Different Collection Tubes in Cardiac Biomarkers Detection

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The role played by different collection tubes is one of the more frequently forgotten parameters, which influences the quality of laboratory results.

We aimed to determine whether there is a significant difference in assay of myoglobin, cardiac troponin I, and CK-MB depending on the presence of anticoagulant and gel separator in the collecting tubes.

Blood samples were collected in 40 consecutive patients admitted for acute coronary syndrome. The samples were taken at the same moment in three different tubes (tubes without anticoagulant and with gel separator, heparin tubes with gel separator and heparin tubes without gel

separator; Venosafe, Terumo Europe, Leuven Belgium). Myoglobin, CK-MB and cardiac troponin I were measured with a double side (sandwich) chemiluminescent immunoassay (Access Myoglobin, Access CK-MB, Access AccuTnI).

Cardiac troponin I was not influenced by the type of collection tube used. On the contrary, myoglobin and CK-MB showed a statistical difference depending on the test tube. In particular, there was a significant difference between tubes without anticoagulant and with gel separator and tubes with heparin and without gel. *J. Clin. Lab. Anal.* 22:391–394, 2008. © 2008 Wiley-Liss, Inc.

Key words: cardiac biomarkers; collection tubes; acute coronary syndrome; anticoagulant; gel separator

INTRODUCTION

The impact of laboratory medicine in clinical cardiology has increased dramatically in recent years (1) and the fundamental role played by biochemical markers in the diagnosis of acute coronary syndrome has been extensively described in the recently published Guidelines of the National Academy of Clinical Biochemistry (2).

Myoglobin is usually determined as an early, but not specific, marker of acute coronary syndrome (3), while cardiac troponin I and T are highly sensitive in detecting myocardial damage and particularly specific for the myocardial muscle (4,5).

Over the past two decades there have been many changes in the tubes used for collection of blood for laboratory tests (e.g., substitution of glass with plastic as primary tube component, polymer gel in evacuated blood collection tubes, clot activator).

Separator gel is a common component of tubes and acts as a barrier between serum and clot or plasma and cells after centrifugation of tubes. Plastic separator gel tubes are widely used in clinical laboratories because of the comfort they provide.

Some studies suggest that separator gel may have an analytical effect on many assays, which usually has no clinical relevance, except in cases of therapeutic drugs monitoring (6,7) and total triiodothyronine (8).

The concentration of cardiac troponin I and T is decreased in heparin plasma compared with serum, especially in the early phases of myocardial injury. This observation suggests that the binding of heparin to troponins could decrease their immunoreactivity and this effect could depend on the kind of antibodies used (9).

The purpose of our study was to determine whether there is any significant difference in assay of cardiac biomarkers (myoglobin, cardiac troponin I, and CK-MB) depending on the presence or not of anticoagulant and gel separator in the collecting tubes.

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MATERIAL AND METHOD

Forty consecutive patients admitted to the coronary intensive-care unit of our hospital for acute coronary syndrome were included in the study. Specialized nurses collected blood samples in three different tubes at the same moment. The three collection tubes were: tubes without anticoagulant and with gel separator (Venosafe, Terumo Europe, Leuven Belgium; Group 1); heparin tubes with gel separator (Venosafe, Terumo Europe; Group 2) and heparin tubes without gel separator (Venosafe, Terumo Europe; Group 3).

The tubes were centrifuged at 3,900 rpm for 10 minutes within 30 min of collection and analyzed shortly afterwards.

Myoglobin, CK-MB and cardiac troponin I were measured with a double side (sandwich) chemiluminescent immunoassay (Access Myoglobin, Access CK-MB, Access AccuTnI). These methods are suitable for use with the Beckman Coulter Immunoassay. All the determinations were performed on the analyzer Access 2 (Fullerton, CA) following the manufacturer's instructions. Cut-off levels in our laboratory are <100 ng/ml for myoglobin, <6 ng/ml for CK-MB, and <0.06 ng/ml for troponin I.

Statistical Analysis

Values are reported as mean and standard deviation. In the comparison between the groups (Group 1, 2, and 3) analysis of variance (ANOVA) for repeated measures was performed. A protection from the type 1 errors of 0.05 was used. Levels of *P* less than 0.05 were considered significant. If the ANOVA was significant, a post hoc analysis with the Holm Test was performed to calculate the differences between the groups. Bland–Altman plots were used to evaluate the differences between groups with significant difference in the ANOVA analysis. Statistical analysis was performed with the Software Minitab Inc Release 14.20.

