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Multi-marker network in ST-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention: When and what to measure

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ABSTRACT

Background: Data on the correlations between biomarkers to suggest cost-effective multi-marker (MM) panels predictive for ST-elevation myocardial infarction (STEMI) patients are lacking. We sought to explore the relationship between cardiac troponin I (cTnI), C-reactive protein (CRP), B-type natriuretic peptide (BNP), and chromogranin A (CgA) accounting for biomarkers' profiles detected within 48 h from successful primary percutaneous coronary intervention (PPCI).

Methods: In 73 STEMI patients cTnI, CRP, BNP, and CgA were measured before PPCI and 6, 24, and 48 h later. STATIS methods generalizing Principal Component Analysis on three-way data sets were employed to extract information about: 1) similarities between patients, 2) contribution of each time of sampling and 3) correlations between biomarkers' profiles.

Results: STEMI patients who underwent successful PPCI emerged to have a homogeneous profile tailored on biomarkers' evaluation within 48 h. Their measurements at 24 h contributed the most variability and information both to patients' and to biomarkers' profiles.

BNP and cTnI were highly correlated and explained the 40.1% of the total variance, whereas CgA resulted independent and explained the 26.3% of the total variance.

Conclusions: Markers' measurements at 24 h after PPCI contributed most information to the definition of patients' profile. BNP and cTnI resulted interchangeable in a MM panel for reporting about the extent of necrosis.

1. Introduction

Several circulating biomarkers have been reported to carry prognostic value in patients with a ST-elevation myocardial infarction (STEMI)[2] undergoing primary percutaneous coronary intervention (PPCI) [1–4]. In addition, a growing body of evidence has supported a multi-marker (MM) strategy, including biomarkers of necrosis, hemodynamic stress, and inflammation, to improve the prognostic assessment of STEMI patients after revascularization [5,6].

Some studies highlighted that in STEMI patients dynamic changes of these markers occurring in the course of the acute event more than baseline concentrations gather relevant information on the infarct size and on reperfusion effects [7–9]. This is in agreement with the fact that in the course of acute event in early reperfused STEMI patients blood concentrations of biomarkers are affected by wide-time dependent modifications [10]. In this perspective, the evaluation of dynamic changes of cardiovascular biomarkers rather than "static" concentrations may better describe the evolution of the acute event. Till now investigators have usually analyzed biomarker profiles one by one, despite that it is noteworthy that biomarkers of necrosis, hemodynamic stress, and inflammation are involved in overlapping pathways and an interrelation should be expected [11–13]. An effective MM panel, implying a minimal set of independent biomarkers, is indeed recommended to improve the prognostic assessment.

However there is a great gap in the current literature whether considering that any prognostic evaluation involving a MM strategy should imply the preliminary investigation of time dependent dynamics, patterns of release and relationships of co-detected biomarkers. In particular to avoid redundant and not cost-effective information it is mandatory to clarify the correlations between the joined profiles of candidate biomarkers.

In this study we aimed to explore the relationship among four cardiovascular biomarker profiles in the evolution of STEMI after PPCI in

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[2] Nonstandard abbreviations: STEMI: ST-elevation myocardial infarction; PPCI: primary percutaneous coronary intervention; MM: multi-marker; cTnI: cardiac troponin I; CRP: C-reactive protein; BNP: B-type natriuretic peptide; CgA: Chromogranin A; PCA: Principal Components Analysis; MACE: major adverse cardiovascular event; PC1,2: first, second principal component; AMI: Acute Myocardial Infarction.

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order to characterize a basic cluster of biomarkers providing independent information about the evolution of acute event. Furthermore, we sought to evaluate if the provided information about patients' profiles might be summarized by the simultaneous detection of these markers performed once. The biomarker profiles derived in the first 48 h after revascularization included cardiac troponin I (cTnI), C-reactive protein (CRP), B-type natriuretic peptide (BNP), and chromogranin A (CgA). As these biomarkers are involved in nested and sequential pathophysiologic pathways developing in the course of acute event (i.e. necrosis, inflammation, hemodynamic stress, and sympathetic activation), complex relations among their profiles are expected. Thus we resorted to STATIS methodology a statistical approach suitable to investigate the patterns of correlations between different *biomarkers*, measured at serial *time points*, on the same set of *patients*, independently by dimensionality. This technique belongs to the Principal Component Analysis (PCA) family, whose goal is to extract relevant information from sets of correlated variables, as biomarkers, without losing information carried by the original data [14]. Although classical multivariable statistical analysis might result of easier interpretation and is widely used in biomarker investigations, it does not enable us to obtain unbiased evidences for our purpose because of the high correlations between serial measurements.

