Optimising the early rule-out and rule-in of myocardial infarction using biomarkers

Raphael Twerenbold, Gimenez Maria Rubini, Nestelberger Thomas, Boedinghaus Jasper, Wildi Karin, Mueller Christian

Cardiovascular Research Institute Basel (CRIB) and Department of Cardiology, University Hospital Basel, University of Basel, Switzerland

Summary

Detailed clinical assessment, the electrocardiogram and cardiac troponin form the three cornerstones of the early diagnosis of myocardial infarction (MI). After the clinical introduction of high-sensitivity cardiac troponin (hs-cTn) assays about 8 years ago, precise quantification of cardiomyocyte injury around the 99th percentile became possible and thereby substantially increased the accuracy of MI diagnosis from blood drawn at presentation to the emergency department. Higher diagnostic accuracy enabled the development and validation of multiple early hs-cTn-based diagnostic algorithms including the ESC 0/1-hour algorithm, which substantially reduced the time required for safe rule-out or rule-in of MI. This algorithm is widely applicable, including patients with renal dysfunction and in the USA, where hs-cTn approval is slightly different from the rest of the world. Apart from hs-cTn, other emerging cardiac biomarkers might have the potential to further improve the early triage of patients with suspected MI.

Key words: high-sensitivity troponin, cardiac myosin-binding protein C, myocardial infarction, rule-out, rule-in, algorithm

High-sensitivity cardiac troponin

Symptoms suggestive of myocardial infarction (MI) are among the most frequent symptoms leading to an emergency department (ED) presentation [1]. Since clinical assessment and electrocardiogram (ECG) alone are not sufficient to diagnose or exclude non-ST-segment-elevation myocardial infarction (NSTEMI) in most patients, the addition of blood tests to serially measure markers of myocardial necrosis form a cornerstone of the early diagnosis of MI. When considering the clinical utility of cardiac biomarkers for the early diagnosis of MI, it is of utmost importance to interpret them only in the appropriate clinical setting of suspected MI and always in conjunction with detailed clinical assessment and thorough interpretation of the ECG (fig. 1).

Until 1990, aspartate aminotransferase, lactate dehydrogenase, creatine kinase (and its isoforms) or myoglobin were used to detect myocardial necrosis. In the 1990s, cardiac troponin (cTn) T and I assays were clinically introduced, providing higher sensitivity and specificity for myocardial necrosis as compared with the previous markers (fig. 2). Recent improvements in the analytical sensitivity of cTn assay have led to a further refinement in clinical ability to detect and quantify cardiomyocyte injury [2–34]. These assays increased diagnostic accuracy at presentation, substantially reduced the sensitivity deficit of cTn at presentation for MI and the associated “troponin-blind” interval, and allowed the recent development of several novel strategies for the rapid rule-out or early rule-in of NSTEMI [2–34]. These improved assays are labelled “sensitive” if they are able to detect cTn in ~20–50% of healthy individuals, and “high-sensitivity” if they detect cTn in >50% of reference (apparently healthy) subjects and have a coefficient of variation of <10% at the 99th percentile upper reference limit of the assay [5]. High-sensitivity assays have the ability to accurately detect cTn at lower levels than older generation assays, giving them higher sensitivity for the detection of MI at presentation, which means that the time interval to the second measurement of high-sensitivity cTn (hs-cTn) can be significantly shortened [2–35].

Both cTnT and I are structural proteins unique to the heart. Thus, cTnT and I are organ-specific, but not disease-specific markers. High-sensitivity and sensitive cTnT and I assays exactly quantify the amount of cardiomyocyte injury [3, 35]. They should be interpreted as quantitative variables and not in a binary fashion (negative/positive) like a pregnancy test. From a diagnostic perspective, it is highly inappropriate to label a patient as “cTn-positive”, as this would lump together patients with only mildly elevated cTn levels barely above the 99th percentile and an associated positive predictive value (PPV) for NSTEMI of only about 40–50%, and patients with markedly elevated cTn levels (e.g., about five times above the 99th percentile) and an associated PPV of 90%. The higher the cTn level, the higher is the likelihood for the presence of MI.

