analysed as continuous variable in the prognostic statistical analysis. When several values of laboratory parameter per patient were collected, they were summarized as means in the prognostic statistical analysis in all studies excepted one.

Illustration of the used method impact

The results of the different analyses are shown in Figs. 2 and 3, without and with stepwise selection, respectively. The results of the association between laboratory parameters and cerebral vasculopathy were different depending on the strategy used, either in terms of significance, i.e. 95% confidence interval (fetal hemoglobin, hemoglobin, leukocyte and neutrophil count) and/or with regard to the meaning of the association, i.e. the same laboratory parameter could be either a risk factor or a protective factor (fetal hemoglobin, leukocyte and neutrophil count). For example, increased fetal hemoglobin was a significant risk factor for CV when the last known value was used (Fig. 2.b, HR 1.13[1.10–1.19]) or the mean of the measurements (Fig. 2.c, HR 1.08[1.04–1.10]), and it was a significant protective factor when repeated measurements were taken into account in a model (Fig. 2.d, HR 0.89[0.83–0.96]) in the same cohort of patients. Conversely, increased hemoglobin was a significant protective factor for CV when the last known value was used (Fig. 2.b, HR 0.64[0.39–0.89]), but had no prognostic value when the mean of the measurements (Fig. 2.c, HR 0.91[0.64–1.39]) or modelisation (Fig. 2.d, HR 0.9”[0.52–1.78]) were used. With stepwise selection, the number and type of variables selected changed depending on the statistical analysis used (Fig. 3).

Discussion

Laboratory parameters are critical prognostic parameters in prognostic studies on SCD. We therefore conducted a review of the methods used in studies in this field to take into account and to analyse laboratory parameters. Our review found a large heterogeneity of the methods used. This led us to assess four different methodological strategies for analysis of laboratory parameters. We found that different methodologies can lead to different conclusions on the prognostic value of the laboratory parameters on CV.

There was an heterogeneity among the studies regarding the age range at the study inclusion. Given the variations in laboratory parameters depending on age [15] [16], as well as the absence of standards according to age and sex in the SCD population, the broad age ranges at study inclusion could weaken the study conclusions by inducing an error in the association estimation between the laboratory parameters and a SCD clinical event. For example, in assessing the association between fetal hemoglobin and CV in children between 18 months and 5 years of age, which is the period of onset of this complication, it is important to bear in mind that blood fetal hemoglobin levels decrease by approximately 25–15% over this age range. Moreover, blood hemoglobin levels vary depending on age with very high levels in newborns, followed by a gradual decrease, with the lowest levels observed between 1 and 6 months of age, followed by a gradual increase and then stabilisation at puberty [15].

Surprisingly, most cohort studies used a single value per patient in the statistical analysis, measured at various ages depending on the studies. When a full set of measures was available, one strategy applied in published studies to determine risk versus protective factor was to take into account the mean of the measures. However, with this strategy, changes over time in the laboratory parameter are not taken into account. For example, in the study conducted by Curtis et al [17], which enrolled 359 patients with sickle cell anaemia, lower baseline levels of fetal hemoglobin were associated with increased mortality, however a significant risk of increased mortality, based on longitudinal changes in percentage of fetal hemoglobin, was not detected.

The course of a laboratory parameter over time may further influence the different conclusions concerning the correlation between the laboratory parameter and the SCD event depending on the method of analysis. This could explain in part the variability of the results in the published studies. For example, fetal hemoglobin was shown to be protective against CV in one study [9] while other studies failed to demonstrate this [6] [7]. The difference in the results might be due to differences in the study populations or to differences in the definitions used, however it could also be due to differences in the methods used to assess the prognostic value of fetal hemoglobin.

To our knowledge, this is the first work that explores how laboratory parameters are used to predict outcome in SCD studies in children. Although this field is sparsely explored, it raises fundamental questions about methodological approaches relevant for clinician researchers. Even if they could request statistician support, they should be made aware of the importance in taking into account longitudinal data in prognostic cohort studies.

One of the strengths of this study is that suggests the use of an under-known method for statistical analysis that takes into account longitudinal data in the research of association with a clinical event, as has been done for other diseases, notably for Human Immunodeficiency Virus [11] [13] [18] [19] [20]. More recently, this method has been adopted in others areas of clinical research, including cancer [21], cardiovascular diseases [22], and kidney transplantation studies [23] [24] .In the field of SCD, this rigorous method was used for the first time only recently [9]; it took into account variations over time for repeated measures throughout the study.