2. Materials and methods

2.1. Patients

The complete study protocol, the enrollment criteria of STEMI patients who underwent successful PPCI and the employed analytical methodologies have been reported in detail elsewhere [10]. Here we briefly summarize the methodology. From 2007 to 2009 a continuous prospective case series of 87 STEMI patients undergoing PPCI were enrolled at the Catheterization Laboratory of Cardiology II of Maggiore Hospital of Novara. PPCI and pharmacological treatment were administered according to American College of Cardiology/American Heart Association/European Society of Cardiology Guidelines [15].

The inclusion criteria were: 1) ischemic chest pain; 2) ST-segment elevation in two contiguous leads on the 12-lead electrocardiogram; and 3) time from onset of symptoms to admission (admission time) <12 h. Exclusion criteria were: 1) successful thrombolysis, unsuccessful PPCI (TIMI from 0 to 2 at the end of procedure), emergency surgery, chest pain lasting more than 12 h; 2) dilated or hypertrophic cardiomyopathy, previous diagnosis of acute or chronic cardiac failure, cardiogenic shock, deep venous thrombosis or acute pulmonary embolism, infection, systemic inflammatory or neoplastic disease; 3) documented renal failure or clinical evidence of renal impairment; 4) Killip index ≥ 2; and 5) age >90 years. The study was approved by the Institutional Review Board and a written informed consent before PPCI was obtained.

2.2. Biomarkers

Venous blood samples were drawn before PPCI (baseline) and 6 h, 24 h, and 48 h after PPCI (time of sampling) into tubes containing EDTA (BNP) or no anticoagulants [cTnI, CRP, CgA, and Cystatin C (CyC)]. Samples were stored in aliquots at −80 °C until measurements. We employed the following assays:

1) TnI-Ultra™ assay with: analytical range of measurement 0–50 μg/L; limit of detection (LoD), 0.02 μg/L; cut-off, 0.04 μg/L; analytical coefficient of variation (CVa), 15% at 0.08 μg/L and 5% at 9 μg/L.
2) BNP immunoassay with analytical range of measurement 0–5000 ng/L; LoD, 2 ng/L; cut-off, 110 ng/L; CVa, 5.3% at 48.2 ng/L, 4.3% at 474 ng/L and 3% at 1797 ng/L.
3) CRP immunoassay with analytical range of measurement 0–163 mg/L; LoD, 1 mg/L; cut-off, 10 mg/L, CVa, 8% at 6.9 mg/L and 7.7% at 25 mg/L.

These assays were carried out on the Advia Centaur platform (Siemens Healthcare Diagnostics).

4) A manual CgA immunoradiometric assay (CIS-BIO), with analytical range of measurement 0–1370 μg/L; LoD, 1.5 μg/L; cut-off, 98 μg/L; CVa, 12.2% at 42.3 μg/L, 8% at 131.6 μg/L and 6% at 294.2 μg/L.
5) An immunonephelometric assay for CyC performed on BN-II nephelometer (Siemens Healthcare Diagnostics), with analytical range of measurement 0–7.58 mg/L; LoD, 0.005 mg/L; cut-off, 1.0 mg/L; CVa, 2.4% at 0.8 mg/L and 2.9% at 7.1 mg/L.

The protocol allowed deriving a complete biomarker release profile for cTnI, BNP, CRP, CgA and CyC [10].

Follow-up data were collected in order to possibly aid the interpretation of patient profiles.

2.3. Statistical methods

The interplay in overlapping pathophysiologic mechanisms of evaluated biomarkers suggested that in the present data complex correlations should be expected. Thus to assess possible associations between biomarker profiles we resorted to multivariate STATIS methodology [16,17]. In fact correlations among profiles of different biomarkers and among their serial measurements at different times of sampling prevented us from the application of classical multivariate statistical analysis.

