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A screening method to spot biomarkers that may warn of serious events in a chronic disease – illustrated by cardiological CLARICOR trial data

https://doi.org/10.1515/cclm-2021-0333
Received March 19, 2021; accepted July 21, 2021;
published online August 12, 2021

Abstract

Objectives: To develop a crude screening method for detecting biomarkers which frequently exhibit a rise (or fall) in level prior to a serious event (e.g. a stroke) in patients with a chronic disease, signalling that the biomarker may have an alarm-raising or prognostic potential. The subsequent assessment of the marker's clinical utility requires costly, difficult longitudinal studies. Therefore, initial screening of candidate-biomarkers is desirable.

Methods: The method exploits a cohort of patients with biomarkers measured at entry and with recording of first serious event during follow-up. Copying those individual records onto a common timeline where a specific event occurs on the same day (Day 0) for all patients, the baseline biomarker level, when plotted against the patient's entry time on the revised timeline, will have a positive (negative) regression slope if biomarker levels generally rise (decline) the closer one gets to the event. As an example, we study 1,958 placebo-treated patients with stable coronary artery disease followed for nine years in the CLARICOR trial (NCT00121550), examining 11 newer biomarkers.

Results: Rising average serum levels of cardiac troponin T and of N-terminal pro-B-type natriuretic peptide were seen prior to a fatal cardiovascular outcome. C-reactive protein rose prior to non-cardiovascular death. Glomerular filtration rate, seven lipoproteins, and nine newer cardiological biomarkers did not show convincing changes.

Conclusions: For early detection of biomarkers with an alarm-raising potential in chronic diseases, we proposed the described easy procedure. Using only baseline biomarker values and clinical course of participants with coronary heart disease, we identified the same cardiovascular biomarkers as those previously found containing prognostic information using longitudinal or survival analysis.

Keywords: biomarkers; cardiology; control charts; personalized medicine; reverse alignment.

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Introduction

For a biomarker to signal a serious event (outcome) during a clinically stable chronic disease, typical patients must show a biomarker change (rise or fall) prior to the event. The assessment of the clinical utility of such a biomarker, however, requires costly and difficult studies where the biomarker is measured regularly and for a long time in each patient. An appropriately designed schedule for interpreting the measurements as they arrive must also be devised. For this purpose, a procedure, known primarily from industry, is available, *viz.* the construction of a *control chart* [1]. For a brief review of the technique, see Box 1.

Box 1: Control charts.

Control charts

A control chart is used for plotting biomarker measurements within a given patient and over time to monitor whether the departures from the current average remain within statistically derived *control limits* [1]. So, to construct a control chart for a manufacturing process (or a patient), measurements must be made at regular time intervals. When enough measurements (say 10–20) have been obtained, the control-chart is constructed showing the mean ± an upper and a lower statistical control limit. If the values scatter at random relative to the mean (the *set point*) and all fall within the control limits, the process is said to be *in statistical control*. If a value falls outside a control limit (say the upper), it is concluded that the patient's set point has changed in an upward direction and a pre-planned procedure should be initiated [1].

However, as mentioned, the assessment of the utility of a control chart is both costly and complicated. Therefore, prior to such a venture, it would be useful to obtain some preliminary evidence that separates promising markers from others that hold little promise. It is for this purpose that we propose a procedure for crude provisional screening of the biomarkers. It utilizes a follow-up cohort of patients in a chronic but stable condition (or healthy people, for that matter) and attempts to extract longitudinal information from data that are epidemiologically speaking non-longitudinal (a single measurement per individual). In epidemiological terms, an inherently longitudinal question is given a preliminary, but low-cost answer, by juggling with data that hold no longitudinal patient records.

Our screening procedure exploits data from a cohort of patients, with records including date of patient entry, baseline biomarker value measured at entry, and date of the first occurrence of an outcome among those studied. Typically, a dataset from a large clinical trial that involves patients in a chronic but clinically stable state could be used. Depending on circumstances, one may then choose to limit attention to a control (placebo) arm as it is done in the example below.

