



Cardiac troponins in diagnostics of equine myocardial injury

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Abstract: Unlike humans, who no longer need to run or walk for surviving, riding is the natural horses' occupation, which justifies their use in many working and recreational activities. Despite a difference in size, the biochemical, biological, anatomic, physiological, physiopathological and pathological characteristics of the heart are almost unvaried in humans and horses. Several lines of evidence now attest that the measurement of cardiac troponin I (cTnI) or T (cTnT) has become the cornerstone for diagnosing cardiac injuries in humans, independently from the ischemic, infective, traumatic, toxic, inflammatory or autoimmune origin, thus paving the way to extend their clinical use in equine cardiology. Post-exercise increments of both cTnI and cTnT are commonplace in active horses and need to be accurately distinguished from pathological increases as in humans. Taken together, current literature data would hence lead us to conclude that the clinical significance of measuring cTnI or cTnT in humans and horses appears nearly overlapping, though some important drawbacks must be recognized. These include preliminary analytical and clinical validation of human methods in horses before introduction into veterinary practice, accurate definition of reference ranges and diagnostic thresholds, which could be method-, breed-, age-dependent, as well as validation of human diagnostic algorithms in horses and appropriate interpretation of post-exercise variations, which are more frequent in equines than in humans. A final consideration shall be made on the use of algorithms for diagnosing myocardial injury. Although these have been largely validated in humans, and equine and human cardiology displays many overlapping features, clinical validation would always be needed before straightforwardly translating human algorithms into equine diagnostics.

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Clinical use of cardiac troponins

The measurement of cardiac biomarkers, namely cardiac troponin I (cTnI) and cardiac troponin T (cTnT), has now become the cornerstone for diagnosing cardiac injury, regardless of its ischemic, infective, traumatic, toxic, inflammatory or autoimmune origin (1,2). The introduction of these biomarkers shall be seen as a real breakthrough in the diagnostics of heart diseases, especially when assayed with the novel high-sensitivity immunoassays, which allow

detecting even physiological concentrations of both cTnI and cTnT in the vast majority of healthy subjects (3,4).

It is now well-established that different forms of physical exercises are associated with substantial biochemical abnormalities (5), including a temporary and almost completely reversible increase of cTnI and cTnT concentrations in humans practicing endurance exercise (6), which appears directly proportional to the amount of cardiac tissue temporarily injured by the increased biological and physical stress placed on cardiomyocytes (7). Although

the relative post-exercise variation of both cTnI and cTnT depends on running intensity and race distance, in the vast majority of people practicing recreational running (i.e., between 5–20 km at low-to-mild intensity) such increase is typically modest (e.g., between 2- and 6-fold from pre-exercise) and persists for less than 12–24 h (8). It is hence reasonable that the regular assessment of these cardiac biomarkers in physically active subjects shall be seen as a reliable and suitable opportunity as preparticipation athletic evaluation, but also for longitudinal monitoring athlete's health as well as for predicting the risk of cardiovascular events and death in regular endurance practitioners (9).

Although this notion is now straightforward and virtually incontestable, many doubts remain as to whether the measurement of cardiac troponins, which have been for long used for diagnosing human cardiac diseases, would also be reliable and clinically informative in veterinary medicine. This holds especially true for horses, one of the animal species which has mostly impacted human history for its stamina and speed (10). Although the use of horses for transportation has significantly declined over the last decades, these beautiful and faithful animals are still largely employed for their most recognized qualities, i.e., power and strength in riding, thus including equestrian performance sports (11). Proactive integration of equine care shall hence be seen as an important strategy for avoiding or mitigating trainer effect and risk of injuries, including that of severe cardiac pathologies, up to sudden death (12). In this perspective, the measurement of either cTnI or cTnT in horses is a potentially valuable perspective, whose leading clinical opportunities and potential drawbacks will be analyzed in the following parts of this article.

Biochemistry and measurement of cardiac troponins in horses

The heart size increases in parallel with body size across most mammalian species, so that horses have a heart that is usually ~10-fold bigger (i.e., 3.5 *vs.* 0.35 kg) than humans. Despite the difference in size, the biochemical, biological, anatomic, physiological, physiopathological (i.e., athlete's heart is commonplace in racehorses) and even pathological characteristics of the heart are almost unvaried among different mammalian species (13), thus leading the way to identify many overlapping areas between human and veterinary cardiology.