Fourth universal definition of myocardial infarction

In August 2018, the fourth universal definition of myocardial infarction was published [2]. This document officially introduced the term “myocardial injury”, describing any cTn concentration above the 99th percentile irrespective of its underlying cause (e.g., MI, congestive heart failure,
Figure 1: Clinical assessment of patients presenting with suspected acute coronary syndrome. The initial assessment is based on the integration of low likelihood and/or high likelihood features derived from clinical assessment (symptoms, vital signs), 12-lead ECG, and cardiac troponin determined at presentation to the emergency department and serially thereafter. “Other cardiac” includes, among others, myocarditis, tako-tsubo cardiomyopathy, or congestive heart failure. “Noncardiac” refers to thoracic diseases such as pneumonia or pneumothorax. Cardiac troponin at presentation and its changes during serial sampling should be interpreted as a quantitative marker: the higher the 0-h level or the absolute change during serial sampling, the higher the likelihood for the presence of myocardial infarction. In patients presenting with cardiac arrest or haemodynamic instability of presumed cardiovascular origin, echocardiography should be performed/interpreted by trained physicians immediately following a 12-lead ECG. If the initial evaluation suggests aortic dissection or pulmonary embolism, D-dimers and multi-detector computed tomography angiography are recommended, according to dedicated algorithms.


Figure 2: Historical timeline of myocardial necrosis markers. Clinical introduction of myocardial necrosis markers over time and their respective biochemical and analytical sensitivity. AST = aspartate aminotransferase; CK = Creatine kinase; CK-MB = Creatine kinase muscle/brain; cTn = cardiac troponin; ESC = European Society of Cardiology; hs-cTn = high-sensitivity cardiac troponin; LDH = lactate dehydrogenase; POCT = point of care testing.
chronic changes in cTn concentrations during serial sampling differentiates acute myocardial injury (e.g., MI, myocarditis, Tako-Tsubo cardiomyopathy, congestive heart failure, cardiac ablation) from chronic types (e.g., chronic kidney disease, structural heart disease). Acute myocardial injury is only considered an MI if there is clear clinical evidence of acute myocardial ischaemia (e.g., symptoms of myocardial ischaemia, new ischaemic ECG changes, development of pathological Q waves in the ECG and/or imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities).

To differentiate MI from other causes of chest pain, absolute rather than relative hs-cTn changes seem to be the best metric [12, 19–21]. The larger the absolute cTn change within 1, 2, or 3 hours, the higher the likelihood for the presence of MI. Continuous medical education and training of physicians in these concepts is essential to avoid inappropriate interpretation of the chronic mild elevations of cTn associated with, for example, heart failure or other structural cardiac disorders as signs of MI.

About false-positive hs-cTn measurements, troponinaemia, troponinitis and other fairy tales

In the absence of overt myocardial ischaemia, elevated cTn levels are often labelled as “false positive” hs-cTn results, troponinaemia or troponinitis. These terms should be avoided, as most of these unexpected hs-cTn elevations are “true-positive” for myocardial injury (rather than MI) and reflect previously undetected or underestimated cardiac disease, including valvular heart disease, heart failure and chronic coronary artery disease. Many cardiac and noncardiac disorders may lead to substantial amounts of cardiomyocyte injury and thereby hs-cTn elevations (table 1) [3, 8]. It is important to note that cTn elevations universally portend a worse prognosis than for otherwise similar patients without a cTn elevation, irrespective of the underlying disease. This is true regardless of whether the patient has heart failure, renal dysfunction, gastrointestinal bleeding, sepsis, respiratory disease, pulmonary embolism, subarachnoid haemorrhage, or stroke or whether the patient is asymptomatic without known cardiovascular disease [36]. Obviously, the medical consequences of cardiomyocyte injury as quantified by cTn elevations will be highly individualised and different from those in patients with MI.

Rapid triage algorithms using hs-cTn

The most important clinical advantage of the new, more-sensitive cTn assays is their ability to substantially reduce the “troponin-blind” interval in the first hours of an MI and thereby to allow novel strategies to rule-out or rule-in NSTEMI early. Multiple troponin-based strategies rely on serial hs-cTn testing. Two of them, the 0/1-hour algorithm and a 0/3-hour algorithm, are currently recommended by the European Society of Cardiology (ESC) with a class I recommendation [3].

