A pilot survey of the use and implementation of cardiac markers in acute coronary syndrome and heart failure across Europe

The CARdiac MArker Guideline Uptake in Europe (CARMAGUE) study

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Abstract

Background: Guidelines on preferred cardiac marker strategies for investigation of patients with acute coronary syndromes (ACS) are available from the laboratory medicine and cardiology communities. Therefore, implementation of these guidelines into daily clinical practice should be a joint effort of laboratory specialists and clinicians. This was investigated in this survey.

Methods: A pilot study was performed sponsored by the European Federation of Clinical Chemistry and Laboratory Medicine. A link to an online questionnaire was e-mailed to 990 laboratories from eight European countries in May 2006. The requested information included tests performed, clinical protocol development, and reference limits.

Results: We obtained a total of 220 responses. Out of these, 208 responses (95%) were from hospitals that provide 24-h admission of patients. The suggested turn-around-time (60 min) was apparently met by 88% for cardiac troponin T/I and for CK-MB mass.

Conclusions: Our survey demonstrated that cardiac troponin is the preferred biomarker for the diagnosis of ACS. Half of the participants had written protocols, mostly as a result of collaboration between laboratory and clinicians.

Keywords: cardiac markers; implementation of guidelines.

Introduction

The diagnosis of acute myocardial infarction (AMI) is based on symptoms and signs, the electrocardiogram (ECG), and laboratory tests. As cardiac troponins (cTn) are currently the most sensitive and specific markers of myocardial damage and are also markers indicating a poor prognosis in acute coronary syndromes (ACS), there was a need to include these modern markers into the diagnostic guidelines. In 1999, both the clinical cardiology (American College of Cardiology (ACC) and European Society of Cardiology (ESC)) and laboratory organizations (International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and National Academy of Clinical Biochemistry (NACB)) established common guidelines for diagnostic criteria of AMI, leading to the redefinition of AMI (1–3). Because critical clinical decisions depend on cardiac marker threshold levels, assay performance and standardization are also of extreme importance. Therefore in 2001, the IFCC Committee on Standardization of Markers of Cardiac Damage (C-SMCD) recommended quality specifications for both analytical and pre-analytical factors of cTn assays (4).

B-type natriuretic peptide (BNP) and the N-terminal prohormone fragment (NT-proBNP) have been shown to be the best and most powerful markers among all tested natriuretic peptides and neurohormones for the diagnosis of acute and chronic heart failure (HF), and have been widely integrated in clinical practice. The IFCC C-SMCD committee presented a recommend-
ation on analytical and pre-analytical quality specifications for these markers in 2005 (7).

As more sensitive and specific assays have become available and even more sensitive ones are to be expected, the discussion on the choice of assays and cut-off levels becomes more pertinent. The increasing diagnostic sensitivity of cardiac troponin assays will result in the detection of more patients with myocardial damage and an increase in the number of AMI cases identified.

A Project Group under European Communities Confederation of Clinical Chemistry and Laboratory Medicine (EC4, now European Federation of Clinical Chemistry and Laboratory Medicine, EFCC) was established to document the current situation on the use and implementation of cardiac markers in ACS and HF in different countries. Apart from an international survey on the use of cardiac markers in 1999 (8), the adherence and implementation of the guidelines have not been studied until now (9–12) and this was, therefore, the subject of the present investigation.

Materials and methods

A Project Group under EC4, now EFCC, conducted an online pilot survey to assess the patterns of use of biochemical markers as well as the cooperation between clinical units and laboratories in the management of ACS and HF. The purpose of this pilot study was to establish an initial baseline set of data, to determine how difficult it would be to perform such a study on a European basis and to test the design of the electronic questionnaire. It is the intention of the Project Group to perform a full-scale survey in the future across all of the European countries and potentially extend this also outside Europe based on the experience of this pilot study.