The STATIS method generalizes PCA [18] to handle three-way data sets like those consisting of several *biomarkers* (variables) serially measured at sequential *times of sampling* in several *patients*. Fig. 1 shows the three-way structure for the present study. In particular, PCA extracts relevant independent information from a more simple two-way matrix of data consisting of *biomarkers* measured on *patients* by studying the pattern of association among biomarkers. Thus PCA identifies independent components (*principal components or axis*) as linear combinations of the original variables. The principal components are ordered on the basis of the amount of data variability they explain. Then PCA allows the visualization of results by a plot displaying projections of variables or patients on the space defined by the first two indentified principal components. As anticipated because of serial measurements, the methodology [16,17] suitable to this specific three-way structure of data is STATIS. This is performed twice: firstly to evaluate similarities between patients (DIRECT STATIS), and then to define biomarker release profiles (DUAL STATIS). Each STATIS procedure consists of two sequential steps namely *Interstructure* and *Compromise*. *Interstructure* evaluates possible correlations among different times of sampling and provides their optimal weights in the definition of patient profiles (DIRECT STATIS) or in the definition of average biomarker profiles (DUAL STATIS). Thus the *Interstructure* plot shows the most correlated times of sampling and in addition those mainly contributing to the definition of the *Compromise*. *Compromise* searches similar patterns among patients (DIRECT STATIS) or an average association structure between biomarkers (DUAL STATIS). To this purpose a standard PCA is performed on an average three-way data set derived from the original one (Fig. 1). *Compromise* results can be visualized by plotting patients (DIRECT STATIS) or biomarkers (DUAL STATIS) on the space defined by the respective first two principal components. These methods are commonly employed to analyze large sets of environmental data and other authors exploited them for cardiovascular framework [14]. All analyses were performed by R software, library *ade4* [19].

3. Results

3.1. Patients

Seventy-three out of 87 subjects entered in the present statistical analysis; particularly, we excluded 13 patients since marker measurements

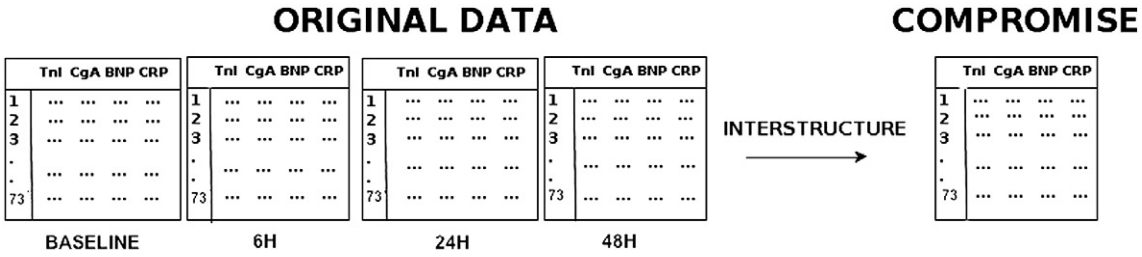

Fig. 1. Three-way structure of the study data:

1. Original data: simple data sets (*patients* × *biomarkers*) at each time of sampling.
2. Data set for *Compromise* assessment: average three-way data set (obtained from *Interstructure*) undergone standard PCA for *Compromise* definition.

were not available at all times and one who underwent unsuccessful revascularization ("no-reflow").

Baseline features of the enrolled patients and marker concentrations at each time of sampling are reported in Tables 1A and 1B. Although the original protocol implied the measurement of CyC, this marker was not further considered in the analysis since in our patients there was no evidence of increasing/decreasing kinetics. In addition, median CyC concentrations detected at each time point were <1.0 mg/L (threshold level reported in literature for considering the clinical relevance of marker concentrations) [10].

The first occurrence of a major adverse cardiovascular event (MACE), defined as cardiac death, recurrence of STEMI (re-STEMI), recurrence of PCI (re-PCI) of the infarct-related artery for in-stent restenosis, and re-hospitalization for occurrence of non ST-elevation acute coronary syndrome in a 1-year follow-up, was recorded. MACEs resulted in 10 patients (13.7%).

3.2. Similarities between patients: DIRECT STATIS

DIRECT STATIS allowed highlighting patient profiles (Fig. 2). Firstly, *Interstructure* investigates whether a common correlation structure between the four times of sampling could be assumed. *Interstructure* results in Fig. 2A suggest that times of sampling at 24 h and 48 h display a high correlation (quite overlapping arrows) measured in STATIS by the correlation coefficient RV = 0.914. Lower correlations resulted between times of sampling at baseline and at 6 h (RV = 0.610) and between those at 6 h and 24 h (RV = 0.735). According to the table of weights in Fig. 2B, times of sampling at 24 h were suggested to contribute most variability to patient profiles. Thus, it is likely that after 24 h the patterns of patients might not considerably change, whereas the main variation is evident from baseline to 6 h after treatment. Furthermore the *Compromise* plot (Fig. 2C) indicated a large homogeneity in patient profiles, with the first principal component (PC1) accounting for 71.3% of total variability and the second one (PC2) for 12.6%.

Only 2 patients (ID 32 and 15) in the middle/upper area of the plot and 2 patients (ID 51 and 55) in the down side of the graph showed a different average profile when compared to the other patients. We looked at the clinical features/biomarker profiles characterizing those patients isolated from the main group that can possibly explain the different behavior. Both patients 51 and 55 were characterized by a late and consistent BNP increase (at 48 h), associated with a typical kinetic for cTnI and to persistently elevated CgA concentrations. Only in the latter case there was a late CgA rising. No other clinical characteristics differentiated these patients from others plotted in the central part of the graph. Patients 32 and 15 differed from the others only because of lower CRP concentrations at all times of the sampling, probably due to a strong pre-admission anti-inflammatory treatment.

