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Translational proteomics in cardiogenic shock: from benchmark to bedside

Oriol Iborra-Egea[1,2], Antoni Bayes-Genis[1,2,✉]

1. Heart Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; 2. Department of Medicine, CIBERCV, Universitat Autònoma de Barcelona, Barcelona, Spain
✉ Correspondence to: abayesgenis@gmail.com
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An acute coronary syndrome can compromise blood flow to certain areas of the heart and cause ischemia and myocardial cell death, which can lead to ventricular dysfunction. If this myocardial injury is large and the ventricular dysfunction is severe, cardiogenic shock (CS) may develop, which results in a life-threatening state of hypoperfusion and critical hypoxia of vital organs. Acute myocardial infarction (AMI) with left ventricular dysfunction is the most common cause of CS (80% of the cases). Other possible causes of CS include myocarditis and acute decompensation of chronic heart failure (HF).[1]

The incidence of CS has increased notably during the last decade, in part due to important macrotrends, such as the aging of the population. The incidence of CS derived from AMI increased from 6.5% in 2003 to 10.1% in 2010, with similar trends observed in patients with congestive HF not preceded by AMI.[2] Nonetheless, in-hospital mortality decreased from 62% in 2004 to 48% in 2014, probably due to significant advances in revascularization and supportive treatment, such as the use of mechanical circulatory support (MCS) devices. However, mortality remains unacceptably high, with one in two patients dying within ninety days.[3]

Overall, CS is estimated to affect between two million people and four million people in more developed countries (Table 1).

The multiorgan failure associated with CS is the source of innumerable circulating molecules that has been of great value in the characterization of CS, since contemporary advances in "omic" technologies provide new insights into a more holistic molecular signature of CS, including the inflammatory response and the involvement of other vital organs (kidney, liver and intestine, among others) beyond the myocardial dysfunction.

Indeed, MCS devices are transforming the management of HF and represent the first life-saving treatment in emergencies like CS. Unfortunately, the implantation of this type of device requires a specialized center and equipment, it is highly invasive, with serious risks for the patient and enormous associated costs. Therefore, deciding which patient needs an MCS and which patient could do well with only standard treatments becomes an absolute necessity. Moreover, this is worsened by the rapid downward spiral evolution of CS and sometimes decision-making can be subjective, which entails greater risks for patients and a greater economic burden for health system. Sadly, molecular tools for CS prognosis have not yet been developed to guide such therapies.[4]

In recent years, two clinical scores (CardShock and IABP-SHOCK II) have been developed to try to aid in the prognosis of CS.[5,6] Both scores are calculated from clinical variables at baseline and associated with short-term mortality. However, the only laboratory parameters included in these risk scores come from classical biochemical tests, glucose and lactate, which are the only metabolic biomarkers used in this pathology since 1950s. In addition, in emergency situations such as CS, the time needed to make a decision is crucial for the patient, and classic clinical variables may take time to reflect alterations in their values, which in turn make the decision-making of the medical team more imprecise and late.

Many studies have explored different predictive

Table 1 Total number of cases of myocardial infarction, other cardiovascular causes of cardiogenic shock and cases of cardiogenic shock, and implied costs of treatments. Other cardiogenic shock causes include decompensated heart failure and myocarditis. Number of cardiogenic shock cases have been estimated accounting for an 8% incidence over myocardial infarction cases plus 5% incidence over other cardiogenic shock cases.

Country	Myocardial infarction incidence	Other causes prevalence	Number of cardiogenic shock
France	67,749	1,500,000	79,940
Germany	232,377	3,320,000	184,590
Italy	130,090	723,977	46,606
Japan	90,757	1,316,790	73,100
Spain	90,891	1,880,000	101,271
The United Kingdom	73,232	999,750	55,846
The United States	805,000	7,293,973	429,099
China	1,603,994	12,986,033	777,621
India	1,944,012	9,291,105	620,076
Total	5,032,102	39,311,628	2,368,150
Spain	**Number of therapeutic mechanical circulatory support**	**Cost of treatment**	**Total cost for hospitals**
Myocardial infarction + Heart failure	1,970,891		
8% + 5% cardiogenic shock	101,271		
10% mechanical circulatory support	10,127.1	22,000	
Total			222,796,200 €

cardiac and extracardiac biomarkers in the CS setting;[7] however, most of them are small-scale studies that have not been validated in large cohorts of patients, did not evaluate the added predictive value of these biomarkers in combination with current clinical practice or have not been able to add prognostic value to this pathology.[8]

Nonetheless, two newly discovered biomarkers pose a real opportunity to tackle this issue.

