Assessment of the Stability of Midregional Proadrenomedullin in Different Biological Matrices

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Abbreviations: MR-proADM, midregional proadrenomedullin; ADM, adrenomedullin; PTV, Policlinico Tor Vergata University Hospital; CBM, Campus Bio-Medico University Hospital.

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ABSTRACT

Midregional proadrenomedullin (MR-proADM) has been shown to play a key role in endothelial dysfunction, with increased levels helping to prevent early stages of organ dysfunction. Recent clinical evidence has demonstrated MR-proADM to be a helpful biomarker to identify disease severity in patients with sepsis as well as pneumonia. This biomarker is helpful at triage in emergency departments to assess risk level of patients. The aim of this study is to evaluate the stability of MR-proADM in different biological matrices. The results, obtained by Bland-Altman and scatter plot analyses, demonstrate that deviation of MR-proADM concentration in serum compared to EDTA plasma unequivocally shows that serum should not be used as a sample matrix. Instead, the excellent correlation of heparin plasma vs EDTA plasma samples shows that heparin plasma can be used without reservation in clinical routine and emergency samples.

The rapid measurement of blood biomarkers is crucial to make early and accurate clinical decisions. The measurement of a panel of different biomarkers from a single blood sample can facilitate faster results and reduce the laboratory time required to prepare different matrices for testing.

One peptide gaining great interest is adrenomedullin (ADM), which has been shown to play a key role in microcirculation and microvascular dysfunction, with increased levels helping to prevent local tissue hypoxia and the early stages of organ dysfunction.[1–3] However, the reliable measurement of ADM is complicated by a number of issues, such as a short half-life, rapid metabolism, low concentration, rapid degradation by proteases, and binding to complement factor H.[4] Accordingly, ADM levels typically can be underestimated. The measurement of midregional proadrenomedullin (MR-proADM) provides a solution to these problems.[5]

MR-proADM is a fragment of 48 amino acids that splits from the proADM molecule in a ratio of 1:1 with ADM and proportionally represents the level and activity of ADM. Its biological inactivity means that it is not involved in the binding to vessel walls and surfaces found with ADM. Its longer half-life of several hours and biological inactivity result in a more accurate estimation of plasma concentration level than using ADM.[4] Recent clinical evidence has shown it to accurately identify disease severity in patients with sepsis[6,7] as well as patients with pneumonia[8] and urinary tract infections.[9] Such findings can help guide treatment strategies and patient disposition decisions.[10–12]

At present, however, the B·R·A·H·M·S MR-proADM sandwich immunoassay is only recommended for use in EDTA blood samples, thus limiting its potential adoption into daily clinical routine. Nevertheless, MR-proADM matrix expansion is beginning to gain interest, with 2 recent studies showing similar serum MR-proADM concentrations or cutoffs in a healthy population of 102 blood donors[13] and 79 noncardiac surgery patients,[14] to preestablished concentration ranges found in EDTA plasma elsewhere for healthy blood donors[15] or mortality prediction.[6] However, a number of limitations could be observed in this comparison, including the use of only 1 blood sample per patient, allowing no direct concentration comparisons to be made between matrices within the same patient. Furthermore, the use of only healthy blood donors did not consider the potential effects of different disease conditions on MR-proADM concentrations among different matrices. A direct comparison was made in a limited set of healthy blood donors[16] that showed a >30% decrease in serum compared to EDTA plasma. However, part of the determinations were around or below the assay limits, leading to great imprecision in the measurement.