In summary, the results from the *Compromise* plot of DIRECT STATIS allowed the identification of a unique profile for STEMI patients who underwent successful PPCI.

3.2.1. Contribution of each time of sampling to patient profiles

The contribution of each biomarker measured at each time of sampling to the definition of PC1 and PC2 of DIRECT STATIS *Compromise* was further investigated. The derived panels (Fig. 3) were obtained by plotting projections of biomarkers on the space of the first two components of the *Compromise* according to the different times of sampling. According to this approach, the coordinates on PC1 had a

Table 1A
Baseline features of the patients.

Variable	Number of patients (%)
Male	68 (80.9%)
Hypertension	52 (61.9%)
Active smoking	43 (51.2%)
Diabetes	11 (13.1%)
Vasculopathy	3 (3.57%)
Family history of coronary artery disease	24 (28.6%)
Hypercholesterolemia	35 (41.7%)
Previous history of coronary artery disease	18 (21.4%)
Anterior MI	33 (39.3%)
Admission left ventricular ejection fraction >40%	65 (77.4%)
Medications	
Aspirin	27 (32.1%)
Plavix	13 (14.5%)
Statins	17 (20.2%)
Beta-blocker	18 (21.4%)
Angiotensin-converting enzyme inhibitor	22 (26.2%)
Calcium-channel antagonists	17 (20.2%)
PPCI features	
Number of treated vessels	
0	6 (7.1%)
1	74 (88.1%)
2	4 (4.8%)
Number of stents	
0	8 (9.5%)
1	47 (55.9 %)
2	17 (20.2%)
3	11 (13.1%)
4	1 (1.2%)
Patients submitted to GPIIb/IIIa inhibitors	45 (53.6%)

Table 1B
Median (25th; 75th percentile) biomarker concentrations at different time points.

Concentrations	Baseline	at 6 h	at 24 h	at 48 h
cTnI (μg/L)	0.43 (0.075;2.41)	74.3 (30.3;213.7)	48.3 (33.5;78.1)	22.2 (14.0;43.4)
BNP (ng/L)	27.8 (14.8;69.6)	71.45 (39.9;132.8)	173.9 (99.9;277.0)	125.7 (69.8;220.6)
CRP (mg/L)	2.6 (1.0;6.7)	3.4 (1.0;8.2)	16 (10.0;29.0)	31 (15.0;66.0)
CyC (mg/L)	0.73 (0.6;0.9)	0.72 (0.62;0.8)	0.74 (0.63;0.92)	0.74 (0.62;0.8)
CgA (μg/L)	72.95 (49.35;116.0)	75.0 (50.0;119.0)	97.0 (64.4;162.9)	127.0 (85.0;183.0)