It is important to consider five points when applying troponin-based strategies in clinical practice. First, they should be used only in conjunction with full clinical assessment. Second, these strategies should be considered triage strategies rather than definite diagnostic strategies, as additional imaging tests such as invasive coronary angiography, stress testing, echocardiography or computed tomography (CT) angiography may be necessary for a definite diagnosis. Third, the percentage of patients eligible for rule-out or rule-in varies widely from ≈9.8% to 77% depending on the underlying algorithm, the cTn assay used and the clinical setting, including the prevalence of NSTEMI [20, 23]. Fourth, these strategies should only be applied after the initial ECG has excluded ST-segment-elevation myocardial infarction (STEMI) since these high-risk patients need prompt identification based on the ECG and immediate reperfusion therapy without the need for cTn testing [2]. Fifth, any triage strategy should be embedded in the local standard operating procedures of the ED.

The main performance metrics of early triage strategies are safety of rule-out (quantified as the negative predictive value [NPV] and sensitivity for NSTEMI), overall efficacy (quantified as the percentage of patients triaged either towards rule-out or rule-in), and accuracy of rule-in (quantified as the PPV and specificity for NSTEMI) if the respective algorithms provides a rule-in strategy.

ESC 0/3-hour algorithm

NSTEMI is ruled-out if hs-cTn concentrations remain in the normal range (below the respective assay-specific 99th percentile) in the blood samples drawn at presentation and 3 h after presentation, and if the patient fulfils two additional requirements: to be pain-free and to be at low-risk of in-hospital mortality as quantified by a Global Registry of Acute Coronary Events (GRACE) score below 140 (fig. 3A) [3]. In patients presenting more than 6 h after chest pain onset, in whom chest pain onset can be reliably quantified, one single blood draw at presentation is considered to be sufficient. Patients are ruled-in if they have a clearly elevated hs-cTn blood concentration at presentation, or if the 3-hour sample shows a relevant change. This approach

Table 1: Conditions other than myocardial infarction associated with cardiac troponin elevations.

<table>
<thead>
<tr>
<th>Tachyarrhythmias</th>
<th>Heart failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive emergencies</td>
<td>Critical illness (e.g., shock/ sepsis/ burns)</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>Tako-tsubo cardiomyopathy</td>
</tr>
<tr>
<td>Ischaemic heart disease (e.g., aortic stenosis)</td>
<td>Pulmonary embolism, pulmonary hypertension</td>
</tr>
<tr>
<td>Aortic dissection</td>
<td>Renal dysfunction and associated cardiac disease</td>
</tr>
<tr>
<td>Coronary spasm</td>
<td>Acute neurological event (e.g., stroke or subarachnoid hemorrhage)</td>
</tr>
<tr>
<td>Cardiac infarction or cardiac procedures (e.g., CABG, PCI, ablation, pacing, cardiac resynchronization, or endomyocardial biopsy)</td>
<td>Hypo- and hyperthyroidism</td>
</tr>
<tr>
<td>Infiltrative diseases (e.g., amyloidosis, haemochromatosis, sarcoidosis, scleroderma)</td>
<td>Myocardial drug toxicity or poisoning (e.g., doxorubicin, 5-fluorouracil, herceptin, snake venoms)</td>
</tr>
<tr>
<td>Extreme endurance efforts</td>
<td>Rhabdomyolysis</td>
</tr>
</tbody>
</table>
| CABG = coronary artery bypass surgery; PCI = percutaneous coronary intervention. Adapted from Twuernbold et al. [4] with permission from the publisher.
has been recommended by the ESC Guidelines since 2011 and is still the most widely used algorithm in Europe [3, 8]. Its use regarding rule-out of MI seems to be acceptably effective and safe for all hs-cTn assays and possibly also some sensitive cTn assays [37]. The exact performance for rule-in cannot be quantified, as no precise definitions of its rule-in cut-off levels are given. Given the average turn-around time for hs-cTn of about 1 h, the hs-cTn measurement performed at 3 h after ED presentation would become available about 4 h after ED presentation and would allow clinical decision making regarding hospitalisation versus outpatient management about 4 h after ED presentation in the majority of patients. In a recent study, this strategy enabled outpatient management in 56% of patients, with a median time in the ED of about 5 h in the overall population, and 4½ h in those patients managed as out-patients [38].

