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INVITED EDITORIAL

A long way to translation: will cMyC survive?

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In the present issue of Basic Research in Cardiology [1], Baker and coworkers report on the time-dependent release of cardiac myosin-binding protein-C (MYBPC3) following cardiac injury. MYBPC3 is a cardiac-specific sarcomere protein involved in regulating cardiac structure and function [2]. Based on the previous experimental work, it is believed that MYBPC3 is being released earlier into the bloodstream compared to other cardiac proteins [3–5]. A potential explanation might be that MYBPC3 undergoes proteolysis following cardiac stress, leading to the production of a 40 kDa NH2-terminal fragment [6] that is abundantly located in an unbound fraction in the cytosolic pool. In a porcine model of permanent ligation of the LAD, MYBPC3 levels were significantly elevated within 3 h after ligation of a branch of the LAD, peaked after 6 h, and returned to the baseline values at 12 h when compared with other cardiac proteins [5]. Concentrations of cTnI, cTnT, measured with a conventional assay, and myosin light chain-3 were not significantly different within the first 3 h but continued to remain elevated at later time points [5]. In a TASH human infarct study, plasma MYBPC3 levels were significantly elevated within 4 h after ethanol ablation; whereas levels of conventional cTnT levels in the same samples were significantly increased only after 6 h [5].

We like the concept of using sarcomeric MYBPC3 as a biomarker for myocardial injury, since MYBPC3 shares many features with cardiac troponin T and I. MYBPC3 is a sarcomeric protein which is—like cTnI or cTnT—encoded by different genes in cardiac, slow skeletal and fast skeletal muscle resulting in the expression of tissue-specific MYBPC3 isoforms. Thus, MYBPC3 holds the potential of being a cardio-specific marker. However, no data are provided in the present manuscript on the true analytical precision, potential interferences and specificity in concomitant skeletal muscle injury of the MYBPC3 ELISA. Thus, to appreciate the potential cardio-specificity, we still need to wait for the results from properly powered and designed clinical trials.

Now, Baker and coworkers [1] compared MYBPC3 release with cTnT using a hs assay in ex vivo and in vivo animal models including a rodent model of permanent ligation of the LAD. In addition, they studied three patient cohorts including 20 patients with a STEMI, 20 patients with hypertrophic obstructive cardiomyopathy undergoing ethanol ablation of septal hypertrophy, and 20 patients who underwent elective CABG and in whom blood was obtained serially to evaluate the cardioprotective effects of ciclosporin. MYBPC3 was quantified by two different technologies, namely either immunoblotting or double sandwich ELISA. They define an early biomarker by its time to peak value and "speculate that cMyC (which is MYBPC3) could enable earlier diagnosis of myocardial infarction and re-infarction in suspected non-STEMI". However, based on the reported data in the present publication, we need to tamper the enthusiasm of Baker and coworkers and must indicate some critical limitations of this interesting work which are important for the readers of Basic Research in Cardiology.

First, Baker and coworkers test the release of MYBPC3 and TnI in cell culture using adult rat cardiomyocytes and in mice manipulated by LAD ligation. Based on these very

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distinct model systems, they conclude that "cMyC is released from rodent myocardium at least as quickly as cTn and can be detected in peripheral blood". However, on rodents, they provide data only on cTnI and not on cTnT. These data are, therefore, only relevant to cTnI since the appearance function of cTnI and cTnT differs markedly in patients with reperfused and non-reperfused myocardial infarction [7]. Furthermore, although they conclude "that the antibodies against cMyC and cTnI reveal similar sensitivity" (or do they mean affinity), they later indicate that "due to the interferences from mouse serum proteins it was not possible to detect cTnI in immunoblot reliably". These data clearly indicate the importance of the methodology used for comparative sensitivity analyses and the critical role of the experimental conditions used. In fact, the authors report that "there is some elution of full-length cMyC but no detectable cTnI in keeping with minimal infarction". However, in clinical care using the high-sensitivity assays, cTnI is detectable in many patients classified as unstable angina and can be measured in blood in the vast majority of presumably healthy subjects. Thus, the data on MYBPC3 and cTnI release in rodents indicate the poor sensitivity of the cTnI detection tools used and question the value of these experimental findings to predict the differential performance of MYBPC3 and cTnI in clinical care.