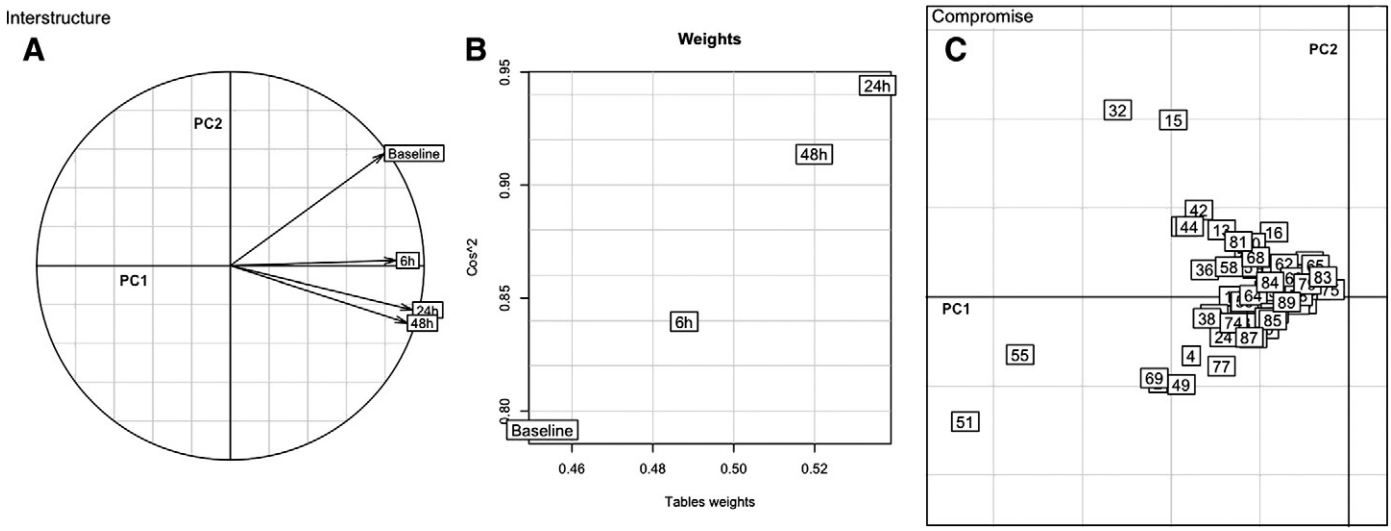

Fig. 2. DIRECT STATIS graphical visualization of results. A) *Interstructure* plot showing the high correlation between sampling at 24 h and 48 h (overlapping arrows). B) Table weights of the four times of sampling (square cosines vs weights) showing the highest importance of 24 h sample (higher squared cosines) in the definition of the *Compromise* plot. C) *Compromise* plot: projections of each patient according to average biomarker profiles on the space of the first two components (PC1 and PC2). This plot shows the high homogeneity among patients' profiles.

negative sign for all biomarkers measured at each time. On PC2 a negative sign was reported for all biomarkers, except for CgA.

Fig. 3 summarizes the contribution of all markers to the evolution of the profile of a patient in the course of the acute event. Across the four time windows, those markers showing relevant changes and mainly contributing to the definition of PC1 were cTnI and BNP. In particular, cTnI measured at 6 h and BNP at 24 h and also at 48 h provided the highest contribution.

The presented results complete the information on the dynamics of single biomarkers in the evolution of the acute event drawn in our previous study [10]. Accordingly the main markers showing dynamic changes across the four time windows were cTnI and BNP. In particular, cTnI shifted at 6 h far from CRP (a more stationary marker), coming back to the original position at 24 h. BNP seemed to follow the cTnI shift, but increasing its distance from cTnI and CRP at 48 h. CRP appeared as the most static marker, whereas CgA tended to slightly move at 48 h.

3.3. Similarities between patterns of markers: DUAL STATIS

DUAL STATIS allowed highlighting relationships between biomarker profiles. Graphical results, analogous to those obtained for DIRECT STATIS, are reported in Fig. 4. Since *Interstructure* extracts a common

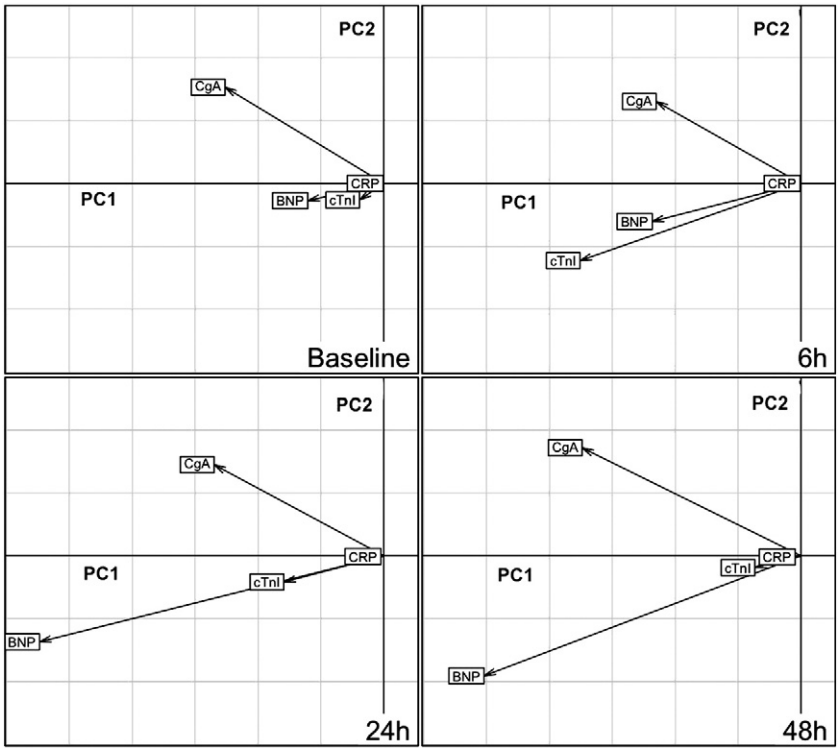

Fig. 3. Dynamics of markers according to times of sampling. PC1 = first principal component; PC2 = second principal component. Note that biomarkers move in a space with PC1 coordinates with negative sign.