**ESC 0/1-hour algorithm**

The concept of the ESC 0/1-hour algorithm is exclusively based on information provided by hs-cTn concentrations at ED presentation and their absolute change within 1 hour, using assay-specific cutoffs (concept depicted in fig. 3B; assay-specific cutoffs for hs-cTnT by Roche®, fig. 3C; assay-specific cutoffs for hs-cTnI by Abbott®, fig. 3D) [3, 20, 29, 30, 39, 40]. The 0/1-hour ESC algorithm results in safe rule-out of NSTEMI (NPV 99–100%) and allows an accurate early triage in about 75% of patients: 60% towards rule-out and in 15% towards rule-in of NSTEMI [40]. The application of the ESC 0/1-hour algorithm is also possible in institutions with a median turn-around time of more than 1 h, since the 1 h only refers to the timing of the serial sample. In these institutions, the second blood draw would simply need to be performed while still awaiting the results from the first blood draw.

**Direct rule-out based strategy on undetectable/very low baseline hs-cTn concentrations**

Undetectable or very low blood concentrations of hs-cTn at presentation to the ED have a very high (98.6–100%) NPV for NSTEMI. This approach has a unique simplicity, as it requires only a single blood draw for an inexpensive and widely available biomarker test. As the lower limit of detection is assay dependent and varies among the clinically available hs-cTn assays, “very low concentrations, e.g., below the 30th percentile of healthy individuals” may be the preferred metrics to identify biological-equivalent values. Four large studies and three recent meta-analyses have provided consistent results for hs-cTnT and hs-cTnI assays [31, 32, 41–46]. As the release of cTn is a time-dependent phenomenon, this approach should only be used in patients with a chest pain onset at least 2–3 h prior to ED presentation, as safety was found to be reduced in very early presenters in a recent observation [45]. In the 2015 ESC guidelines, this approach is recommended in combination with the 0/1-hour algorithm as the preferred rule-out strategies owing to their excellent balance between speed and accuracy [3].

**Strengths and limitations of the different rapid triage algorithms**

There are some important differences between the above listed algorithms that have to be mentioned. First, direct rule-out strategies rule-out patients with a single hs-cTn measurement at ED presentation, whereas the other algorithms require serial sampling at 1 or 3 hours. Whereas the ESC 0/1-hour algorithm incorporates both the hs-cTn concentration at ED presentation and its absolute change during resampling, the ESC 0/3-hour algorithm relies on the assay-specific 99th percentile only. The integration of absolute changes in the ESC 0/1-hour algorithm has the potential to improve safety and efficacy compared with the ESC 0/3-hour algorithm. However, as direct head-to-head comparisons are still lacking, their impact could not be quantified yet. Second, while the 0/1-hour and 0/3-hour algorithms allow for triage towards rule-out and rule-in of NSTEMI, the other described algorithm (direct rule-out strategy) can only be used for early rule-out of NSTEMI. Third, by incorporating the time since chest pain onset, the ESC 0/1-hour algorithm takes advantage of patients pre-
senting very early after chest pain onset, a subgroup of patients requiring particular attention in order not to miss late rises in hs-cTn [3, 45].

Early rule-out algorithms are helping to guide clinicians identifying patients at very low risk for NSTEMI and major adverse cardiovascular events (MACE). However, the strategy for rapid triage of suspected MI in clinical practice must be chosen by each institution individually, depending on the locally used cTn assay (sensitive versus high-sensitivity), wish for additional rule-in guidance and individual preferences regarding targeted balance between safety and efficacy.

Additional helpful steps in the observe zone

The ESC 0/1-hour algorithms [3, 20, 29, 30, 39, 40] provide detailed guidance for rule-in of NSTEMI in addition to a rule-out strategy. In addition, an intermediate-risk group has been created, leaving up to one third of patients in this observe zone [3, 18, 20, 29, 30, 39, 40, 47, 48]. These patients are typically elderly men with pre-existing coronary artery disease and have been shown to have increased long-term mortality [49]. Detailed clinical assessment, additional hs-cTn measurement at 3 h and cardiac imaging are key for accurate diagnosis in these patients. The clinical interpretation of mildly abnormal hs-cTn levels is crucial for physicians in the ED, since up to one third of patients triaged to the observe zone are finally diagnosed with NSTEMI or unstable angina. Therefore, hs-cTn should be retested at 3 h to better differentiate an acute cardiac disease (such as NSTEMI) associated with a dynamic hs-cTn course from a chronic cardiac disease reflected by stable hs-cTn levels. Coronary angiography (in those with high likelihood for NSTEMI), echocardiography, and functional stress imaging (in those with low likelihood for NSTEMI) seem to be the preferred tests in observe patients [49].