Second, Baker and coworkers then analyze cTnT time to peak value (and not cTnI) and the slope of MYBPC3 rise in patients with STEMI to proove that MYBPC3 is an earlier marker than cTnT. However, time to peak value is not a surrogate of early sensitivity of a biomarker. Early sensitivity is defined as the time for first detection of a significant elevation of a biomarker in blood following onset of myocardial injury/infarction. The time to early peak of cTnT is highly dependent on the quality of micro-vascular perfusion and the kinetic changes in blood of cTnT on day 1 post MI are actually well-established clinical indicators of success of reperfusion therapy. Thus, there is no peak of cTnT in patients with non-reperfused MI on day 1 but only a late peak on days 4–6, whereas there is a marked first peak in patients with excellent reperfusion amounting to values of cTnT up to 10,000 μg/L at 12 h after symptom onset. Furthermore, cTnT concentrations in blood were determined by ELISA; while MYBPC3 release was determined by densitometry of immunoblots. These are two different analytical modalities which may not necessarily provide comparable results. It would have been preferable to compare ELISA measurements of both MYBPC3 and cTnT or if immunoblotting is the preferred method to compare the immunoblot results of MYBPC3 with cTnT using the very same monoclonal antibodies which are employed in the final ELISA.

Third, inappropriate assay sensitivity of the MYBPC3 assay precludes conclusions on time-dependent release and clearance of this marker. The present immunoassay has a limit of detection at 116 ng/L, a limit of quantification (concentration that can be measured with a CV $\leq$10 %) of 640.0 ng/L, and a coefficient of variance <20 % for the concentration range of 640–400,000 ng/L [8]. In this context, even selective inclusion of large STEMI as defined by a peak hsTnT concentration >2000 ng/L at 12 h after admission did not prevent that 2 of 20 STEMI patients did not disclose detectable MYBPC3 concentrations, at all. Previous findings [5] on time release of MYBPC3 versus cTnT and CKMB following TASH demonstrate an early release of MYBPC3 reaching a peak at 4 h with a mean concentration below 600 ng/L, a concentration that cannot be quantified with an acceptable imprecision of less than 20 % [7]. The issue of imprecision might explain the rationale to use the time to peak concentration and slope rather than the time to concentrations defining an MI.

Fourth, another important drawback of this study is patient selection, restricting quantification of MYBPC3 only to patients with large STEMI and measurement of MYBPC3 only in off-target study populations such as STEMI, TASH-related MI and type V MI post-elective CABG. In clinical practice, troponin testing in STEMI is discouraged unless information on infarct size or prognosis is pursued [9]. Conversely, non-inclusion of patients with NSTEMI, the established target population for troponin testing, is an important limitation for the evaluation of clinical performance of MYBPC3 for both clinical sensitivity but also for clinical specificity.

Finally, we appreciate the investigation of release kinetics of cardiac biomarkers in patients with hypertrophic obstructive cardiomyopathy (HOCM) undergoing a TASH procedure. However, this model does not reflect release kinetics in ACS patients. TASH is a model of permanent micro-vascular obstruction due to persistent septal artery occlusion following ethanol injection, a condition only rarely found in the clinical care of ACS patients. As indicated, the perfusion-dependent release of unbound cTnT on day 1 is entirely absent under these conditions of persistent micro-vascular obstruction and as such it is not surprising that MYBPC3 will peak earlier than cTnT. However, it is not the time to peak that matters for early sensitivity but the detection of an analytically robust elevation of biomarker in blood compared to blood levels of the very same biomarker in a large clinical cohort without myocardial injury.

There is an additional point that may be raised against the use of TASH treated HOCM patients to assess MYBPC3 as a potential biomarker of myocardial injury. HOCM in European populations is in more than 26 % of cases caused by mutations of MYBPC3 [10]. It would thus be interesting to know how many patients of the current study group suffered from MYBPC3 disease and whether there was any interference of antibody binding in mutated

MYBPC3 or differences in recovery of MYBPC3 by the developed double sandwich assay, which requires preservation of two epitopes in a mutated protein for dual antibody binding.

Thus, in summary, we share the excitement with Baker and coworkers that MYBPC3 may be an interesting biomarker for myocardial injury. However, we have seen many carefully selected biomarkers believed to replace or partner with the cardiac troponins which were lost in translation. Therefore, despite the interesting data provided by Baker and coworkers, the added value of MYBPC3 in clinical care remains speculative and much more robust work is needed to better understand the potential role of MYBPC3 as biomarker of myocardial injury.

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