Materials and methods

Materials (illustrative dataset)

To illustrate the application of our method we have chosen an example from cardiology using the placebo-treated cohort from the CLARICOR trial of participants with stable coronary artery disease (see Supplementary File 1) [2–8]. The 19 biomarkers [2–5] that we measured at randomisation were eight standard biochemistry variables (C-reactive protein/mg/L (CRP), glomerular filtration rate/mL/min/1.73 m^2 (GFR), ApoA1 lipoprotein/g/L (ApoA1), ApoB lipoprotein/g/L (ApoB), cholesterol-high density lipoprotein/mmol/L (Ch_HDL), cholesterol-low density lipoprotein/mmol/L (Ch_LDL), cholesterol/mmol/L (cholesterol), triglyceride/mmol/L (TG)), and 11 recently introduced cardiological biomarkers (N-terminal-pro-B-type natriuretic peptide/ng/L (NTproBNP), high sensitivity cardiac TnT/ng/L (cTnT), endostatin/ng/mL (endostatin), osteoprotegerin/ng/L (OPG), soluble TNF receptor 1/ng/L (sTNFreceptor1), soluble TNF receptor 2/ng/L (sTNFreceptor2), YKL40/µg/L (YKL40), neutrophil gelatinase-associated lipocalin/ng/L (NGAL), cathepsin-B/µg/L (Cath B), cathepsin-S/µg/L (Cath S), calprotectin/mg/L (calprotectin), see Table S2.1, Supplementary File 2).

The selection of 1,958 informative nine-years records from the 2,200 records of placebo-treated participants is detailed in Supplementary File 1.

We studied the first outcome event observed during follow-up, distinguishing between fatal composite cardiovascular outcome, non-fatal composite cardiovascular outcome, and non-cardiovascular death.

In this process we combined hospital information from the Danish National Patient Registry ('Landspatientregistret') with data from the Danish National Registry of Causes of Death ('Dødsårsagsregisteret') [9–12].

A composite cardiovascular (CV) outcome was defined as an admission with acute myocardial infarction (AMI), unstable angina pectoris (UAP), or a cerebrovascular disease (CeVD). It is classified as fatal if the participant died a CV death within 90 days and non-fatal if he/she was alive on day 91. The third outcome is a non-cardiovascular death prior to which no composite CV outcome was recorded.

Some participants died during follow-up without having had a CV outcome resulting in a hospital contact. In this situation we used the registry cause of death. If cardiovascular, the outcome counted as a fatal composite CV outcome; if not, it counted as a non-cardiovascular death.

Ethical approval: This research complies with the relevant regulations and institutional policies in concordance with the tenets of the Helsinki Declaration. Ethics approval and consent to participate was given by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015. Informed consent was obtained from all individuals included in the trial.

Methods (proposal)

The procedure we propose differs from a survival analysis where the predictors are ascertained at baseline ('Day zero') and the dependent variable (survival time) is regressed on the independent variable, the biomarker. In our method Day 0 is the day of outcome occurrence. For each outcome studied we delimit the group of patients in whom this outcome occurred as the first one following the participant entry. We then regress the biomarker value on time of biomarker measurement (which must be negative since the biomarker specimen was sampled prior to the occurrence of the outcome). Here, therefore, the dependent variable is the biomarker value, and the independent variable is the time of the collection of the specimen used for the biomarker assay. The regression line then depicts how the group biomarker mean changed prior to the occurrence of the outcome. For instance, if the slope of the line is >0, it will show that the closer one gets to Day 0, the higher was the group mean.

Thus, our method only requires a single baseline value of one or more biomarkers to be available per participant. The price one pays is that what can be studied as a function of time is only a group mean. However, if the outcome is preceded by a change in mean level, it suggests that the marker may be useful for patient monitoring purposes, and an added motive for further studies using the control-chart method is obtained.

Note, however, that there is no guarantee. From the shape of the mean curve one can neither prove nor disprove that the biomarker has an alarm-raising potential (or has prognostic implications). It all depends on what happens in individual cases – and when (relative to clinical events).