Coronary computed tomography angiography (CCTA) seems a suitable imaging modality in only a minority [50]. A randomised controlled trial recently showed no benefit of routine CCTA over standard optimal care encompassing hs-cTnT in patients with suspected acute coronary syndrome regarding identification of significant coronary artery disease (CAD) requiring revascularisation within 30 days, duration of hospital stay or direct discharge from the ED [51]. Functional rather than anatomical testing is mandatory to differentiate coronary lesions resulting in myocardial ischaemia and acute chest pain at rest from lesions that are innocent bystanders in the acute chest pain episode leading to ED presentation [49].

Overruling a triage recommendation

Hs-cTn-based triage algorithms must always be used in conjunction with detailed clinical assessment and thorough interpretation of the ECG. This synthesis may well result in overruling a “rule-out” recommendation provided by the hs-cTn-based algorithms in some patients perceived to be at high-risk of NSTEMI. Overruling should then lead to the identical process described for patients assigned the observe zone and should always include an additional hs-cTn measurement at 3 h.

Rule-out for MI does not always equal outpatient management: the novel strategies were developed to safely rule-out NSTEMI, but not other disorders that still may require hospital admission, such as unstable angina, pulmonary embolism, aortic dissection or severe sepsis from pneumonia. Accordingly, the percentage of patients who can possibly be managed as out-patients is smaller than the percentage of patients ruled-out for NSTEMI. Standard operating procedures should be in place to ensure appropriate follow-up of patients rapidly discharged from the ED, which often will include outpatient functional cardiac stress testing.

Hs-cTn in patients with renal dysfunction

Patients with suspected NSTEMI and renal dysfunction are at substantially higher risk of NSTEMI as compared to patients with normal renal function [4, 52–54]. Accurate rule-out and rule-in of NSTEMI is of paramount importance since patients with renal dysfunction are more prone to adverse events related to cardiovascular medication (e.g., anticoagulation), as well as to cardiovascular procedures including coronary angiography and coronary intervention [3, 35]. However, rapid and accurate diagnosis of NSTEMI is challenging in this vulnerable patient subgroup, since they often present with chronically elevated troponin levels (10–20% using s-cTn, up to 70% using hs-cTn) even in conditions other than acute myocardial ischaemia, which are still associated with poor prognosis [54, 55]. The underlying pathophysiological mechanism is poorly understood and not primarily explained by reduced glomerular filtration rate [56–59].

In general, the high diagnostic utility of hs-cTn can be maintained in patients with renal dysfunction if adjusted decision levels higher than the assay-specific 99th percentiles are considered [54]. A recent international analysis addressed the question of whether the ESC 0/1-hour algorithm can safely be used in patients with renal dysfunction [60]. High safety of rule-out (sensitivity 100% for hs-cTnT, 98.6% for hs-TnI) was documented, supporting the use of the ESC 0/1-hour algorithm also in patients with renal dysfunction (fig. 4 depicting performance of the 0/1-hour algorithm using hs-cTnT in different stages of renal dysfunction). However, specificity of rule-in and efficacy of rule-out were decreased as fewer patients with renal dysfunction presented with low hs-cTn blood concentrations. Modifications of the rule-in and rule-out thresholds did not improve the specificity or overall efficacy of the 0/1-hour algorithm.