RESULTS

In Group 1 the concentration of myoglobin ranged from a minimum value of 24.5 ng/ml to a maximum value of 2,431.7 with a mean of 321.2 ± 558.3 ; CK-MB ranged from a minimum value of 2.9 ng/ml to a

maximum value of 470.4 with a mean of 90.5 ± 116.4 ; cardiac troponin I ranged from a minimum value of 0.1 ng/ml to a maximum value of 317.9 with a mean of 26.9 ± 52.3 .

In Group 2 the concentration of myoglobin ranged from a minimum value of 23.8 ng/ml to a maximum value of 2,325.6 with a mean of 316.1 ± 545.6 ; CK-MB ranged from a minimum value of 3 ng/ml to a maximum value of 453.5 with a mean of 89.1 ± 114.2 ; cardiac troponin I ranged from a minimum value of 0.12 ng/ml to a maximum value of 318.8 with a mean of 27.12 ± 52.4 .

In Group 3 the concentration of myoglobin ranged from a minimum value of 22.4 ng/ml to a maximum value of 2,251.2 with a mean of 306 ± 532.5 ; CK-MB ranged from a minimum value of 2.8 ng/ml to a maximum value of 437.2 with a mean of 87.4 ± 111.1 ; cardiac troponin I ranged from a minimum value of 0.1 ng/ml to a maximum value of 317.9 with a mean of 26.67 ± 50.8 . These results are summarized in Table 1.

ANOVA for repeated measurements did not show any significant difference among the three groups for the determination of cardiac troponin I, whereas there was a statistical difference in the determination of myoglobin and CK-MB.

Statistical analysis using the Holm Test showed a significant difference between groups 1 and 3 both for myoglobin and CK-MB assay ($P < 0.05$) and no significant difference between the other groups. The difference between the groups was further evaluated using the Bland–Altman plot (Figs. 1 and 2).

DISCUSSION

Laboratory results can be affected by preanalytical errors such as incorrect identification or mishandling of samples and by interference factors such as hemolysis, lipids, heterophile antibodies, or drugs. The role played by the different collection tubes is one of the more frequently forgotten parameters that influence the quality of laboratory results in daily activity. Mass spectrometry has shown that many types of tubes shed polymeric materials into samples and these may interfere with some types of analysis such as protein profiling (10).

TABLE 1. Descriptive Measures (Mean $\pm$ Standard Deviation) for the Concentration of Myocardial Markers in Tubes Without Anticoagulant and With Gel (Group 1), Heparin Tubes With Gel Separator (Group 2) and Heparin Tubes Without Gel (Group 3)

	Group 1	Group 2	Group 3	ANOVA
Myoglobin (ng/ml)	321.2 ± 558.3	316.1 ± 545.6	306.0 ± 532.5	0.002*
CK-MB (ng/ml)	90.5 ± 116.4	89.1 ± 114.2	87.4 ± 111.1	0.007*
Cardiac troponin I (ng/ml)	26.9 ± 52.3	27.1 ± 52.4	26.6 ± 50.8	0.27

*Significant difference between Group 1 and Group 3 (Holm Test).

Fig. 1. Percentage mean difference between Group 1 and Group 3 levels of myoglobin, analyzed using a Bland–Altman plot. The solid line indicates the mean percentage difference among tubes; dashed lines indicate the 95% confidence interval for the differences.

Fig. 2. Percentage mean difference between Group 1 and Group 3 levels of CK-MB, analyzed using a Bland–Altman plot. The solid line indicates the mean percentage difference among tubes; dashed lines indicate the 95% confidence interval for the differences.

These inappropriate results could lead to inappropriate therapeutic decisions with devastating consequences (11). On the contrary, facilitating the collection procedure by simplifying the process using the same collection tube for different analysis could reduce not only the time needed to obtain results but also the economic costs.

The importance of the type of collection tubes has already been extensively evaluated in the literature (12–14), but to our best knowledge it has only been done on troponin in acute coronary syndrome, among the myocardial markers.

Particularly relevant is the possibility of using different collection tubes in the emergency setting in order to facilitate the clinical management of the patients (15). Furthermore, from a purely laboratory point of view, working with collection tubes with gel separator is easier and safer.

Our data suggest that cardiac troponin I, which is the most specific myocardial marker, is not influenced by the type of collection tube used. On the contrary, myoglobin and CK-MB showed a statistical difference depending on the test tube. In particular, there was a significant difference between tubes without anticoagulant and with gel separator (Group 1) and tubes with heparin and without gel (Group 3).