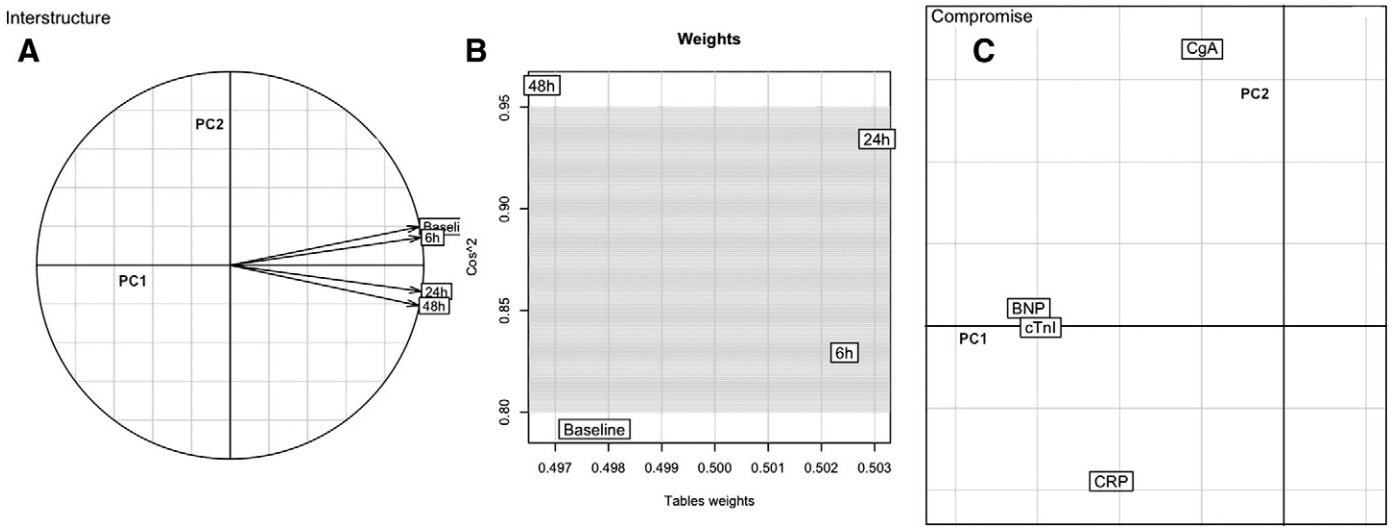

Fig. 4. DUAL STATIS graphical visualization of results. A) *Interstructure* plot showing the high correlation between sampling at 24 h and 48 h (overlapping arrows) and between sampling at baseline and 6 h (quite overlapping arrows); B) Table weights of the four times of sampling (squared cosines vs weights) showing the highest importance of 24 h in the definition of the *Compromise* plot. C) *Compromise plot*: projections of each biomarker according to average patient's profile on the space of the first two components (PC1 and PC2). This plot shows that the first principal component (PC1) is mainly explained by BNP and cTnI.

correlation structure between the four times of sampling, the plot (Fig. 4A) reported relevant associations between measurements at baseline and at 6 h and between those at 24 h and 48 h. The estimated RVs showed a high level of correlation among all times of sampling (RV >0.9), whereas the time of sampling mainly contributing to the definition of the *Compromise* and thus to average biomarker profiles was again 24 h (Fig. 4B).

In the *Compromise* analysis the first two principal components accounted for 66.4% of the explained variance; in particular, PC1 explained 40.1%. According to the *Compromise* plot (Fig. 4C), PC1 was mainly associated to BNP and cTnI, with a minor influence by CRP. Conversely, PC2 was mainly explained by CgA and CRP with coordinates of opposite signs, whereas the contribution of BNP was quite absent. Accordingly, we can speculate that BNP may explain a pathophysiologic mechanism that is orthogonal to that referable to CgA. From this analysis, BNP and cTnI provided quite overlapping information to patients' profiles and they may be considered as interchangeable in a MM panel. From the pathophysiologic point of view, PC1 may explain the extension of necrosis. Independent information was mainly contributed by CgA (orthogonal to previous markers) and less by CRP, which partially overlapped to BNP and cTnI. CgA and CRP seemed to have correlated, but opposite effects (positive vs negative sign). There is a common pathophysiologic mechanism (in sympathetic activation and inflammation) involving both markers that seemed to be positively triggered by CgA release and negatively depicted by CRP.