The questionnaire “Use and implementation of cardiac markers in acute coronary syndrome and heart failure” was implemented as a standard web-form hosted on a website of Helsinki University Central Hospital. The link to this form was e-mailed to 990 laboratories from eight European countries. The method of questionnaire distribution to potentially participating laboratories was chosen by the authors in their own countries. The mailing lists of national external quality scheme providers and known audit groups were used as well as personal contacts. The questionnaires were sent to the head of the laboratory and directed towards the laboratory scientist responsible for cardiac marker measurement. The authors in each country sent two reminders to all laboratories. The best way to achieve the highest response rate was to personally contact colleagues in the laboratories. Unfortunately, this is only possible in small countries with fewer laboratories, such as Finland. This pilot study did not aim to achieve 100% coverage of all the laboratories in each country. It was the intention to obtain a representative sample of laboratories in this pilot study, collect experience and feedback, and revise the questionnaire for the future.

The questionnaire comprised a total of 123 questions on different aspects of the use of cardiac markers. The questions covered areas, such as clinical protocol development, menu of tests performed, preferred marker, turn-around-time (TAT), sample characteristics, reference limits and decision limits for diagnosis and management of ACS and HF. The questionnaire is displayed at http://www.carmague.fi/1/ in exactly the same form as it was used in the survey.

The collected data were stored with a Perl script into a flat file database and downloaded to a personal computer for further processing. The results were analyzed using Microsoft Excel 2003 (Redmont, WA, USA) and custom software developed by one of the authors (J.S.). The custom software we used is a dynamic link library (DLL) created with Borland Delphi to add custom functionality to Excel. Excel macros written in Excel’s own macro programming language, visual basic for applications, were used as an interface between the custom DLL and Excel. The data analysis was a descriptive tabulation of the numbers of different responses and combinations of responses for each question. Some data were pre-processed before counting the numbers of different responses to take into account variations in spelling in free-text form fields, e.g., different variations in the name of a country were standardized before the final analysis.

Results

Participating laboratories

A total of 220 out of 990 (22%) laboratories responded: 54/350 (15%) from the United Kingdom, 39/150 (26%) from Hungary, 29/240 (12%) from the Netherlands, 28/35 (77%) from Finland, 27/53 (51%) from Austria, 24/114 (21%) from Germany, 14/24 (50%) from Denmark, and 5/20 (25%) from Croatia. The respondents represented 58 university hospitals (26%), 63 central hospitals (29%), 86 district hospitals (39%), 10 primary care hospitals (5%), and three did not answer this question. The best response rates were seen in smaller countries where local contact between laboratories could be used to encourage questionnaire completion. Not all respondents provided answers to every question, so total responses to some parts of the survey were less than 220.

The vast majority, 208 responses (95%), were from hospitals that provide 24-h admission of patients. Among our respondents, 82 hospitals (37%) had a separate chest pain unit for less than 12 h stay, 184 hospitals (84%) had an emergency unit for more than 12 h stay, and 192 hospitals (87%) had a coronary care unit. Laboratory service was available 24 h a day in 207 hospitals (94%).

To understand the clinical services of the hospitals supported by the laboratory, we sought to identify the number of patients admitted with suspected ACS. This information was received from 133 hospitals. There were 32 hospitals with less than 500 patients, 37 hospitals with 500–999 patients, 16 hospitals with 1000–1500 patients, and 48 hospitals with at least more than 1500 patients with suspected ACS per year.

Concerning the number of patients with final diagnosis of AMI, we obtained answers from 120 hospitals. Among these hospitals, 77 hospitals had less than 500 patients, 20 hospitals had 500–999 patients, eight hospitals had 1000–1500 patients, and 48 hospitals had at least more than 1500 patients with a final diagnosis of AMI (ICD codes I21–I22) per year.

A total of 93 hospitals provided information on the number of patients with suspected HF or a final diagnosis of HF admitted each year. The reported numbers for suspected HF were less than 500 patients in
21 hospitals, 500–2000 patients in 46 hospitals, 2000–5000 patients in 21 hospitals, and more than 5000 patients in five hospitals.