0/1-hour algorithm according to the hs-cTnT approval in the USA

Although hs-cTn assays have been widely used since 2010 in Europe and many other countries outside the USA, the first hs-cTn assay has just received approval by the Food and Drug Administration (FDA) for clinical use in the USA in spring 2017. However, the FDA-approved use of hs-cTnT differs in two important details from its contemporary use in most other countries: First, low concentrations are reported only down to 6 ng/l as compared to 3 ng/l, and second, a higher age-matched 99th percentile upper reference limit of 19 ng/l is suggested, as compared with 14 ng/l. Both changes could potentially impact the safety and/or efficacy of rapid triage algorithms defined previous-
ly in a non-FDA setting. A recent analysis aimed to quantify the impact of the FDA-approved use of hs-cTnT on the safety and efficacy of the ESC 0/1-hour algorithm [61]. The original ESC 0/1-hour algorithm was minimally adapted to the FDA setting by lifting the direct rule-out cutoff at presentation from <5 ng/l to <6 ng/l, since hs-cTnT levels are only reported down to 6 ng/l in the USA. Rule-out safety as well as rule-in accuracy of the original and the modified algorithm were very high and comparable (NPV 99.8% versus 99.9%, PPV 76.9% versus 76.7%). Both algorithms allowed rapid rule-out and rule-in of NSTEMI in three patients out of four.

Cardiac myosin-binding protein C as a potential alternative to hs-cTn

With the advent of more sensitive cardiac troponin assays, biomarkers previously used to detect myocardial infarction, such as creatine kinase, creatine kinase MB isoenzyme or myoglobin, became less relevant owing to their decreased sensitivity and specificity compared with hs-cTn [2, 10]. Hs-cTn assays represent the current gold standard and the other cardiac biomarkers are no longer supported by most current guidelines [2]. Recently, a novel cardiac biomarker indicating myocardial injury has been described: cardiac myosin-binding protein C (cMyC), a cardiac-specific protein of the contractile apparatus of the myocardium (fig. 5). Preliminary preclinical and clinical data suggested a quicker release into blood after myocardial infarction than for hs-cTn [62]. In an European multicentre diagnostic study, 1954 patients presenting to the ED with suspected NSTEMI were analysed. Surprisingly, the diagnostic accuracy of cMyC was similar to that of hs-cTnT and hs-cTn in the overall population, but statistically superior compared with hs-cTnT in patients presenting early (within the first 2 hours after chest pain onset) [62]. Whether the advantage of this novel biomarker is of sufficient clinical relevance to replace the established hs-cTn assays needs to be examined in further studies.

Conclusions

Hs-cTn assays improve the safe and rapid triage of patients with suspected NSTEMI and complement assessment of clinical presentation and the ECG. Reduction of the “troponin-blind” interval allows delay in serial hs-cTn re-measurements to be substantially shortened. Many factors other than acute myocardial ischaemia may cause cardiomyocyte injury and therefore mild hs-cTn elevations. Dynamic changes of hs-cTn during serial sampling help to distinguish acute from chronic causes of chest pain and troponin elevations. To maximally profit from hs-cTn assays in clinical practice, they should best be used embedded in an institutional standard operating procedure of...
the ED and in conjunction with a rapid triage algorithm enabling rapid decision making within a few hours. Such an approach will not only increase patients’ safety as compared with conventional, less sensitive cTn assays, but also substantially reduce duration of stay in the ED and costs. Hs-cTn is the current gold standard biomarker for the detection of myocardial injury. Whether other novel emerging cardiac biomarkers such as cMyC have the potential to replace hs-cTn in the future needs to be explored in further studies.

Disclosure statement
Dr. Twerenbold received research support from the Swiss National Science Foundation (P300PB_167803), the Swiss Heart Foundation, the University Hospital of Basel and the University of Basel as well as speaker honoraria/consulting honoraria from Roche, Abbott, Siemens, Singulex and BRAHMS Thermo Scientific. Dr. Rubini received speaker honoraria from Abbott. Dr. Boeddinghaus received research support from the University Hospital of Basel (Division of Internal Medicine), the Swiss Academy of Medical Sciences, the Gottfried and Julia Bangerter-Rhyner Foundation, and speaker honoraria/consulting honoraria from Siemens. Dr. Mueller received research support from the Swiss National Science Foundation, the Swiss Heart Foundation, the KTI, the Stiftung für kardiovaskuläre Forschung Basel; Abbott, Alere, Astra Zeneca, Beckman Coulter, Biormerieux, BRAHMS Thermo Scientific, Roche, Siemens, Singulex, Sphingotec, and the Department of Internal Medicine, University Hospital Basel, as well as speaker honoraria/consulting honoraria from Abbott, Alere, Astra Zeneca, Biormerieux, Boehringer Ingelheim, BMS, BRAHMS Thermo Scientific, Cardiorentis, Novartis, Roche, Siemens, and Singulex. All other authors declare no conflicts of interest.

References