Before we proceed let us define three types of prognostic biomarker behavior that must be distinguished.

A long-term prognostic marker behavior is characterized by a stable linear increase (decrease) of its level that reflect the rise in severity (or decline in severity) of the disease as time goes by, and the time course is terminated by a specified event, e.g. the patient's death. Or e.g. the patient's remission etc. It all depends on the clinical context. Alternatively, the marker is characterized by a level that is stable over time, but prognostic across patients.

An alarm-rising prognostic behavior is characterized as follows: a time course of the marker values that (1) includes a clear lead time where all values would fall within the control limits of a control chart, and (2) is followed by a period where values outside control limits occur, and which is of sufficient length to be clinically useful and (3) is terminated by a specified event of clinical interest.

Either criterion should be met in typical, but not necessarily all, patients. A marker may satisfy both criteria or none of them.

Statistical analysis

Statistical analyses were done using the SAS version 9.4 and SPSS version 25. The analysis as described in the following is univariate with one dependent and one independent variable. Exceptions from this rule are dealt with in the Discussion. The analytical procedure comprises three steps.

Step 1: As explained above, for the study of each of the selected events, a pertinent study group of participants is selected from the data material. Each study group comprises participants in whom the event to be studied occurred as the first following the patient's entry into the cohort.

Step 2: Within each study group and following the outcome alignment, the records are subjected to a regression analysis designed to answer the question whether the mean level of one or more markers tends to increase, or decrease, during the months or years leading up to the event. We argue that, given that such a trend typically exists at the individual level, it leaves a trace at the group mean level when those who are in the same pre-outcome time phase have their measurements aligned as explained above.

Thus, if the outcome occurred 60 days following the biomarker specimen sampling day, the latter took place on Day−60. Figure 1 provides a graphical illustration. Using data from the applied example the Figure shows 199 participants who suffered a fatal cardiovascular outcome as their first event during follow-up. The biomarker value is plotted against time/day of participant's entry expressed using the new common timeline. Thus, the rightmost points correspond to participants who met their fatal event right after entry (blood sampling). The *leftmost* points represent patients who lived nearly to the end of their entire nine years (3,287 days) of follow-up.

Inspecting the estimated regression line, one notes that the mean biomarker value is seen to rise as we move from left to right, i.e., as we turn our attention to those patients who entered the cohort closer and closer to the event date. Since the relationship appears linear and covers several years, the slope of the regression line reveals how much the average of the log biomarker level increases per year as the distance between specimen sampling day and event day (the S-to-E period) decreases.

The key feature of the regression setup is this. The group mean signal may become statistically detectable as indicated by a statistically significant slope, despite the non-longitudinal character of the individual data records. That is likely to be possible if noise (i.e., the inter-individual variation in marker level) is low, whereas the size of the S-to-E period varies considerably, as is often the case. The underlying assumption is that the sampling date (cohort entry date) is itself non-informative, as it will be when participants are enrolled on a random day during a clinically stable phase. More specifically, the enrolment should not be triggered by day-to-day clinical fluctuations that are statistically associated with the marker nor by premonitory signs of the impending outcome.

Step 3: The regression graph (exemplified by Figure 1) is mandatory. It may suggest a simple transformation of the biomarker, especially if accompanied by an increase in variance; or a curved regression may indicate that marker changes do not occur until shortly before the event, so the analysis may be restricted to a shorter time range. These steps must be guided by existing knowledge and clinical plausibility. As said, the behavior of the biomarker in individual patients cannot be inferred from an analysis of the time course of the group mean. However, the biomarker depicted in Figure 1 is well established as a marker of left ventricular