Markers of cardiac tissue damage

The respondents were asked to indicate which biochemical tests their laboratories offer for ACS with a choice of one or a combination of the following tests: AST (aspartate aminotransferase), HBDH (hydroxybutyrate dehydrogenase), LDH (lactate dehydrogenase), total CK (creatine kinase), CK-MB activity, CK isoforms, total CK/CK-MB ratio, myoglobin, CK-MB mass, cTnI (cardiac troponin I) and cTnT (cardiac troponin T). We obtained the answers from 219 laboratories. The menu of laboratory tests showed a large variation. In total, 10 laboratories offered only cTnI, 10 laboratories offered only cTnT, and 199 laboratories offered different tests. Of these, 38 laboratories offered two tests, 25 laboratories offered three tests, 30 laboratories offered four tests, and 106 laboratories offered five or more different tests for ACS.

There was less variation in the choice of the preferred marker for diagnosis of ACS: cTnT for 105 laboratories, cTnI for 98 laboratories, CK-MB mass for five laboratories, total CK/CK-MB ratio for four laboratories, total CK for two laboratories and myoglobin for two laboratories. In response to the question whether they usually combine their preferred marker with another cardiac marker, 155 laboratories indicated that they do, while 62 laboratories indicated that they do not. Only 52 laboratories specified their answers by stating that their secondary marker of choice was CK-MB mass for 18, CK for 15, CK-MB activity for six, total CK/CK-MB ratio for six, cTnT for four, and myoglobin for two respondents. There were no marked differences in the choices of preferred cardiac markers used between participating countries.

Most laboratories (n = 183) indicated that they do not plan any changes regarding biochemical markers of AMI in the near future, while 34 laboratories did have such plans. Similarly, 192 laboratories did not plan any changes regarding the decision limits of biochemical markers of ACS in the near future, while only 23 did.

Decision limits

The methods used for assessing the clinical decision limits for the preferred marker indicate that the majority of laboratories use the package inserts.

Implementation of protocols

Most laboratories (59%) had a written protocol for use of cardiac markers and in 90% of the cases the written protocol was the same for the emergency and the coronary care unit. In total, 80% of the written protocols were developed as a joint effort between the clinicians and the laboratorians. Cardiac troponin is the preferred marker for the diagnosis of ACS in 94% of the hospitals. The remaining hospitals preferably use CK-MB mass, CK/CK-MB ratio, CK or myoglobin. Most laboratories (71%) still combine the preferred marker with a second marker.

There were marked differences between participating countries in having written protocols: 100% of the Danish laboratories reported having written protocols for the use of cardiac markers, while only 27% of Austrian laboratories had written protocols (Table 1).

Cardiac troponin I

A total of 107 laboratories measured cTnI and it was the preferred marker in 98 laboratories. In total, 17% of these laboratories performed less than 1000, 26% 1000–5000, 30% 5000–10,000, and 26% performed more than 10,000 cTnI tests per year. Out of the 104 laboratories that reported the clinical indications for which cTnI tests were used, 84% used cTnI for diagnosis of AMI. Table 2 shows that the Abbott (Abbott Laboratories, Abbott Park, IL, USA), Beckman (Beckmann Coulter Inc., Fullerton, CA, USA), Bayer and Dade Behring (now both Siemens Diagnostics, Tarrytown, NY, USA) methods were most widely used for cTnI assay. Heparin plasma was the preferred sample type (56/111, 50.5%), but serum was used almost equally (54/111, 49.5%). The samples were generally tested in stat format.

The TAT was defined in this study as a time interval from the arrival of the sample in the laboratory to the sending of the results to the laboratory information system. In this study, TAT for cTnI of routine samples was less than 60 min in 49.5% of the 109 laboratories

Table 1 Implementation of written protocols for the use of cardiac markers.

