[4] Farkas J, Nabb S, Zaletel-Kragelj L, et al. Self-rated health and mortality in patients with chronic heart failure. Eur J Heart Fail 2009;11:518–24.
[5] Shadbolt B, Baresi J, Craft P. Self-rated health as a predictor of survival among patients with advanced cancer. J Clin Oncol 2002;20:2514–9.
[6] Farkas J, Kosnik M, Flezar M, et al. Self-rated health predicts acute exacerbations and hospitalisations in patients with COPD. Chest 2010;138:323–30.
[7] Johansson P, Broström A, Dahlström U, et al. Global perceived health and ten-year cardiovascular mortality in elderly primary care patients with possible heart failure. Eur J Heart Fail 2008;10:1040–7.
[8] Maggioni A, Anker SD, Dahlstrom U, et al. Are hospitalized or ambulatory patients with heart failure treated in accordance with ESC guidelines? Evidence from 12,440 patients of the ESC Heart Failure Long-Term Registry. Eur J Heart Fail 2013;15:1173–84.
[9] McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2012;14:803–69.

0167-5273/$ – see front matter
http://dx.doi.org/10.1016/j.ijcard.2013.12.229

Genetic polymorphisms of human cardiac troponins as an unrecognized challenge for diagnosing myocardial injury

Giuseppe Lippi [a,*], Gianfranco Cervellin [b]

[a] Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy
[b] Emergency Department, Academic Hospital of Parma, Parma, Italy

ARTICLE INFO

Article history:
Received 5 December 2013
Accepted 30 December 2013
Available online 8 January 2014

Keywords:
Genetic polymorphisms
Single nucleotide polymorphism
Cardiac troponin
Myocardial injury
Myocardial infarction

Human cardiospecific troponin I (cTnI), the inhibitory subunit of the troponin complex, is encoded by the *TNNI3* gene, located on chromosome 19q13.4. The final transcript is a 200 amino acid protein (Fig. 1), with a predicted molecular mass of 24 kDa [1]. Human cardiospecific troponin T (cTnT), the tropomyosin-binding subunit of the troponin complex, is instead encoded by the *TNNT2* gene, located on chromosome 1q32. The final transcript is a 298 amino acid protein (Fig. 1), with a predicted molecular mass of 35.9 kDa [1].

The measurement of cardiospecific troponins, thus entailing cTnI and cTnC, has now become the cornerstone for diagnosing a wide spectrum of ischemic and non-ischemic cardiac injuries. In particular, their assessment is pivotal for diagnosing non-ST elevation myocardial infarction (NSTEMI), where electrocardiographic findings are understandably lacking. The recent development of novel and more analytically sensitive immunoassays for measuring these important biomarkers (i.e., contemporary-sensitive and high-sensitivity methods), has substantially improved the efficiency of cardiac injury detection, wherein modestly increased concentrations of cTnI and cTnT can now be detected more efficiently and precociously than with previous methods [2]. Although, these technical advancements have allowed to achieve a final diagnosis of acute myocardial infarction (AMI) within 2–3 h from the onset of symptoms in the vast majority of patients [3], the sensitivity and negative predictive value of contemporary-sensitive and high-sensitivity immunoassays remain suboptimal, and a variable percentage of patients – ranging from 6 to 23% – has normal levels of cardiospecific troponins at presentation, even when measured with high-sensitivity immunoassays [4].

Various hypotheses have been proposed to explain the modest but meaningful number of "false negative" cardiospecific troponin tests in patients who are then finally diagnosed as having an AMI, which include preanalytical issues (i.e., sample misidentification, spurious hemolysis), analytical errors (instrument malfunctioning, reagent deterioration) and interference (e.g., heterophilic antibodies and rheumatoid factor) [1]. Another potential and virtually unrecognized source of false negative test results is then represented by genetic polymorphisms of cardiac troponin genes. The various contemporary-sensitive and high-sensitivity immunoassays contain a heterogeneous cocktail of (monoclonal) antibodies for the capture and detection of the molecules (Table 1), which specifically react against targeted domains of the proteins [5]. It is hence predictable that natural variants of both cTnI and cTnT containing single nucleotide polymorphisms (SNPs) that involve (and structurally modify) any of the epitopes recognized by these antibodies, would decrease or even impair the binding properties of the commercial antibodies. We have hence performed a search on the database of UniProt, which is a comprehensive and high-quality resource of protein sequence and functional information [6], to identify all SNPs in the *TNNI3* and *TNNT2* genes that may involve antibody-binding domains of either cTnI or cTnT. This search identified 19 and 4 of such polymorphisms in the sequence of *TNNI3* and *TNNT2*, respectively. We also found that 71% (i.e., 12/17) of commercial cTnI immunoassays, as well as all four cTnT immunoassays, contained antibodies targeting SNP-containing domains in the *TNNI3* and *TNNT2* genes (Table 1) [7–16]. In some cases, the monoclonal antibodies recognized regions of the proteins containing more than one single SNP. Even more interestingly, the vast majority of these SNPs were associated with inherited cardiac disorders, namely idiopathic dilated cardiomyopathy, familial hypertrophic cardiomyopathy and familial restrictive cardiomyopathy, whereas two SNPs of the *TNNT2* gene have been reported to be clinically silent so far (Table 2). It is noteworthy that the vast majority of these

* Corresponding author at: U.O. Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14, 43126 Parma, Italy. Tel.: +39 0521 703050; fax: +39 0521 703791.
E-mail addresses: glippi@ao.pr.it, ulippi@tin.it (G. Lippi).