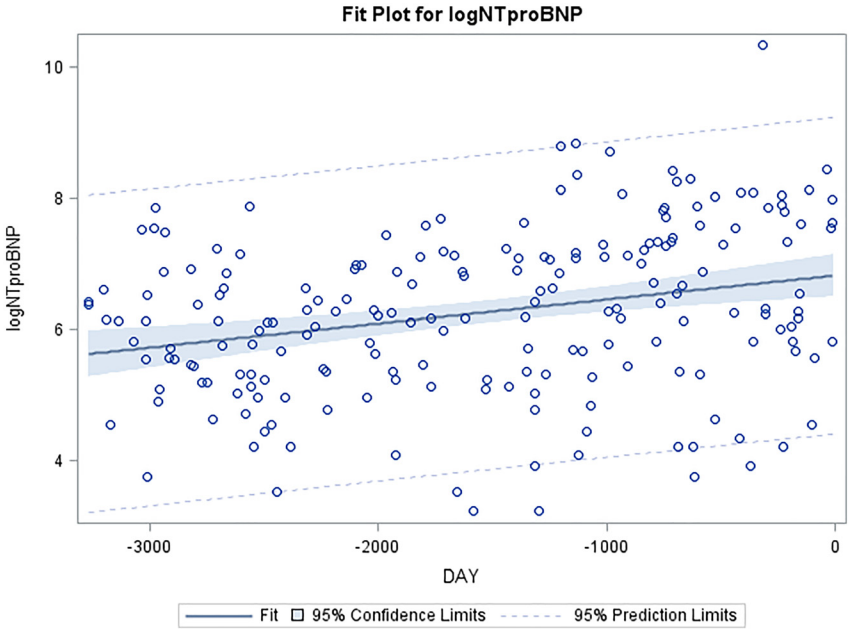

Figure 1: Proposed regression procedure illustrated by CLARICOR data (NTproBNP in Group 1, see later section).
The timeline shifts all outcome events to Day 0. The logNTproBNP is the natural logarithm of N-terminal-pro-B-type natriuretic peptide/ng/L. DAY: the day of specimen sampling relative to Day 0 (day of outcome).

function with prognostic implications. Thus, the most likely interpretation of Figure 1 is that it confirms that NTproBNP is a long-term prognostic marker rather than an alarm-raising marker (see definitions of the various biomarker behaviors in the Methods proposal section).

Results (methods proposal applied to example)

Step 1: Formation of the three study groups of participants

We identified three groups of participants, one for each type of outcome (see Table 1) and 872 participants who were alive after the follow-up and did not experience any cardiovascular outcome during the follow-up (see Table 2). The biomarker mean of the latter group may serve as a kind of reference.

Group 1 comprised 199 participants who all experienced a first and fatal composite CV outcome; Group 2 comprised 670 participants whose first outcome was a non-fatal composite CV outcome; Group 3 comprised 217 participants who all died a non-CV death without previously experiencing a non-fatal composite CV outcome during follow-up. Outside the three groups are the above-mentioned 872 participants.

Table 1 shows the distributions of the Sample-to-Event (S-to-E) intervals in each of the three groups and in each of the three subgroups of Group 2 as defined by the first occurring component of the composite CV-outcomes.

Step 2: The regression analyses of the three outcome groups

The upper part of Table 2 concerns the three study groups. Results are presented in part in the table and fully (all 19 biochemical markers) in Supplementary File 2, Table S2.1. Bold font p-values were significant using a Bonferroni adjusted significance level of 0.00088. The table shows the group mean *at baseline* and the annual percent increase or decrease in the group mean calculated from the slope of the regression line of the mean value on time.

We subjected each of the components of the non-fatal composite CV outcome of Group 2 to a separate regression analysis. Results are shown in the bottom section of Table 2 (and fully in Supplementary File 2, Table S2.2). Interesting findings were highlighted using an exploratory threshold of $p<0.010$.

Step 3: Appraisal of regression findings

In the upper section of Table 2 four trends were significant according to the Bonferroni-adjusted criterion. NTproBNP and cTnT are implicated in cardiac outcome and CRP in non-CV mortality. The significant and estimated annual change in NTproBNP in patients suffering a fatal composite CV outcome is 14.3%. This supports the contention by Mishra et al. [13] that a change in NTproBNP signals a CV death.