<table>
<thead>
<tr>
<th>Country</th>
<th>Yes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>27 (7/26)</td>
</tr>
<tr>
<td>Croatia</td>
<td>40 (2/5)</td>
</tr>
<tr>
<td>Denmark</td>
<td>100 (10/10)</td>
</tr>
<tr>
<td>Finland</td>
<td>52 (14/27)</td>
</tr>
<tr>
<td>Germany</td>
<td>29 (7/24)</td>
</tr>
<tr>
<td>Hungary</td>
<td>55 (21/38)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>65 (17/26)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>85 (45/53)</td>
</tr>
</tbody>
</table>

Table 2 cTnI methods used in laboratories participating in the CARMAGUE survey.

<table>
<thead>
<tr>
<th>Method</th>
<th>% used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access, Beckman Coulter</td>
<td>11.0</td>
</tr>
<tr>
<td>Advia Centaur, Bayer</td>
<td>17.4</td>
</tr>
<tr>
<td>AiO, Innotrac</td>
<td>1.8</td>
</tr>
<tr>
<td>Architect, Abbott</td>
<td>16.5</td>
</tr>
<tr>
<td>AxSym, Abbott</td>
<td>16.5</td>
</tr>
<tr>
<td>Dimension R&amp;L, Dade Behring</td>
<td>10.1</td>
</tr>
<tr>
<td>Immulite 2000, Immulite 2500, Immulite, DPC</td>
<td>8.3</td>
</tr>
<tr>
<td>Liaison, DiaSorin</td>
<td>4.6</td>
</tr>
<tr>
<td>Stratus CS, Dade Behring</td>
<td>1.8</td>
</tr>
<tr>
<td>Tosoh AIA</td>
<td>0.9</td>
</tr>
<tr>
<td>Triage, Biosite</td>
<td>0.9</td>
</tr>
<tr>
<td>Vidas, bioMerieux</td>
<td>5.5</td>
</tr>
<tr>
<td>Vitros ECI</td>
<td>1.8</td>
</tr>
<tr>
<td>Others</td>
<td>2.3</td>
</tr>
</tbody>
</table>
which responded to this question. The TAT for STAT (emergency) request was better, in 88.3% (98/111) of cases it was less than 60 min. However, it should be noted that the question on TAT in the survey was not unequivocal. Therefore, we cannot draw definite conclusions on TAT for TnI. In addition, we do not know whether and how TAT was measured. Most laboratories did not have any criteria for the frequency and number of samples to be collected. Almost all laboratories participated in external quality assessment (EQA) schemes at the national level, but only very few participated in European or international schemes. However, seven laboratories (6.7%, 7/105) reported not having any type of EQA. In Denmark, Finland, Hungary, and the UK all the laboratories who responded to this question reported of having national or international level EQA program, and in Germany and the Netherlands only one laboratory in each country reported of having no EQA at all.

An important issue of the survey was to find out how the decision limits used in the laboratories were determined and how they were chosen. The decision limits used for cTnI were mostly (42.4%, 42/99) determined by reference interval (99th percentile of the reference population distribution) and assay imprecision (concentration at 10% coefficient of variance (CV)) was used less often (36.4%, 36/99). Most laboratories (55%, 59/107) obtained their decision limits from cTnT package inserts. Only eight (7.5%) laboratories reported that they used the existing guidelines and 16 laboratories (15.0%) used peer-reviewed articles for determining the decision limits.

**Cardiac troponin T**

A total of 113 laboratories measured cTnT and it was the preferred marker in 105 laboratories. The majority of them (83.2%) used Roche immunochemistry analyzers for the measurement of cTnT and only a few laboratories (14.2%) reported using point-of-care testing. Most laboratories (60.5%, 69/114) analyze more than 5000 tests a year, only 14.9% analyze less than 1000 tests annually. cTnT tests were mainly used for diagnosis of AMI. Heparin plasma was used as a sample in 44 laboratories, even though it is known that heparin interferes with cTnT results (13–16). Serum was the main sample type used (54/111) and EDTA plasma was used in 13 laboratories. Most of the participating laboratories (94%, 29/31) in the UK used serum as sample material, whereas most of the Finnish laboratories (88%, 15/17) reported using heparin plasma and only 12% used EDTA plasma. Other countries used all sample types in almost equal amounts. The samples were mainly tested in stat format. The TAT (from receiving in the laboratory) was less than 60 min in 38.6% (27/70) of laboratories. The TAT for STAT request was better, less than 60 min in 89.3% (67/75) of laboratories. Again, this question on TAT was not unambiguous, so one cannot draw definite conclusions on TAT for cTnT.