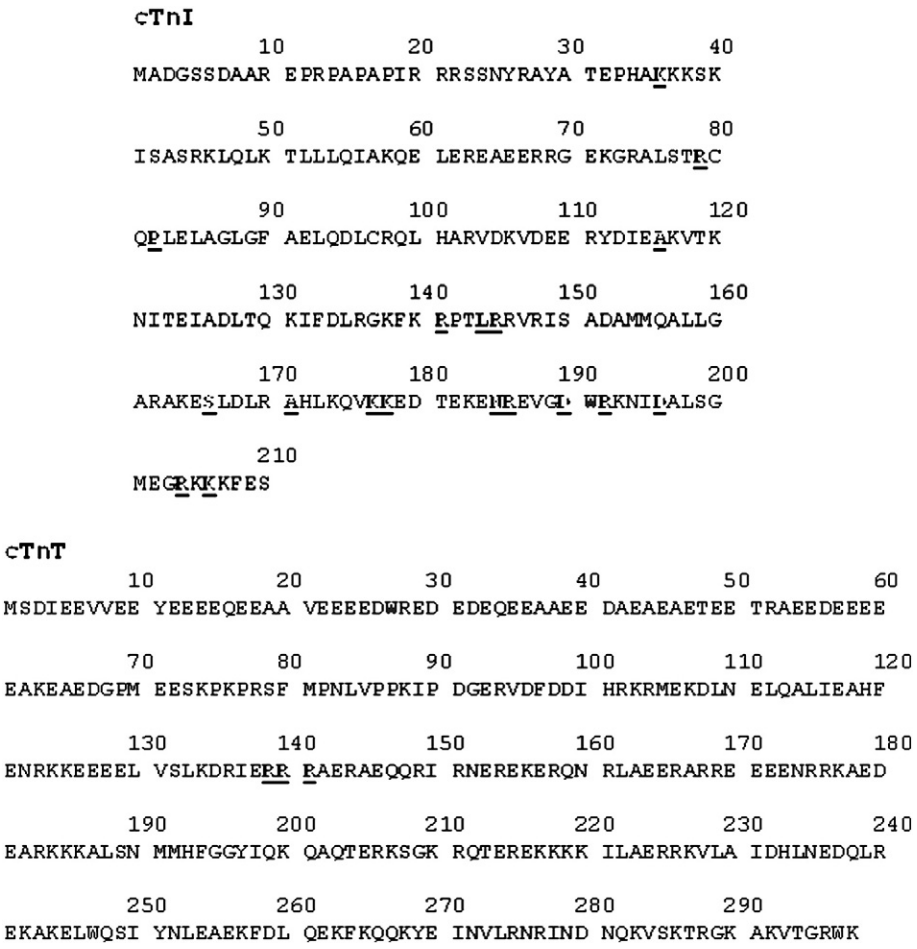

Fig. 1. Amino acid sequence of cardiospecific troponin I (cTnI) and cardiospecific troponin T (cTnT). The single nucleotide polymorphisms (SNPs) that may impair the immunoreactivity of the molecules are shown in bold and underlined.

inherited conditions are characterized by incomplete penetrance, variable expressivity and seldom late onset (i.e., not rarely after the fifth–sixth decade of life) [17].

According to this data, it is hence nothing but unlikely that carriers of one or more of these SNPs may suffer from AMI or other acquired cardiac injuries earlier than the onset of symptoms of cardiomyopathy, and may hence be misdiagnosed and potentially mistreated due to a false negative cardiospecific troponin test. It may hence be advisable that the next generation of cardiospecific troponin immunoassays should be designed to contain antibodies reacting not only with the more stable region of the proteins, but also against domains that do not contain known SNPs (Fig. 1).

References

[1] Lippi G, Targher G, Franchini M, Plebani M. Genetic and biochemical heterogeneity of cardiac troponins: clinical and laboratory implications. Clin Chem Lab Med 2009;47:1183–94.
[2] Aldous SJ. Cardiac biomarkers in acute myocardial infarction. Int J Cardiol 2013;164:282–94.
[3] Lippi G, Cervellin G. Challenges of serial troponin testing: an unfinished symphony. Int J Cardiol 2013;168:4397.
[4] Hoeller R, Rubini Giménez M, Reichlin T, et al. Normal presenting levels of high-sensitivity troponin and myocardial infarction. Heart 2013;99:1567–72.
[5] Apple FS, Collinson PO, IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem 2012;58:54–61.
[6] UniProt. Available at:http://www.uniprot.org/ [Last access: 5 December 2013].
[7] Carballo S, Robinson P, Otway R, et al. Identification and functional characterization of cardiac troponin I as a novel disease gene in autosomal dominant dilated cardiomyopathy. Circ Res 2009;105:375–82.
[8] Mogensen J, Kubo T, Duque M, et al. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. J Clin Invest 2003;111:209–16.
[9] Niimura H, Patton KK, McKenna WJ, et al. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. Circulation 2002;105:446–51.
[10] Millat G, Bouvagnet P, Chevalier P, et al. Clinical and mutational spectrum in a cohort of 105 unrelated patients with dilated cardiomyopathy. Eur J Med Genet 2011;54:e570–5.
[11] Erdmann J, Daehmlow S, Wischke S, et al. Mutation spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. Clin Genet 2003;64:339–49.
[12] Richard P, Charron P, Carrier L, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation 2003;107:2227–32.
[13] Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet 2005;42:e59.
[14] Kimura A, Harada H, Park JE, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. Nat Genet 1997;16:379–82.
[15] Song L, Zou Y, Wang J, et al. Mutations profile in Chinese patients with hypertrophic cardiomyopathy. Clin Chim Acta 2005;351:209–16.
[16] Mogensen J, Murphy RT, Shaw T, et al. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 2004;44:2033–40.
[17] Tester DJ, Ackerman MJ. Genetic testing for potentially lethal, highly treatable inherited cardiomyopathies/channelopathies in clinical practice. Circulation 2011;123:1021–37.