4. Discussion

A large body of literature has shown that blood concentrations of various cardiovascular risk biomarkers (i.e., markers of myocardial necrosis, hemodynamic stress/ischemic burden, inflammation, and sympathetic activation) detected after a STEMI are associated with clinical prognosis [1–5]. Due to compelling pathophysiologic assumptions, a MM strategy based on the simultaneous detection of different biomarkers seems to offer incremental prognostic value to common clinical risk scores [2,6,20]. However, only sparse data are available about those candidate markers to be combined in an effective MM panel for improving the risk profile of patients [6,9,21]. It is likely that some biomarkers may contribute redundant and partially overlapping information as the global biochemical milieu refers to a complex sequence of interacting and correlated pathophysiologic mechanisms developing during the acute event [9,11]. In this framework, two aspects should be stressed. First, few information is available about the relationship of different biomarkers in STEMI patients and this mainly threatens the selection of biomarkers for making reliable prognostic algorithms [6]. Secondly, STEMI is a dynamic phenomenon in which biomarker releases assume specific kinetics [10]. As a consequence, a single marker concentration detected at admission or just before revascularization is unlikely to capture the more relevant pathophysiologic changes, underlying STEMI evolution (i.e., infarct size, reperfusion effect, and inflammatory response), and greatly influence the patient's prognosis.

This concept has been well clarified by a wide literature reporting as the shape of the pattern of cTnI release within 48 h from revascularization carries a relevant prognostic impact. In particular, a rapid cTn increase, peaking at ~14 h, followed by a fast decrease mirrors the sharp washout of necrosis markers and a successful reperfusion [22]. According to the preliminary evidence on cTnI, in STEMI patients who underwent PPCI it is mandatory to investigate the release patterns of those biomarker candidate to a MM panel together with their correlations. Before evaluating the prognostic power of a MM panel and promote survival studies in these patients, a strong background on the joint behavior of the included biomarkers is currently required to be built up. For a reliable MM approach it is first mandatory to characterize a minimum cluster of biomarkers that may be selected whether contributing independent information about the pathophysiologic background of STEMI. Furthermore, it is relevant to define whether the biomarker profiles might be synthesized by one single measurement (traditionally assumed as the peak level) [3]. Thus, the optimal time of sampling should be assessed to gather most information from the simultaneous quantitation of different markers performed once. To this purpose, our study was performed on a homogeneous population of STEMI patients who underwent successful PPCI. In these patients, four relevant cardiovascular risk biomarkers (BNP, cTnI, CRP, and CgA) were measured before PPCI and 6, 24, 48 h later in order to derive complete biomarker profiles [10]. We identified firstly those markers contributing independent information to the assessment of average patients' profiles. Furthermore, we defined the optimal time of sampling to perform the simultaneous detection of markers contributing the largest amount of information to the patient's profile. Dealing with a great matrix of expected correlated data (i.e., correlations between intra-patient measurements of each biomarker and between concentrations of all biomarkers), we resorted to a complex PCA analysis that, with a more simple approach and explorative purposes, has already been applied for quantitative

profiling of large sets of biomarkers in the cardiovascular framework [14]. Despite its complexity, the STATIS method is recommended instead of the classical multivariate analysis to extract unbiased evidence from a three-way data set (*patients* x *biomarkers*×*times of sampling*) with a high grade of correlation between data.

The PCA analysis highlighted a high grade of similarity between average marker profiles in our patients. Most patients were characterized by quite overlapping mean profiles and this suggested that evolving STEMI after successful PPCI may be represented by a rather homogenous phenomenon, despite the complexity of the pathophysiologic background and the wide biomarker changes detectable across the four time frames (particularly for cTnI and BNP). Furthermore, it was relevant to define that the sampling time contributing most information from biomarker measurements to the definition of patients' profile was 24 h after PPCI. Thus, one single determination of all markers at this time may be enough, whereas marker determinations at baseline resulted less informative. This is not a marginal result since several studies assessed the prognostic contribution of cardiovascular biomarkers by accounting marker concentrations at admission or, alternatively, at a variable time before/after reperfusion, likely losing a great amount of information contributed by biomarker detection [3,12,20,21]. Increasing evidence has recently suggested that selection of the optimal time of sampling is critical to define the prognostic contribution of biomarkers in STEMI [8–10]. In fact, the marker release follows a specific dynamic, implying wide changes in concentrations within the first 48 h from the onset of the acute event [10]. This concept was further confirmed in this study: cTnI and BNP appeared as highly dynamic markers in contrast to CRP and, particularly, to CgA, which may be assumed as the most static ones. cTnI seems the early marker mirroring, between 6 and 24 h after PPCI, the perturbation of the cardiovascular system due to infarct and following immediate reperfusion. At 48 h, cTnI envisages the restoration of the original balance as it comes back to baseline concentrations. BNP followed the cTnI shift, as it reports overlapping information concerning infarct size, angiographic reflow and ST-segment resolution [23–25]. Persistent high concentrations of BNP at 48 h may be associated to additional pathophysiologic aspects that are not detectable by pure necrosis markers (such as ischemic burden, hemodinamic stress, left ventricular dysfunction, sympathetic activation, and counter-regulatory system activation) [26,27]. Quite unexpectedly, in the studied patients neither inflammatory response to infarct size (mirrored by CRP increase) nor sympathetic activation (by CgA) seemed to be relevant.