The decision limits used for cTnT were mostly (41.7%, 40/96) determined by assay imprecision (concentration at 10% CV). The reference interval (99th percentile of the reference population distribution) was used less often (28.1%). Most laboratories (48.5%, 49/101) obtained their decision limits from cTnT package inserts provided by the manufacturer. Out of 101 laboratories, 10 (9.9%) used the existing guidelines and 18 laboratories (17.8%) used peer-reviewed articles for determining the decision limits. Half of the laboratories (52/103) did not have any criteria for the frequency and number of samples to be collected. Almost all laboratories participated in EQA schemes, 83.5% (81/97) in national programs and 5.2% (5/97) in either European or international programs. However, 11 laboratories did not have any type of EQA program, but all the Finnish, German, and UK laboratories had national level EQA programs.

**CK-MB mass**

CK-MB mass is widely used in laboratories in Europe (74 laboratories). Out of these laboratories, 43.6% perform more than 5000 tests a year. The main manufacturers of CK-MB mass tests are Roche and Abbott (52 and 74). CK-MB mass tests were mainly used for the diagnosis of AMI, but 18% (13/72) of the laboratories reported its use for evaluation of re-infarction. Plasma was the preferred sample type (46/75), but serum was also used. The patient samples were mainly tested in stat format. The TAT (from receiving in the laboratory) was less than 60 min in 38.6% (27/70) of laboratories. The TAT for STAT request was better, less than 60 min in 89.3% (67/75) of laboratories. Again, this question on TAT was not unambiguous, so one cannot draw definite conclusions on TAT for CK-MB.

Very few laboratories who responded to the questionnaire reported on the decision limits of CK-MB mass. Of the laboratories that did report, almost all used the upper reference limit as the decision limits and they were mainly (51.6%, 33/64) chosen by the manufacturer’s kit insert. Locally derived decision/reference limits were used in 21.9% of the laboratories and peer-reviewed literature was used in 14.1% of the laboratories. Most laboratories (68.1%, 47/69) did not have any criteria for the frequency and number of samples to be collected. Almost all laboratories (81.5%, 53/65) participated in national EQA schemes, but four laboratories reported having no EQA programs at all.

**Cardiac markers of heart failure**

A total of 200 laboratories (91%) responded to the latter part of the questionnaire concerning diagnostics of HF, out of these laboratories only 112 (56%) measured natriuretic peptides. A total of 42 laboratories (37.5%) measured BNP, whereas 70 (62.5%) measured the N-terminal split product of proBNP, NT-proBNP. The Abbott, Bayer, and Biosite (Biosite; San Diego, CA, USA) methods were used for BNP assays (41/49). NT-proBNP was measured by the Roche assay (65 laboratories) (Roche, F. Hoffman – La Roche Ltd.,...
Basel, Switzerland. Out of the laboratories providing natriuretic peptide measurements, 65 (58%) offered the stat facility for measurement of these markers. Out of 103 laboratories, 84 indicated that the TAT was less than 60 min in the case of an emergency request.

Of the laboratories measuring BNP or NT-proBNP, 34% used EDTA plasma (38/111), 39% (43) used heparin and 27% (30) used serum. This information is possibly worrying, as BNP should have been measured in EDTA plasma in 2006, when this survey was performed. The 4th generation BNP assays which allow the use of heparin plasma were launched after this survey. Our questionnaire was inadequate in that we combined both BNP and NT-proBNP in the same question, so we really cannot draw any definite conclusions on the use of sample type for these analytes.

Table 3 presents the methods of choice of the decision limits for cardiac markers of HF by different laboratories. The fact that age-dependent reference limits are implemented by only 8.5% of the laboratories (9/106) is interesting.