Dipeptidyl peptidase 3 (DPP3) has been shown to modulate cardiac contraction and kidney hemodynamics in severe HF in a mice pre-clinical model,[9–11] and circulating concentrations of DPP3 are elevated in different types of shock.[12,13] More specifically, Takagi K, *et al*.[13] performed an ancillary analysis of the OptimaCC trial (Unique Identifier: NCT01367743), and found that patients with refractory CS displayed higher levels of cDPP3 than those with non-refractory CS up to 48 h (P = 0.027). Moreover, CS patients with high cDPP3 levels (≥ 59.1 ng/mL) at inclusion presented with higher severity, lower cardiac index, and lower estimated glomerular filtration rate. Additionally, Deniau B, *et al*.[12] found that high DPP3 levels in plasma were also associated with increased short-term mortality risk [hazard ratio = 1.4 (1.1−1.8)] and severe organ dysfunction in 174 CS patients from the CardShock cohort.

On the other hand, our research group defined the Cardiogenic Shock 4 Proteins (CS4P) risk score in 2019.[14] Through a quantitative proteomic analysis using mass spectrophotometry, 2,654 proteins were analyzed in patients diagnosed with CS in the RutiShock cohort (n = 48), of which 51 proteins differed in their abundance between surviving and non-surviving patients at ninety days. Finally, a combination of 4 proteins presented the best classification results (CS4P). The European CardShock cohort (n = 97) was used to cross-validate the obtained protein classifier, which is based on circulating levels of liver fatty acid binding protein, beta 2-microglobulin, aldolase fructose-bisphosphate B, and complement inhibitor C1. The CS4P score improved the ninety-day mortality prediction versus the CardShock score [area under curve (AUC) = 0.76] both independently (AUC = 0.83, CS4P) and in combination (AUC = 0.84, P = 0.03; CS4P + CardShock). More importantly, the CS4P shows a notable benefit in reclassifying patients, with a classification improvement of 0.49 (P = 0.020) compared to the CardShock score, which translates into an improved reclassification of 60% of patients. Next, we seeked to validate our results using a translational assay available in clin-

ical laboratories. Thus, the classification power of CS4P was confirmed by the enzyme-linked immunosorbent assay (ELISA), with no statistical differences compared to mass spectrometry, indicating clear transferability to the clinical setting.

Here, we want to discuss the translational process of the CS4P score towards a clinically available solution.

By developing a simple assay containing the measurements in serum/plasma of the 4 proteins contained in the CS4P score, clinicians could rapidly and effectively assess each patient development in a personalized manner. Thus, clinicians could take more informed decisions in an already stressful situation, decreasing therapy risks and likely reducing CS-associated costs.

In the translational path towards clinical implementation, we filed a European Priority patent for CS4P (EP19382126.1). Although this is not always necessary, it is crucial to develop a strong intellectual protection strategy to ensure the project can be developed safely; whether this involves a patent (or a family of patents), an industrial design, copyright agreements or industrial secrets depends exclusively on the project and the tech transfer responsible(s). Finding a great intellectual protection law firm to delineate such strategies and execute properly and timely should be of the utmost importance for the team, especially in the biomedical field.

It is important to emphasize avoiding a technology push to market, and instead find a true pull from the market to solve a real pain. In other words, a technology looking for a market is potentially a death knell. Here, the pull needs to come either from the patient (who benefits from the technology), the physician (end user of the technology), a BioPharma company (who solves a strategic need) or public healthcare providers (who reduce costs and/or improve societal pains).