To complete the regression study, we have checked the model fit. The biomarkers show a reasonable fit of the

Table 1: Distribution of sampling-to-event (S-to-E) periods, i.e., days from sampling of biomarker-specimen and until occurrence of outcome in the three study groups.

Study groups	First event after randomisation	Distribution of S-to-E periods							
		Mean	5%	25%	50%	75%	95%	Min	Max
Group 1, n=199	Fatal composite cardiovascular outcome	1,542	154	713	1,385	2,463	3,016	12	3,270
Group 2*, n=670	Non-fatal composite cardiovascular outcome	1,227	73	496	1,082	1,864	2,894	0	3,186
Group 3, n=217	Non-cardiovascular death	1,733	222	1,027	1,764	2,552	3,146	45	3,276
*Subsets from Group 2	Non-fatal acute myocardial infarction, n=247	1,245	62	483	1,057	1,974	2,918	2	3,186
	Non-fatal unstable angina pectoris, n=227	1,076	62	444	990	1,558	2,588	13	3,025
	Non-fatal cerebrovascular disease, n=196	1,379	98	570	1,292	2,079	3,062	0	3,181

univariate linear model, with one exception, as is visible in Figure 2. The residuals of the regression of logcTnT on the revised timeline in Group 1 were right-skewed (probably because of the conventional low-level truncation at the measurability limit of 3 ng/L (log cTnT=1.10)). So far, we will assume that the biomarker level rises linearly over the whole range of time. A carefully conducted monitoring study is needed to decide what type of prognostic biomarker logcTnT really is.

As noticed by an anonymous reviewer, it appears that a substantial proportion of Group 1 has concentrations above the 99th percentile of the assay. The authors are not aware of any technical or geographical factors that may have caused a shift of the distribution. Therefore, the conclusion appears to be that, when chronic CAD patients while in stable phase show up for enrolment and cTnT testing, their cTnT levels are higher than in a healthy reference population (very plausible!); however, the prognostic impact of a high value is then much less gloomy than when the patient arrives in the emergency room with acute symptoms of coronary trouble as assumed by the European Society of Cardiology (ESC) guidelines [14] (again more than plausible!). Furthermore, the very fact that Group 1 is selected by outcome may explain the elevation: their cTnT level is about twice that of the event-free participants (exp(2.56) =13 vs. exp(1.76)=6 ng/L, Table 2).

As to the estimated rise in cTnT and NTproBNP in front of non-CV death (see Table 2, upper part), they are roughly as large as in the neighbouring cells, suggesting that they may represent facts after all (i.e., suspected type 2 error). Otherwise, most of the cells are far from statistical significance, with sTNF2 as a borderline exception (see Supplementary File 2, Table S2.1).

In the lower section of Table 2 we note that the average NTproBNP, cTnT, endostatin, and cathepsin B levels showed an increase prior to a stroke, or to some types thereof. A rise in ApoA1 and Ch_HDL was noted for acute myocardial infarction. We have no plausible explanation of these latter findings. In case of unstable angina pectoris all trends were minor with p-values greater than the threshold set by us.

In reports on the cardio-prognostic power of our biomarkers, OPG won a third place after NTproBNP and cTnT, but its prognostic impact appeared to be a longer-term one [5, 15, 16]. In the present analysis OPG, tellingly, failed to come out significant. This confirms that OPG is a long-term but stable risk marker and therefore unlikely to be fit for patient monitoring.

Discussion

To identify biomarkers which have a monitoring and alarm-raising potential we have proposed a simple and easy procedure, which is also almost cost-free when cohort data and baseline biochemical analyses are available.

We have illustrated the basic version of the procedure, but many variants are imaginable. With minor modifications the procedure can clearly be adapted to other types of cohorts, such as multi-stage cohorts, or to simultaneous change signals from several markers and clinical variables.

If laboriously collected cohort data are left unscreened, researchers risk overlooking a good marker in case its predictive merits lie not in its level but in its pattern of time change.