Criteria regarding frequency of testing were formulated by 67% (75/112) of the laboratories measuring natriuretic peptides. Most of these laboratories preferred no test repetition (61%, 46/75) at all, whereas 29% suggested repeated analysis only after 24 h. Remarkably, 21% (22/101) of the laboratories did not have any form of EQA for their natriuretic peptide analyses. This was observed in participating laboratories in all of the countries in the study.

**Discussion**

ACS is one of the most frequent causes for attendance in hospital as an emergency case in almost all European countries. The mortality data and outcome of disease are influenced by the interval between the onset of symptoms of disease and the time when diagnosis is made and treatment is started. In recognizing this fact (since well before 1999), professional organizations have continuously been involved in preparation and implementation of guidelines for diagnosis of AMI. The recently updated document published in 2007 provides comprehensive recommendations on biomarkers to be used for diagnosis of AMI together with derivation decision limits.

In laboratory medicine, we are often confronted by the fact that although more sensitive and specific in vitro diagnostic assays are available and guidelines for their use are discussed among professionals, implementation of these tests into routine clinical practice sometimes takes several years. There is no rational explanation for this conservative behavior and therefore there is a need to promote and advocate the implementation of new biomarkers for AMI actively. Also, the adherence to these guidelines should be followed up.

This pilot study was designed and conducted with the aim to obtain an overview on adherence of laboratories to NACB/ACC/ESC guidelines and recommendations for AMI in clinical practice in eight European countries. Obviously, the adherence also reflects the interaction and the discussion between the laboratory and the main clinical units or hospitals. The pilot study was performed in 2006, when the most recent guidelines and recommendations were not yet published. Based on the methodology presented above, the clinical and technical aspects of the results obtained should be interpreted with respect to the use of biomarkers of AMI for diagnosis and prognostic assessment in different healthcare systems. When evaluating the results of this study, one has to keep in mind that the healthcare systems and the management process reflecting the roles of primary healthcare vs. specialized care are different in different countries.

Based on the evidence that the outcome of treatment of ACS strongly correlates with the early diagnosis and onset of treatment, ACS patients are treated in a separate chest pain unit or emergency rooms. Apart from a variety of practices regarding the duration of patient observation on admission to hospital, most of the hospitals surveyed (87%) had a separate coronary care unit.

An essential requirement for early AMI rule-out is frequent ECG testing and blood sampling for the measurement of cardiac biomarkers. Patients with negative test results by serial biomarker testing and an ECG without evolving or dynamic changes are unlikely to have an AMI. They may, however, have unstable angina or other forms of acute cardiovascular disease. Establishment of a clinical practice guideline for the evaluation of patients with chest pain will reduce the variability of practices among physicians and institutions, and at the same time improve the accuracy of clinical decision making (12). The majority of laboratories which were included in this pilot study were located in hospitals and perform a 24-h service for emergency or chest pain units. In this study, the choices of biomarkers or combination of biomarkers used showed a large variation. The most surprising fact was that 106/220 laboratories provide five or more different tests for ACS including enzymes, such as LDH, AST, and total CK or myoglobin beside the preferred marker. The most preferred markers for the diagnosis of ACS in the majority of hospitals were cTnT or cTnI and the second most preferred marker was CK-MB mass. This finding suggests good compliance with the recommendation of the NACB/ACC/ESC guideline. The results shown above strongly suggest the need for closer cooperation between professionals involved in rational use and treatment and appropriate timing of laboratory tests.

**Table 3** Methods of choice of BNP/NT-proBNP decision limits chosen by laboratories participating in the CARMAGUE study.

<table>
<thead>
<tr>
<th>Method of choice</th>
<th>BNP/NT-proBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Package insert</td>
<td>59% (63/106)</td>
</tr>
<tr>
<td>Peer-reviewed literature</td>
<td>24% (25/106)</td>
</tr>
<tr>
<td>Age and gender related reference limits</td>
<td>8.5% (9/106)</td>
</tr>
<tr>
<td>Locally derived decision/reference limits</td>
<td>8.5% (9/106)</td>
</tr>
</tbody>
</table>
in emergency units. Two additional points for discussion are the choice of sample types and the decision limits chosen for test interpretation.