Moreover, being unique and first in class is not immediately an asset *per se*, as it demands you to create the strategic roadmap instead of following an established, more secure one.

Recently, we have identified and characterized the preliminary product by exploring assay components via screening, identifying and evaluating critical technologies and components, and characterized the lead design. We have demonstrated the preliminary assay with simplified samples and the sensitivity and specificity with spike/recovery studies in the appropriate settings.

All of this has allowed us to develop a proprietary chemiluminescence immunoassay (CLIA) with much higher sensitivity than standard ELISA tests. Although both methodologies are based on the sandwich type immunoassay reaction in which the antigen is immobilized between a pair of antibodies, in the case of the ELISA, the detection antibody is coupled with an enzyme that catalyzes a chemical to produce a change in color, but in the CLIA test, the same antibody catalyzes a chemiluminescent reaction to produce a change in the intensity of the light observed.

Once developed, these antibodies need to be validated analytically.

First, we need to ensure the antibodies developed correlate well with the previous results obtained with commercially available ELISA assays. Here, follow the Guidance for Industry on Bioanalytical Method Validation:

ISR result variability (%) = (repeated value – original value)/mean × 100%

Where mean = (repeated value + original value)/2

The ISR is considered successful if > 67% of the samples meet the criteria.

Model calibrations require using the Hosmer-Lemeshow test, and patient discrimination and reclassification are recommended to be evaluated using the Harrell C-statistic and continuous net reclassification improvement.

To avoid interference or reproducibility problems related to external factors, when comparing both methodologies, the same technical staff has to be involved in the evaluation of the two methods at the same time for each protein of the CS4P panel, following the guidelines of good laboratory practices and developing standard operating procedures that will be duly completed and stored.

Next, we need to characterize the stability of both the antibodies and the analyte within the sample matrix of interest. Understanding storage conditions, the linearity range, limit of detection and limit of quantification, accuracy and precision of the antibodies is essential for their distribution and use in emergency laboratories, and demonstrating that results obtained with frozen samples (which is the

standard condition of most clinical trials) can translate efficiently to fresh samples is required to match the results to the clinical reality in which the assay will be used.

Finally, all of these validations need to follow a strict regulatory roadmap that ensures the applicability and commercialization of the assay.

The European Union's new in vitro diagnostic regulation (IVDR) of medical devices 2017/746 is drastically impacting the in vitro diagnostic industry at varying levels. Under this directive, all products that stay on the market following IVDR transition must be reassessed for compliance to ensure that products are fit for purpose and safe for use.

Under the new set of regulations, a quality management system (QMS) gains even more importance. A QMS is a standardized system that documents processes, procedures, and responsibilities for achieving quality policies and objectives, which helps coordinate and direct the necessary activities to meet customer and regulatory requirements and improve its effectiveness and efficiency. For medical devices, pharmaceutical drugs and in vitro diagnostics, the ISO 13 485 is the most recognized and implemented QMS.

It is very important to follow a regulatory strategy from early on in the research and development process, so the testing can be planned and performed to comply. This also helps when submitting applications to Health Regulatory Agencies.

Regulations are meant to ensure the reproducibility, safety and correct application of medical products and services. For these very reasons, every country has its own regulations, which are constantly being reviewed and require specialized audits before obtaining the final registration.

In Europe, this registration is the Conformite Europeenne (CE) mark, which after notifying the European Union Competent Authority (of the country where the legal manufacturer, or the European Union Authorized Representative, has its legal entity), the company needs to request the certificate of free sale, certifying that the products listed in the certificate are CE marked, they are compliant with the IVDR and they are approved for export and trade in international countries.

All of this information must be evidenced in a technical file which may need to be submitted to a Notified Body for review (dependent on device classification) to enable CE marking and market access.

With this article, we aim to present our journey in developing a new biomarker for a condition with a very marked unmet clinical need, as well as to provide a short guide for other researchers that want to pursue similar projects and lack references to advance their translational research.

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