Limitations

The dominant obstacle in the type of analysis here proposed is the unavoidable inter-patient biological variation, i.e., the variation among the participants' stable-state marker levels (or 'set points'). A control chart uses the patient as his/her own control and does so in two ways: it

Table 2: Regression analyses of baseline biomarker on time of entry relative to time of outcome.

Analyses of the three study groups using Bonferroni-adjusted significance level=0.00088				
Biomarker (quantities)	**Group 1 Fatal composite cardiovascular outcome (n=199) Mean/unit, % change/year, p**	**Group 2 Non-fatal composite cardiovascular outcome (n=670) Mean/unit, % change/year, p**	**Group 3 Non-cardiovascular death and no cardiovascular outcome (n=217) Mean/unit, % change/year, p**	**Survivors without cardiovascular outcomes (n=872) Mean/unit**
High sensitivity C-reactive protein, mg/L log[a]	1.23 + 4.1%	1.07 – 1.1%	1.36 + 12.7%	0.86
	0.19	0.50	**0.0004**[b,c]	
N-terminal-pro-B-type natriuretic peptide, ng/L log	6.26 + 14.3%	5.34 + 6.2%	5.72 + 5.9%	4.88
	<0.0001	0.0046	0.09	
High sensitivity cardiac TnT, ng/L log	2.56 + 9.0%	2.05 + 5.0%	2.42 + 6.1%	1.76
	0.0001	**0.0001**	0.0048	
Exploratory analyses of the components of the non-fatal composite cardiovascular outcome (Group 2) using a threshold of p<0.01				
Quantities	**Acute myocardial infarction (n=247) Mean/unit, % change/year, p**	**Unstable angina pectoris (n=227) Mean/unit, % change/year, p**	**Cerebrovascular disease (n=196) Mean/unit, % change/year, p**	**Survivors without cardiovascular outcomes (n=872) Mean/unit**
ApoA1 lipoprotein, g/L	1.68 + 1.6%	1.65 – 0.20%	1.71 – 0.003%	1.71
	0.0009	0.75	0.99	
Cholesterol-high density lipoprotein, mmol/L	1.00 + 2.5%	0.99 + 0.46%	1.03 + 0.66%	1.03
	0.0012	0.63	0.44	
N-terminal-pro-B-type natriuretic peptide, ng/L log	5.36 + 5.3%	5.20 + 0.98%	5.48 + 14.6%	4.88
	0.14	0.85	**<0.0001**	
High sensitivity cardiac TnT, ng/L log	2.11 + 5.7 %	1.95 + 3.5%	2.08 + 6.8%	1.76
	0.014	0.11	**0.0017**	
Endostatin, ng/mL log	10.3 + 0.92%	10.3 – 0.04%	10.3 + 2.6%	10.2
	0.30	0.97	**0.0091**	
Cathepsin-B, µg/L log	10.7 – 0.27%	10.6 + 0.70%	12.0 + 4.2%	10.6
	0.80	0.61	**0.0007**	

[a]Log indicates that the quantity was log-transformed using natural logarithms. [b](logCRP example in Group 3). The regression coefficient is 0.1198 natural log units/year. This yields a factor=EXP[0.1198]=1.127, i.e., an estimated increase of 12.7% per year. [c]Bold font indicates that the p-value when Bonferroni-corrected was significant (< 0.05, which requires the uncorrected p to be < 0.5/57 = 0.00088), or p < 0.010 as the threshold for exploratory analyses.