The optimal timing of sampling for measurement of biomarkers for the diagnosis of AMI is derived from both the properties of the available biomarkers and patient-related factors, such as timing and duration of symptoms, plus overall probability of AMI. CK-MB begins to rise within 2–3 h after the onset of myocardial injury and falls to normal ranges by 48–72 h. Cardiac troponin rises with a time course similar to CK-MB, but can remain increased for up to more than 10 days. Most laboratories in this study used heparin plasma or serum; the minority used EDTA plasma for cTnI or cTnT. Most performed testing as stat analyses with TAT within laboratory below 60 min for cardiac troponins and CK-MB mass. Interference of sample type might influence the results. For cTnT, a negative bias in patient data has been observed when heparin plasma is used. When measuring cardiac troponins with different methodologies within the same hospital or between hospital and general practitioners, sample type and assay type should be harmonized or a strategy implemented to avoid interpretative confusion by clinicians. However, troponin harmonization is still problematic (16). Therefore, within one institution or hospital a single cardiac troponin assay is recommended.

It is well known that different manufacturers of tests for cardiac troponin assays do not use the same antibodies, primary reference material, or secondary standards. Standardization of cTnI assays has not been achieved, and therefore evaluation of test performance includes assessment of clinical decision limits based on distribution of values of a healthy reference population, statistical determination of the 99th percentile cut-off value for the reference population, and determination of the concentration corresponding to the 10% CV (total imprecision). Half of the laboratories in this survey used the information supplied by the test package insert as the source of their decision limit. The manufacturers report a 99th percentile reference limit which can be used to define the decision limit (17), but this differs from manufacturer to manufacturer. Only 4% of laboratories in this study used NACB recommendations in validation of decision limits according to the IFCC. Others use peer-reviewed literature or locally derived reference limits. Lack of standardization of cardiac troponin assays is accompanied by the absence of a standard method to determine the 99th percentile reference limit, which should be required by regulatory agencies and provided by the manufacturers. This significantly contributes to the great differences between the 99th percentile limits for the different cardiac troponin assays.

Almost all laboratories participate in EQA schemes for cardiac markers, although all the laboratories should use both internal and EQA programs. This is equally as important for harmonization of laboratories as for clinical decisions. However, we did not inquire in this questionnaire if the laboratories performed internal quality assessment, because it was assumed that this would be normal practice in all laboratories. Having these results on EQA, it has become evident that the question of internal quality assessment needs to be included in the next survey.

The third preferred cardiac marker, CK-MB mass, is used not only for diagnosis of AMI, but also for follow-up of patients and detection of re-infarction. As for cardiac troponin, the 99th percentile upper reference limit was also used as a decision limit for AMI. This marker is recommended for use in follow-up of therapy, particularly in patients after bypass surgery or percutaneous coronary intervention (PCI) for early recognition of myocardial necrosis.

The measurement of more than one specific biomarker for ACS is generally not necessary for diagnosis of AMI and is not recommended. However, this study showed a major discrepancy between the guidelines and recommendations accepted by both professions: even after 7 years of the release of the guideline, several parallel and also clearly non-specific markers were used. It would be interesting to study what is the principal reason for this lack of implementation during subsequent studies.