Eligible studies were identified through one important database and only major journals were screened. We consider that we have found sufficient evidence to draw robust conclusions on the potential impact of the heterogeneity of measurement methods and strategies on the analysis of laboratory parameter.

Standardization of practices and methods in prognostic studies in children with SCD would help to avoid so-called vibration of effects, whereby results can differ, i.e. vibrate over a wide possible range, depending on how the analysis was conducted [25]. Standardization and homogenization of methods could lead to more transparent reporting in these studies and enhance the reliability of published data [26]. Large consortia and collaborations would allow investigators to use a common language for clinical definitions, laboratory measurements and statistical analysis.

Conclusion

This work revealed a considerable heterogeneity among studies that investigate factors associated with clinical events in SCD in terms of analysis methods and in terms of results. We showed that different methods on the same data set can lead to different conclusions as to the prognostic value of the laboratory parameters in predicting SCD morbidity, illustrating how important it is to apply appropriate statistical methods. We suggest that assessing values and monitoring their changes over time may be an improved way of assessing SCD prognosis. This may have important implications in disease stratification for therapeutic interventions, particularly for those interventions that have significant morbidity/mortality. A collaborative work on methods, including clinicians and statisticians to obtain the most reliable results and their feasibility in terms of collection in the context of observational studies should be initiated. It seems reasonable to strive to move towards greater standardisation of practices, especially in cohort studies, in order to limit variability and to strengthen the result reliability, so as to allow for comparisons across studies, and to ensure transparent reporting.

Abbreviations

CI: Confidence interval

CV: Cerebral vasculopathy

HR: Hazard ratio

SCD: Sickle cell disease

Declarations

Ethics approval and consent to participate

Verbal information about the database was provided to the parents and non-opposition reported on the medical chart. This was approved by the Ethics Committee of the Groupe Hospitalo-Universitaire Nord (#IRB: 00006477).The database including laboratory parameters was approved by the French National Committee for Computerized Databases (CNIL number 1299665). Data does not compromise anonymity or confidentiality or breach local data protection laws.

Consent to publish

Not available.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was used for this study

Author's contribution

All authors meet the criteria for being contributing authors.

JS designed the study, screened the papers and collected the data of the systematic review, realized the statistical analysis and wrote the manuscript. EL participated to the study design, screened the papers and collected the data of the systematic review, participated to manuscript writing. BK participated to manuscript writing and critically reviewed the manuscript. ZH collected the laboratory data, and participated to their analysis. DM participated to statistical analysis. AB critically reviewed the manuscript. MB participated to results analysis, and participated to manuscript writing and critically reviewed the manuscript. CA participated to study design, statistical analysis design and critically reviewed the manuscript.

All authors were involved in the discussion and interpretation of data and all approved the final version.

Acknowledgments

The authors thank Graham Smith for editorial assistance and Celine Feger and Tomoe Sugiura for her help.

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Tables

Table 1. Study characteristics

	N=31	%
Journal title		
British Journal of Haematology	12	(39)
Blood	6	(20)
Haematologica	4	(13)
The Journal of Pediatrics	4	(13)
American Journal of Hematology	2	(6)
Pediatrics	2	(6)
Archives of Pediatrics & Adolescent Medicine	1	(3)
Region		
North America	19	(61)
Europe	11	(36)
Africa	1	(3)
Type of study		
Cohort	16	(52)
Cross sectional	13	(42)
Case-control	2	(6)
SCD clinical event studied		
Neurological complications*	16	(52)
Vaso-occlusive crises	4	(13)
Acute chest syndrome	3	(10)
Growth	3	(10)
Mortality	2	(6)
Pulmonary arterial hypertension	2	(6)
Others**	8	(26)
Number of patients included		
Median (Q1; Q3)	208 (93; 381)	
(Range)	(37; 1041)	
Age range at inclusion (years)		
Median (Q1; Q3)	9,0 (2,7;13,1)	
(Range)	(0,3;17,0)	

* Neurological complications are cerebral vasculopathy, stroke, silent infarct or headaches ** Others are quoted once time: developmental function, retinopathy, exercise-induced hemoglobin oxygen desaturation, left ventricular dysfunction, alloimmunization, priapism, osteomyelitis, blood pressure

Table 2. Characteristics of laboratory parameters in the 16 cohort studies : measurement and analysis