The essential premise for using human diagnostic tests in veterinary medicine is that these techniques shall be both

analytically and clinically validated in the target animal species, as recently endorsed by the American Society for Veterinary Clinical Pathology (ASVCP) (14), and cardiac biomarkers would make no exception to this rule. Concerning the biochemical structure of cardiac troponins, Rishniw and Simpson sequenced the cTnI gene in equines and compared it with the equivalent genes in other species, including humans (15). Although it could be observed that the cTnI epitope region identified by most commercial cTnI methods was highly conserved among different animal species (i.e., humans, horses, dogs, and cats), eight amino acid differences could be found. Even more importantly, equine cTnI was found to be deprived of 6 amino acids in the N-terminal region, whilst displaying 3 highly horse-specific polymorphisms which may ultimately affect the affinity of monoclonal antibodies for the target protein moiety. This hypothesis has been recently confirmed by Rossi *et al.*, who published the results of an interesting study aimed at validating the use of some cTnI immunoassays in horses (16). A comprehensive analytical protocol was defined and completed by the authors, who finally concluded that two out of the six methods tested should be considered unsuitable in horses due to problems in accurately detecting equine cTnI. Significant heterogeneity was also found in reference ranges and diagnostic thresholds among the different cTnI immunoassays so that these methods would require comprehensive local validation before being introduced into clinical veterinary practice. Unfortunately, no clear information has been published on the structure of the gene encoding for cTnT across mammalian species, except data retrievable from the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/gene/7139>), highlighting that the human (*Homo Sapiens* TNNT2—18,667 bp DNA) and horse (*Equus caballus* TNNT2—14,880 bp DNA) cTnT genes encode for a homologous protein, which displays some important differences between these two species. As for cTnI, a thoughtful analytical validation protocol was defined and completed in horses by Shields and co-authors (17), who finally concluded that the commercial high-sensitivity cTnT immunoassay can be reliably used for equine cardiac diagnostics. This is mostly due to the fact that the epitopes identified by the human cTnT method are also present (with perfect homology) in horses (18). Interestingly, Shields *et al.* also found that the 99th percentile upper limit of the reference range URL) of cTnT was nearly 40% higher in racing thoroughbreds than in a population of non-competition horses (i.e., 23.2 *vs.*

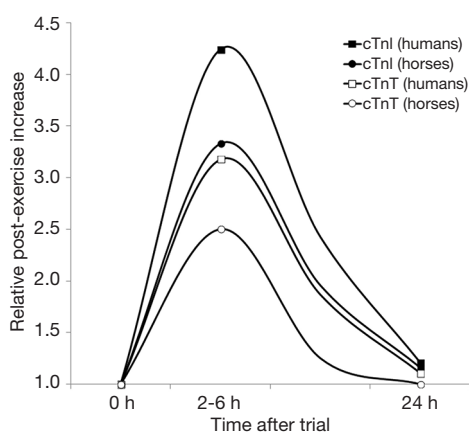


Figure 1 Relative post-exercise increase of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) in humans and horses (24-27).

16.2 ng/L) (17).

In another interesting study, including as many as 586 healthy horses aged between 2–14 years, Slack *et al.* reported that cTnI concentration is not apparently influenced by sex and gait, whilst a weak positive correlation could be found with age ($P=0.03$) and some potentially significant differences could also be observed across different horse breeds (19). Unlike what could naturally envisaged from human and equine anatomy (i.e., horse's heart being nearly 10-time bigger than the human counterpart) (13), the circulating values at rest of both cardiac troponins appears very similar between these two species, since the 99th URL of cTnI is ~30 ng/L in horses and ~15 ng/L in humans, whilst the 99th URL of cTnT is ~14 ng/L in humans and ~23 ng/L in horses, respectively. This can be explained by the fact that these reference ranges have been calculated in animals that are nearly 5-time younger than humans (i.e., 2–8 vs. 20–80 years), so that the effect of the age may contribute—at least partially—to attenuate the difference.

As specifically regards the use of commercial human cardiac troponin immunoassays in equine cardiac diagnostics, several studies have now allowed to definitely establish that enhanced (i.e., diagnostic) values of these biomarkers are found in serum or plasma of animals with various forms of cardiomyopathies (i.e., ischemic, inflammatory or toxic), structural heart disease (e.g., ventricular septal defects, valvular heart disease or aortopulmonary fistula), toxic myocardial injury and even arrhythmias (18,20,21).

Taken together, these findings would lead us to conclude

that once an analytical and clinical validation of both cTnI and cTnT immunoassays has been completed, these methods can be clinically used in equine diagnostics. Moreover, as recently confirmed by Van Der Vekens *et al.* (20), and in keeping with previous findings in humans (22), both cTnI or cTnT can be independently used for detecting and diagnosing myocardial injury in horses.

Post-exercise increase of cardiac troponins in horses

Since horses are mostly born to run, and their anatomic and biologic apparatus has evolved accordingly (23), the identification and appropriate interpretation of exercise-related variation of both cTnI and cTnT is essential in equine like in human medicine.