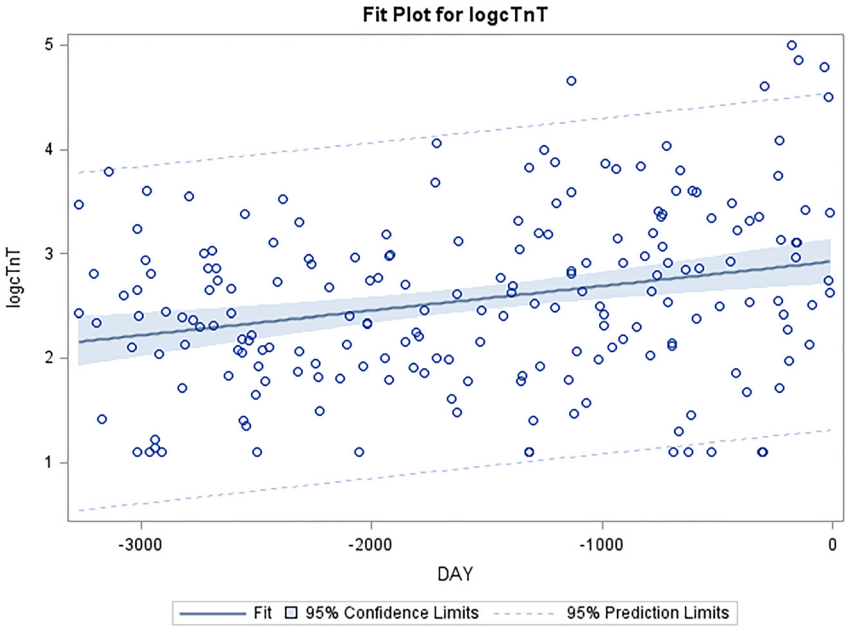

Figure 2: The natural logarithm of high sensitivity cardiac TnT/ng/L vs. day of sampling of specimen used for the biomarker assay in 199 CLARICOR placebo participants whose first cardiovascular event during follow-up was fatal (Group 1). The logcTnT is the natural logarithm of high sensitivity cardiac TnT/ng/L. DAY: the day of specimen sampling relative to Day 0 (day of outcome).

relates a change in biomarker level to the patient's set point, and not to a group average, and it judges the deviation in the light of within-patient random variation (the smaller the latter, the better the marker). In the present type of analysis, on the other hand, both individual set points and within-individual variance are inaccessible as they are buried in inter-patient variation.

Since age and sex often contribute to interindividual variation, it is natural to try to eliminate their influence on the precision of the estimated regression slope. We have repeated our analyses of logNTproBNP and logcTnT in all three study groups now adjusted by age (at patient entry into the cohort) and sex, to the extent that they contribute significantly ($p<0.05$) to model fit. Table 3 compares the unadjusted (Model 1) and the adjusted model (Model 2). The error SD fell by –9 to –36%, implying a similar sharpening of the slope estimate (DAY coefficient). Formally, this is clearly a post hoc analysis, but the results suggest that adjustment by age and sex should be regarded as an integral part of our procedure.

Our screening analysis cannot guarantee that a marker will be statistically fit, let alone clinically useful. It offers absolutely no information about the probability of false positive alarm (i.e., a loss of statistical control which is not followed by the exacerbation in question) or of a missed alarm (an outcome that is not preceded by a deviation from 'statistical control'). That is why the subsequent analysis of the biomarker's clinical utility may not be that easy and may be quite costly. As we have stressed, that is why we recommend a preliminary screening of the biomarkers before launching any longitudinal studies.

Steps towards subsequent confirmation of candidate biomarkers

Thus, screen identification of a potentially useful biomarker should be followed up by new studies in which one obtains the longitudinal biomarker series necessary to construct a control chart, or some essentially equivalent alarm schedule, and optimises the balance between delayed, missed, and false alarms or prove that the marker signal is too faint to be useful. In this situation it becomes important to identify confounding characteristics, especially those that affect eligibility, because they can be associated with an increase in a certain biomarker and thus influence the decision-making process. For example, suppose a control chart is being used to warn against myocardial infarction. In the case of NTproBNP, the onset of atrial fibrillation may cause a marker increase and hence a false alarm.

Ultimately, whenever one wants to introduce new biomarkers for monitoring and treatment-guidance, randomised clinical trials are needed to prove that their benefits trump any harms, and the final benefit-harm balance must be established through systematic reviews of the randomised and observational evidence [17–21].