The second PCA analysis focused on the evaluation of correlations between biomarkers, showing that STEMI may be explained by two orthogonal pathophysiologic aspects. The first one is mainly described by BNP and cTnI determinations that were strictly related. According to specificities of these markers, this aspect should be most closely identified with myocardial necrosis, although BNP appeared to contribute further information to cTnI, being also influenced by previously described additional mechanisms. Our evaluation of biomarker profiles in the first 48 h after PPCI was able to throw a strong correlation between BNP and cTnI in contrast to other studies, which, resorting to baseline marker concentrations, found only a modest relationship [28,29]. CRP was shown to add only minimal independent contribution to the characterization of the pathophysiologic mechanism explained by cTnI and BNP, despite its partial correlation with both biomarkers. Although the relationship of CRP with traditional necrosis markers is well known and this result was rather expected, recent studies have reported that systemic inflammation (evaluated by CRP concentrations at baseline) may also enhance BNP expression independently of myocardial stretch and ischemia [12]. This unexpected pathophysiologic relationship may further complicate the biochemical substrate and potentially increase BNP informative power over the cTnI one.

A second pathophysiologic aspect is mainly depicted by CgA that resulted fully independent from BNP and cTnI. CRP partially contributed to this second component, but with an opposite effect with respect to CgA. Although some evidence about a correlation between CgA and BNP is available in literature [30], our data showed that these markers gathered independent information, in agreement with more recent findings [31]. Although CgA is considered as an index of increased sympathetic activity, its pathophysiologic role in acute myocardial infarction (AMI) should be yet characterized [4]. The complex neuroendocrine and immune activation following acute ischemia and associated left ventricular dysfunction was first suggested to trigger CgA production [32,33]. However, the lack of evident relationship between CgA and BNP has suggested considering this hypothesis with caution. In addition, alternative valid pathophysiologic explanations may be found if we consider the increase of the pro-hormone CgA as predictive for the increase of the derived bioactive peptides vasostatin and catestatin [34,35]. Their elevation following AMI and the possible pathophysiologic implications has been discussed [36,37]. Particularly, these peptides were found to be involved in various counter-regulatory processes, such as protection against the extension of myocardial infarction exerting a cardioprotective influence under infarct/reperfusion conditions against excessive excitatory sympathetic challenges [36,37]. It is relevant to highlight that catestatin exerts a direct protective effect on myocardium, independent of its anti-adrenergic and/or endothelial effects [36]. In this perspective, CgA profiles/measurements may explain a pathophysiologic mechanism orthogonal to the one explained by BNP and opposite to inflammatory response detected by CRP.

Despite the complexity of the statistical approach, a number of papers in the cardiovascular framework have recently resorted to methods generalizing PCA to handle MM panels and serial measurements [14,38,39]. This meets with expert opinions remarking that traditional regression analyses is possibly not sufficient to reliably explore the complex interplay between different biomarkers especially in a dynamic pathological framework. Nowadays, the application of more appropriate statistical methods is required to provide unbiased evidence [40]. As a limitation of this study, we are unable to evaluate the prognostic impact of those markers and of the time of sampling providing the main independent information to patients' profiles. The present case series is quite limited in the number of subjects and MACEs in order to permit survival analysis. On the other hand, this was not the aim of our study as presented findings are preliminary and basic to further prognostic studies requiring a different design, a higher sample size and incidence of MACEs [41]. In addition, we are aware that PCA extracts only linear combinations of available variables and that nonlinearity might be relevant in investigating biomarker profiles. However, our goal was to explore the contribution to overall variability of a set of correlated biomarkers serially measured and not to define a functional pattern to describe the biomarker profile across the different times of sampling. To our aim, linear combinations of biomarkers can contribute most information.

In conclusion, this study shows that BNP and CgA measurements at 24 h after PPCI may synthesize the main independent pathophysiologic mechanisms underlying STEMI. Our findings support previous data showing the complex implications of sympathetic activation [33–37,42–44] and the greater prognostic power of BNP, when compared with cardiac troponins in these patients [6,28,45,46]. These results may contribute a valuable tool to further optimize post-PPCI strategies in patients still at high risk for adverse events, despite the progress in reducing mortality made over the past decade [47,48].

Conflict of interest statement

None declared.

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