Laboratory parameter	Hemoglobin		Reticulocyte count		Leukocyte count		Fetal hemoglobin	
	N	%	N	%	N	%	N	%
Measure of laboratory parameter	**16**	***(100)***	**14**	***(88)***	**14**	***(88)***	**11**	***(69)***
One measure per patient	*7*	*(44)*	*7*	*(50)*	*6*	*(43)*	*5*	*(46)*
Fixed age	7		6		6		4	
Last known value	0		1		0		1	
Several measures per patient	*6*	*(37)*	*5*	*(36)*	*6*	*(43)*	*4*	*(36)*
All values during follow-up	3		3		3		2	
Values measured at fixed range of age	3		2		3		2	
*NR**	*3*	*(19)*	*2*	*(14)*	*2*	*(14)*	*2*	*(18)*
Analysis of laboratory parameter	**15**	***(94)***	**13**	***(81)***	**12**	***(63)***	**11**	
One value per patient	*8*	*(53)*	*8*	*(61)*	*5*	*(42)*	*5*	*(45)*
Continuous variable	6		6		4		5	
Categorical variable	2		2		1		0	
Several values per patient	*5*	*(33)*	*4*	*(31)*	*5*	*(42)*	*4*	*(36)*
Continuous variable summarized	4		3		4		3	
- as means	1		1		1		1	
- by modelization								
*NR**	*2*	*(13)*	*1*	*(8)*	*2*	*(16)*	*2*	*(18)*