Aspects related to statistical theory

As a somewhat crude exploratory technique for sifting markers and other clinical quantitative variables, the

Table 3: Improvement of regression model fit of logNTproBNP and logcTnT following adjustment by age and sex. Groups as in previous tables.

Quantity	Group	Independent variables in the models	p of model	Residual error (as standard deviation)	Percent change in error from model 1 to 2
logNTproBNP	1	Model 1 DAY	<0.0001	1.48	−15%
		Model 2 DAY AGE	<0.0001	1.29	
	2	Model 1 DAY	0.0046	1.71	−30%
		Model 2 DAY AGE SEX	<0.0001	1.32	
	3	Model 1 DAY	0.094	1.57	−18%
		Model 2 DAY AGE	<0.0001	1.33	
logcTnT	1	Model 1 DAY	0.0001	0.661	−9%
		Model 2 DAY AGE	<0.0001	0.608	
	2	Model 1 DAY	0.0001	0.604	−32%
		Model 2 DAY AGE SEX	<0.0001	0.458	
	3	Model 1 DAY	0.0048	0.590	−36%
		Model 2 DAY AGE SEX	<0.0001	0.434	

proposed method operates in a field where unbiasedness in a strict sense is hardly definable, let alone attainable.

Critical, however, are the assumptions that allow the timeline to be meaningfully redefined to have a common reference point at the occurrence of the outcome. As detailed above, this requires the blood-sampling date to be non-informative as regards any connection between marker level and outcome, an unverifiable assumption that can only be approximated by judicious choice of cases. At least, the condition studied should be clinically stable, and enrolment based on acute disease or disease events is banned.

To make the patients compatible it is necessary to operate with a well-defined time window. In our example, for instance, only 23 participants (11 + 12, see Supplementary File 1) had problematic nine-year records, and we discarded them. If there had been numerous such records, one would have had to consider to what extent their shortened time windows might affect the conclusion and how to deal with them.

As regards the *competing outcomes* problem, one outcome (say a non-CV death) may block the observation of another outcome (say an infarction) that would have occurred some weeks later, had the patient not died from non-cardiovascular causes. Provided that this is a common constellation, and infarctions are frequently preceded by a rise in the level of the biomarker studied, the rise may end up being wrongly attributed to factors underlying the observed non-CV death. However, from the point of view of this paper (early warnings, alarm-raising) there is no risk of wrong attribution, because we are assembling the data appropriate for warning purposes, not for studying pathophysiological relationships. To be specific, we study measurements made during (and thus representative of monitoring of) a clinical steady state which prevails until a 'first event' occurs, be it the event under current scrutiny or a competitor event that breaks off the data collection.

Conclusions

To identify biomarkers with an alarm-raising potential, we here proposed a simple and easy screening procedure, which is low cost when cohort data are already available, despite offering just one marker measurement per patient. When tested on CLARICOR data, it rediscovered the potentials of three much researched markers. But we emphasize that subsequent careful monitoring studies, followed, if successful, by control chart evaluation and randomised verification, are necessary to decide whether encouraging findings with our procedure carry the seeds of a clinically useful alarm-raising scheme.

Research funding: This study was funded by the Copenhagen Trial Unit, Centre for Clinical Intervention Research; the original funders of the CLARICOR trial [please see references 2, 3, and 7], and The Swedish Research Council, Swedish Heart-Lung Foundation; Thuréus Foundation; Marianne and Marcus Wallenberg Foundation, Dalarna University; and Uppsala University.

Author contributions: PW conceived the idea of the method and JH and PW reviewed, discussed, and improved the statistical rigor of the proposed methodology. PW wrote the programs used for computation of the tables and figures. JH, PW, CG, and JCJ prepared the manuscript which was subsequently reviewed by the cardiologists of the CLARICOR trial. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in the CLARICOR trial.

Ethical approval: Ethical and regulatory approval was obtained by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015.

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Supplementary Material: The online version of this article offers supplementary Material (https://doi.org/10.1515/cclm-2021-0333).