An interesting study was published by Rossi *et al.* (24), who measured cTnI with a high-sensitivity immunoassay in 5 healthy racehorses, which carried out a warm-up of 28.8 ± 8.4 min, and then run a mile at near-race intensity, with a mean time of 2:09 min (range, 2:02–2:23 min), thus averaging an overall ~30 min endurance exercise. Blood samples were drawn immediately before and after exercise, up to 24 h afterwards. The baseline mean concentration of cTnI (1.38 ± 0.6 ng/L) increased by up to 3.3-fold between 2 to 6 h after the trial and returned to values similar to the baseline after a mean period of 9.0 ± 1.15 h post-exercise. A similar study was carried out by Shields *et al.* (25), who instead measured cTnT with a high-sensitivity immunoassay in 38 healthy racehorses, which completed a 5/8th mile Chuckwagon race. Despite the race distance was completed in a relatively short time (i.e., 72.7 seconds), the concentration of cTnT increased by approximately 2-fold between 2 and 6 hours after the end of the trial (i.e., from 4.0 to 8.0 ng/L), and returned to baseline values nearly 12 h afterwards.

The comparison of both cTnI and cTnT variation after exercise in horse and humans is shown in Figure 1. Although the race distance is different, and thereby the relative increase cannot be straightforwardly compared among species, the pattern is virtually identical, with a significant post-exercise increase, which peaks in both humans and horses between 2–6 h post-exercise, and values returning to the baseline level almost within 12–24 h after exercise (26,27). Importantly, neither in humans nor in horses the post-exercise increase of both cTnI and cTnT seemingly exceeds the species-specific 99th URL. This evidence would lead us to conclude that the assessment of

Table 1 Important issues for diagnostic use of human cardiac troponin immunoassays in horses

Validation (analytical and clinical) of the specific method in horses
Accurate establishment of reference ranges according to:
Method
Breed
Age
Training
Appropriate interpretation of post-exercise elevations
Validation of human diagnostic algorithms in horses

either cardiac troponin retains identical clinical significance in humans and horses, so that the basic concepts underlying cardiac troponin usage in human cardiac pathology may be straightforwardly translated to equine diagnostics.

The way forward

In ancient times entire economies have been dependent on equines, whereby these powerful and intelligent animals have been—and still are in certain parts of the modern world—essential for many human activities. Due to their anatomy and physiology, horses are not only suited to canter, trot, and gallop, reaching speeds approaching 90 km/h, thus being the second-fastest land-animal, only preceded by the Cheetah (28), but can also perform other working- and entertainment-related tasks. The innate equine inclination to run has been for long exploited by humans, whereby horses have been first domesticated around the 3500 BC, and have then been used for transportation, agricultural work, and warfare, always privileging—and even refining through selection—their extraordinary running performance (10). Like every other living organism, horses are also vulnerable to a kaleidoscope of pathologies, including heart injuries, for which early and accurate diagnosis is always advisable.

Nearly 40 years after their first clinical application, both cTnI and cTnT have now virtually become the biochemical gold standard for diagnosing cardiac injury in humans (29). Due to the substantial homology between the human and equine cardiovascular system, which only differ for some minor variations in ventricular septal blood supply (30), the measurement of both cTnI and cTnT using commercial (preferably high-sensitivity) immunoassays can now be seen as a workable opportunity for diagnosing cardiac injuries

also in horses. Taken together, the current literature data would lead us to conclude that the clinical significance of measuring these biomarkers in humans and horses appears nearly overlapping, though some important drawbacks must be recognized and overcome (*Table 1*). First and foremost, the use of commercial methods targeting human cardiac cTnI and cTnT must be preliminarily validated, both analytically and clinically, in horses before they can be introduced into veterinary practice. This would allow preventing the generation of false-negative test results by using kits that inaccurately detect equine proteins, but will also enable an appropriate definition of reference ranges and diagnostic thresholds, which could be largely method-, breed- and age-dependent. Unlike humans, who no longer need to run—neither even to walk—for surviving (31), riding is an almost normal occupation for many horses. Equine heart tends to adapt to endurance exercise, displaying a plastic response to intense athletic training, increasing the size and releasing larger amounts of both cTnI and cTnT during resting conditions. Post-exercise increments of both cTnI and cTnT are hence commonplace in active horses and need to be accurately distinguished from pathological increases, more or less like we need to do in humans (32). This would not be inherently easy since—unlike humans—horses cannot plentifully describe their recent physical activity, nor the owner or the veterinary can always follow the animal lifestyle. Nevertheless, the paradigmatic features of post-exercise variation of cardiac biomarkers (*Figure 1*) would enable distinguishing between and exercise-related release (almost modest and transitory) and increases following structural cardiac injuries (considerable and persisting for up to 1 week). A final consideration shall be made on the use of diagnostic algorithms. Although these have been largely validated in humans (33), and equine and human cardiology displays many overlapping features (30), clinical validation would always be needed before straightforwardly translating human algorithms into equine diagnostics.

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Footnote

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