** NR: Not Reported*

Figures

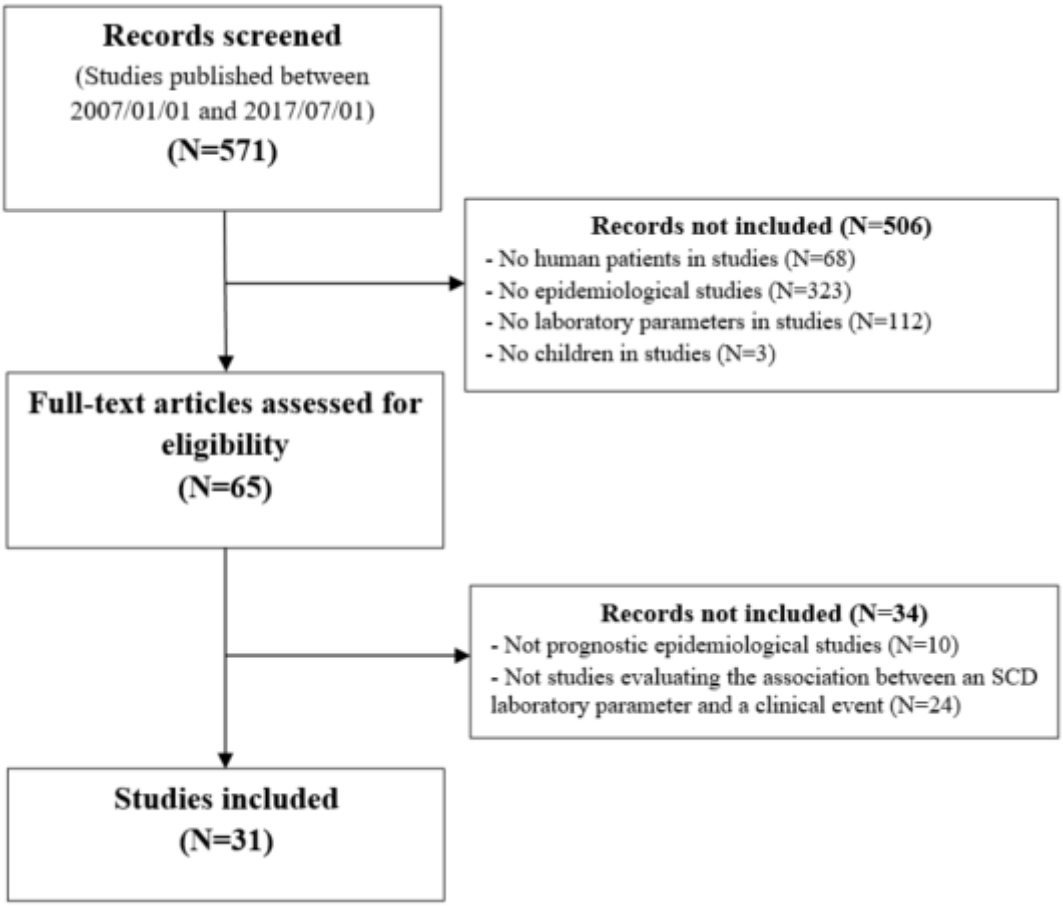

Figure 1

Flowchart

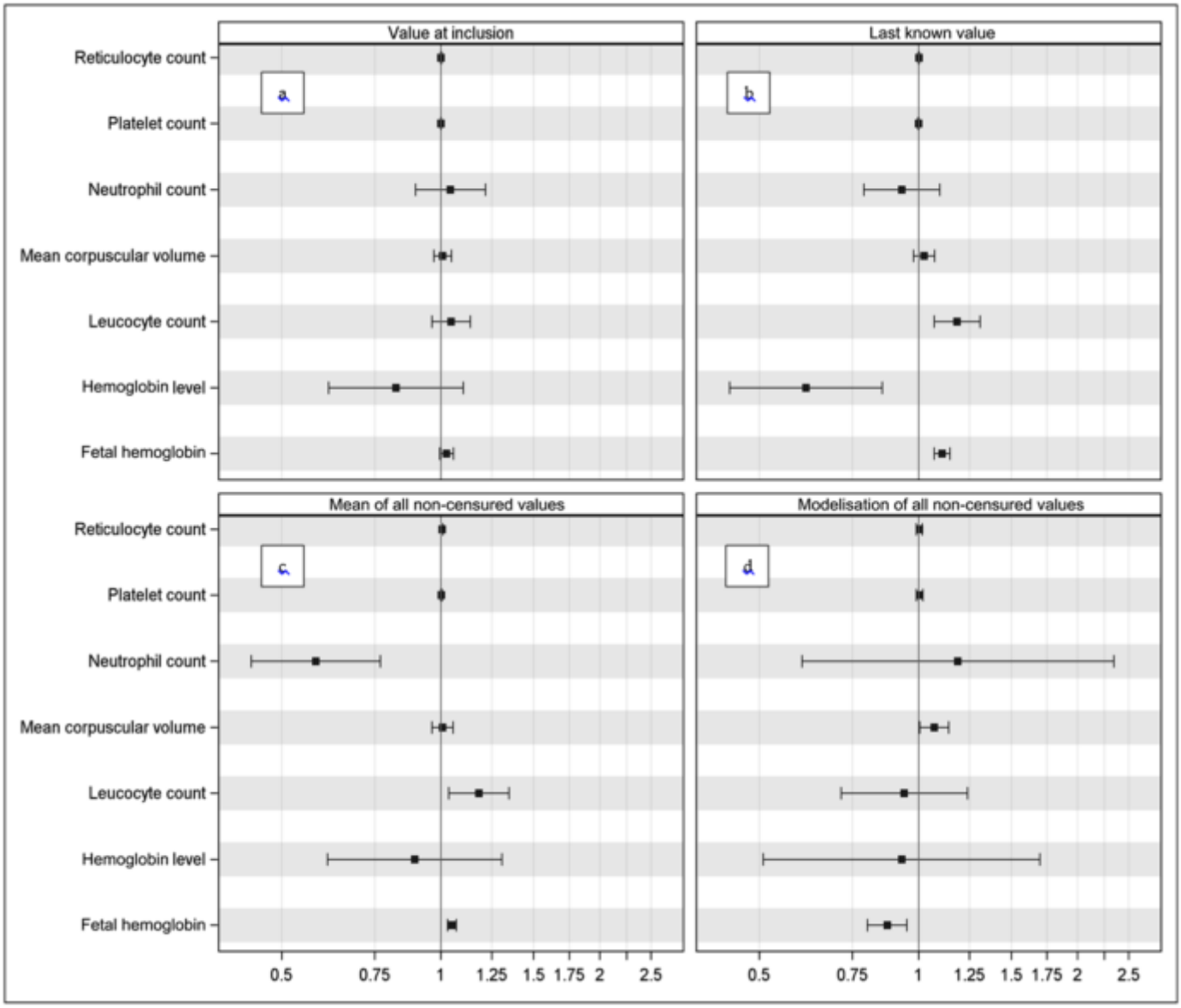

Figure 2

Results of multiple analyses without stepwise selection Each graph represents one of the four methods used in multivariate analysis, i.e. a: one value at inclusion in the study; b: last known value; c: mean of all non-censored values per patient; d: modelisation of all non-censored values. Hazard ratios and 95% confidence interval for each laboratory parameter are represented by square and horizontal lines, respectively. There was a significant association between the laboratory parameter and CV when the horizontal line does not cross the vertical line corresponding to one in abscissa; as a protective factor when the square is to the left of the line and as a risk factor when it is to the right.