The analytical difference observed in our study does not correspond to any significant clinical difference, at least at pathological concentration as suggested by the Bland–Altman plot.

The strange finding in this study is that there was a difference in the determination of myoglobin and CK-MB concentration between tubes without anticoagulant and with gel separator (Group 1) and tubes with heparin

and without gel (Group 3). No difference was found between tubes without anticoagulant and with gel separator (Group 1) and heparin tubes with gel separator (Group 2). Therefore, the difference found between Group 1 and Group 3 could be linked to the separator gel and not to heparin.

These results are discordant with previous observations about the negative interference of heparin in cardiac troponin assay (9), while they could suggest an active role of the separator gel, as recently reported in the determination of free fatty acids (16).

The mechanism of interference of the gel separator with the myoglobin and CK-MB assay is not clear. An unknown substance released from the gel could mildly affect the generation of the chemiluminescent signal or it could affect the interaction between the antibodies used in the assay and myoglobin or CK-MB, but further studies are required.

CONCLUSION

Although a number of interference factors can affect the laboratory test results, our data show that cardiac troponin I could be safely dosed on different collection tubes without altering the results.

On the contrary, CK-MB and myoglobin showed a statistical difference depending on the test tubes used for blood collection, which does not have any clinical relevance.

REFERENCES

1. Clerico A. The increasing impact of laboratory medicine on clinical cardiology. *Clin Chem Lab Med* 2003;41:871–873.
2. National Academy of Clinical Biochemistry Laboratory Medicine practice guidelines. Clinical characteristics and utilization of biochemical markers in acute coronary syndromes. *Clin Chem* 2007;53:552–574.
3. Panteghini M, Pagani F, Bonetti G. The sensitivity of cardiac markers: an evidence-based approach. *Clin Chem Lab Med* 1999;37:1097–1106.
4. Hamm CW. Acute coronary syndromes. The diagnostic role of troponins. *Thromb Res* 2001;103:63–69.
5. Panteghini M. Acute coronary syndromes. Biochemical strategies in the troponin era. *Chest* 2002;122:1428–1435.
6. Hill BM, Laessig RH, Koch DD, Hassemer DJ. Comparison of plastic versus glass evacuated serum-separator (SST™) blood-drawing tubes for common clinical chemistry determination. *Clin Chem* 1992;38:1474–1478.
7. Boeynaems JM, De Leener A, Dessars B, et al. Evaluation of a new generation of plastic evacuated blood-collection tubes in clinical chemistry, therapeutic drug monitoring, hormone and trace metal analysis. *Clin Chem Lab Med* 2004;42:67–71.
8. Bowen RAR, Chan Y, Cohen J, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assay. *Clin Chem* 2005;51:424–433.
9. Gerhardt W, Nordin G, Herbert A, et al. Troponin T and I assay show decreased concentrations in heparin plasma compared with serum: Lower recoveries in early than in late phase of myocardial injury. *Clin Chem* 2000;46:817–821.
10. Drake SK, Bowen RAR, Ramaley AT, Hortin GL. Potential interference from blood collection tubes in mass spectrometric analysis of serum polypeptides. *Clin Chem* 2004;50:2398–2401.
11. Cole LA, Rinne KM, Shahabi S, Omrani A. False-positive hCG assays results leading to unnecessary surgery and chemotherapy and needless occurrences of diabetes and come. *Clin Chem* 1999;45:313–314.
12. Oliveira LC, Kawasato KH, Otta MS, Lima LP, Okay TS. Collection tubes with or without gel separator do not interfere with detection of rubella virus antibodies IgM and IgG. *J Clin Lab Anal* 2007;21:330–334.
13. Lowrey I, Smith G. Elevated results in vitamin B12 assay when using serum separator blood collection tubes. *Ann Clin Biochem* 2003;40:560–562.
14. Bowen RA, Chan Y, Cohen J, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assays. *Clin Chem* 2005;51:424–433.
15. Wang YS, Feng CF, Er TK, et al. Comparison of cardiac troponin I levels in serum and sodium-citrate plasma using the Access 2 immunoassay. *Ann Clin Lab Sci* 2005;35:453–454.
16. Bowen RAR, Vu C, Remaley AT, Hortin GL, Csako G. Differential effect of blood collection tubes on total free fatty acids (FFA) and total triiodothyronine (TT3) concentration: a model for studying interference from tube constituents. *Clin Chim Acta* 2007;378:181–193.