During the past 5 years the measurement of natriuretic peptides has gained an important role in both diagnosis and management of acute and chronic HF. In contrast to the implementation of measurement of cardiac troponin, which took approximately 10–15 years to become an established marker, the measurement of natriuretic peptides has become well integrated into the clinical practice of HF within 2–3 years. This is partly caused by the fact that there was no existing alternative cheaper marker available. However, from this survey it is clear that 44% of the laboratories still do not offer natriuretic peptide measurements to support the diagnostic process of HF as recommended by the guidelines (7, 18). The choice between BNP (37.5%) or NT-proBNP (62.5%) measurement is in favor of NT-proBNP. The latter might be caused by the sample characteristics, in that NT-proBNP can be measured both in serum and plasma, whereas plasma is a prerequisite for BNP. EDTA anticoagulated whole blood or plasma appears to be the only acceptable choice for BNP. Although serum, heparin plasma or whole blood are all specimens of choice for NT-proBNP, EDTA plasma yields a consistent negative bias compared to matched serum samples (18). Additionally, the longer in vitro stability of NTproBNP (up to 7 days) compared to BNP (a few hours) is favorable when there may be delays in sample delivery (19–22). Although several studies reported an age and gender dependency of BNP and NT-proBNP concentrations in healthy as well as in chronic HF patients (20, 23–27), most laboratories used the decision limits recommended by the manufacturers based only on one cut-off concentration for HF. In the case of acute HF, a single decision limit is legitimate, because high natriuretic peptide concentrations are not influenced by age and gender (28–32). However, for the diagnosis of chronic HF age and gender dependent reference concentrations should be offered. Furthermore, since the different...
BNP assays do not show exact agreement in absolute values, the same assay should be used for the follow-up of the patient. Approximately only a third of the laboratories measuring natriuretic peptides repeat the analysis. If repeat analysis or follow-up measurement is carried out for BNP or NT-proBNP, at least a 7-day interval is recommended to reflect significant increases or decreases and to avoid misinterpretations (31–33). The reason for this is that substantial intra-individual weekly biological variations, up to 59%, are reported for NT-proBNP and BNP in healthy subjects (32, 33).

A fifth of laboratories measuring natriuretic peptides did not participate in EQA programs, which may be due to a lack of national EQAs for natriuretic peptides so far or that point-of-care devices may be used. However, for good laboratory practice, assays, including point-of-care assays, should be evaluated regularly to report correct concentrations to the clinicians. If no national EQA is available, participation in international EQAs or EQAs from other countries/nations could be considered.

In conclusion, most of the participants had a separate coronary care unit and results of cardiac troponins were reported within less than 60 min (after receipt in the laboratory). However, there was still a lack of documented protocols for the use of cardiac markers. Such protocols are essential for optimal patient care. In addition, they avoid the measurement of non-specific markers, such as LDH or AST, which are both unnecessary and not recommended for the diagnosis of ACS. The use of different specimens for cardiac troponin measurement, the use of different assays, and different decision limits result in different absolute values even for the same analyte. This is confusing for the clinicians and may hamper patient follow-up. Laboratories should include brief information on the method used for the particular analyte in their result reports. In contrast to cardiac troponins, natriuretic peptides were not offered in almost half of the laboratories. Decision limits were mostly taken from the package insert, which are based on one cutoff value for acute HF. However, there is clear evidence of an age and gender dependency of natriuretic peptides, which should be considered in chronic HF patients.

It is alarming that not all laboratories that reported providing cTnI, cTnT or natriuretic peptides responded to the questions about participation in EQA programs. Approximately 10% of these did not provide responses for the EQA questions (10%, 13%, and 2% of these did provide cTnI, cTnT, and natriuretic peptides, respectively). We assume that these non-responders did not participate in any EQA program or were not aware of it.

Conclusions

The measurement of cTnI or cTnT is the biochemical test of choice for detecting myocardial damage in Europe. For the detection of HF the evidence is not that clear, but the measurement of BNP or NT-proBNP appears to be becoming the biochemical gold standard. At the present time there is no common standardization, within either a single country or within Europe. More efforts should be undertaken to improve standardization of the implementation of cardiac marker testing regardless of the site where cardiac markers are measured. This pilot study on the use and implementation of cardiac markers provides a preliminary view of the situation in Europe, A more extensive study based on the experience of this study is in progress to further verify these results.

References

13. Hermens D, Apple F, Garcia-Beltran L, Jaffe A, Karon B, Lewandowski E, et al. Results from a multicenter eval-