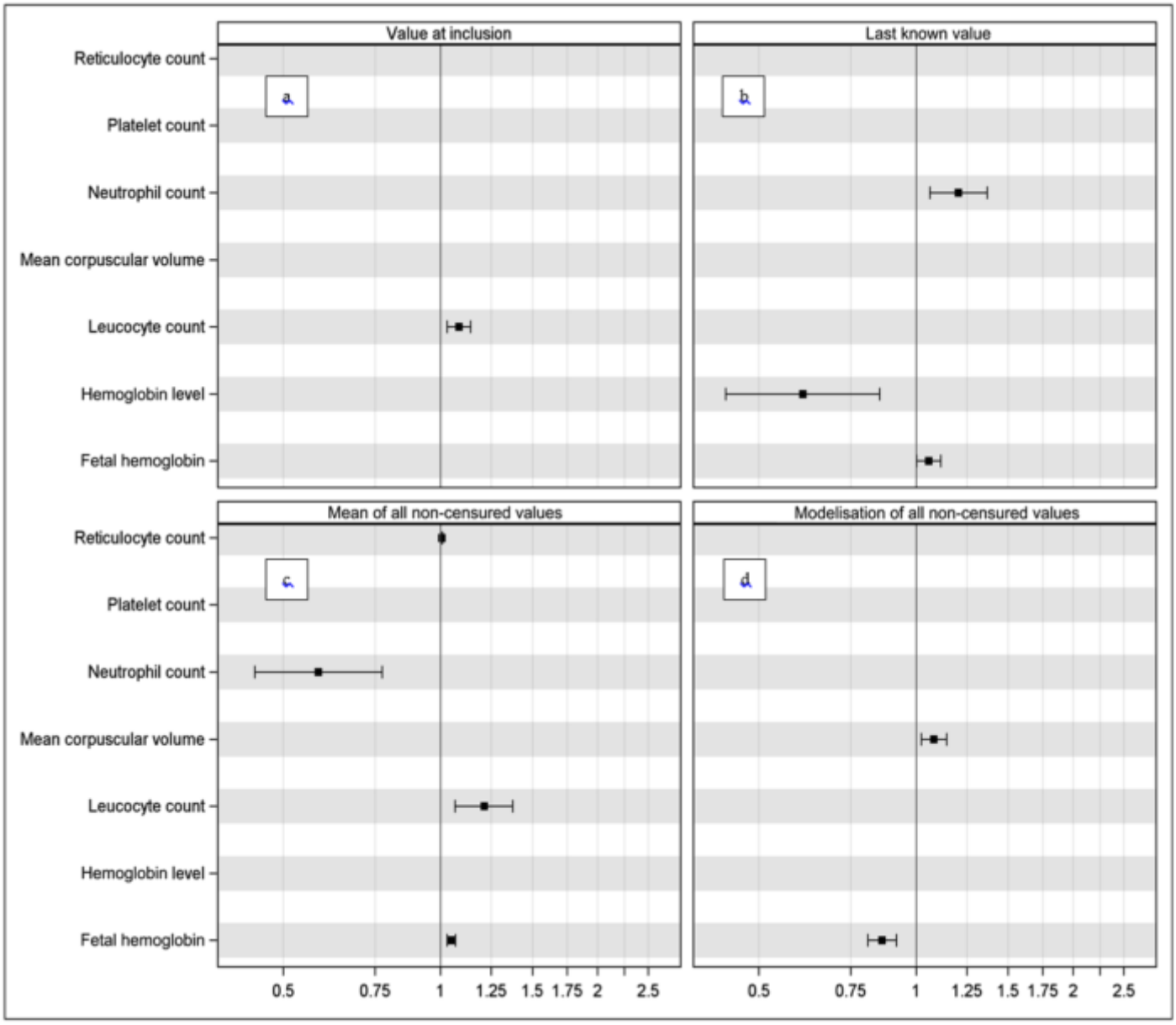

Figure 3

Results of multiple analyses with stepwise selection Each panel represents one of the four methods used in multivariate analysis, i.e. a: one value at inclusion in the study; b: last known value; c: mean of all non-censored values per patient; d: modelisation of all non-censored values. Hazard ratios with 95% confidence intervals for each laboratory parameter are represented by square and horizontal lines, respectively. There was a significant association between the laboratory parameter and CV when the line does not cross the vertical line corresponding to one in abscissa: as a protective factor when the square is to the left of the line and as a risk factor when it is to the right. Stepwise selection is a widely used statistical method in which statistically significant prognostic variables are selected using an automated procedure to build a multivariable model. When this procedure is applied, only variables with a 5% level of significance are captured.