Therefore, this technical evaluation aims to test the preanalytic stability of MR-proADM in serum and heparin plasma compared to the recommended EDTA plasma in freshly collected blood samples of the

TABLE 1. Agreement and Correlation of Serum and Heparin Plasma in Comparison to EDTA Plasma

	EDTA vs Serum	EDTA vs Heparin Plasma
Median bias	−21.3% (−25.9 to −15.0)	−2.6% (0.3 to 4.2)
Spearman correlation	0.97	1.00
Passing-Bablok		
Intercept (CI)	−0.03 (−0.09 to 0.02)	−0.00 (−0.02 to 0.01)
Slope (CI)	0.83 (0.76 to 0.90)	0.98 (0.96 to 1.00)
Agreement at 0.87 nmol/L		
Positive (>0.87 nmol/L)	78%	100%
Negative (≤0.87 nmol/L)	100%	100%
Total	87%	100%
Agreement at 1.50 nmol/L		
Positive (>1.50 nmol/L)	78%	100%
Negative (<1.50 nmol/L)	100%	100%
Total	93%	100%

same patient, both before and after centrifugation. This serves to make an assessment of whether heparin plasma and/or serum could be used reliably for measuring MR-proADM concentration in clinical routine, thus potentially decreasing the number of blood samples taken from a patient for diagnostic testing.

Material and Methods

Study Design

Samples were collected at the Policlinico Tor Vergata University Hospital (PTV) and the Campus Bio-Medico University Hospital (CBM). The local ethics committee approved the study design (R.S. 116/18), and participating patients gave written consent.

Samples were collected in the morning during routine blood draws, using BD Vacutainers without gel for EDTA plasma and serum (CBM) or BD Vacutainers without gel for EDTA plasma and lithium heparin (PTV). All samples were centrifuged within 90 minutes after collection. First

FIGURE 1. Bland-Altman plot of serum vs EDTA plasma (A) and heparin plasma vs EDTA plasma (B).

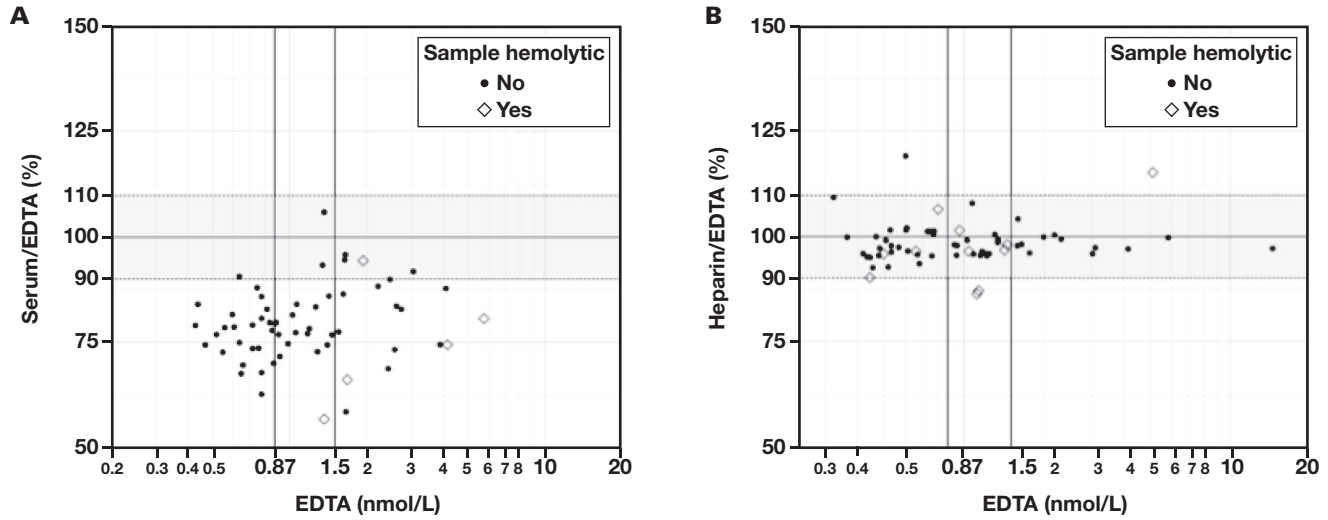


FIGURE 2. Scatterplot of serum vs EDTA plasma (A) and heparin plasma vs EDTA plasma (B).

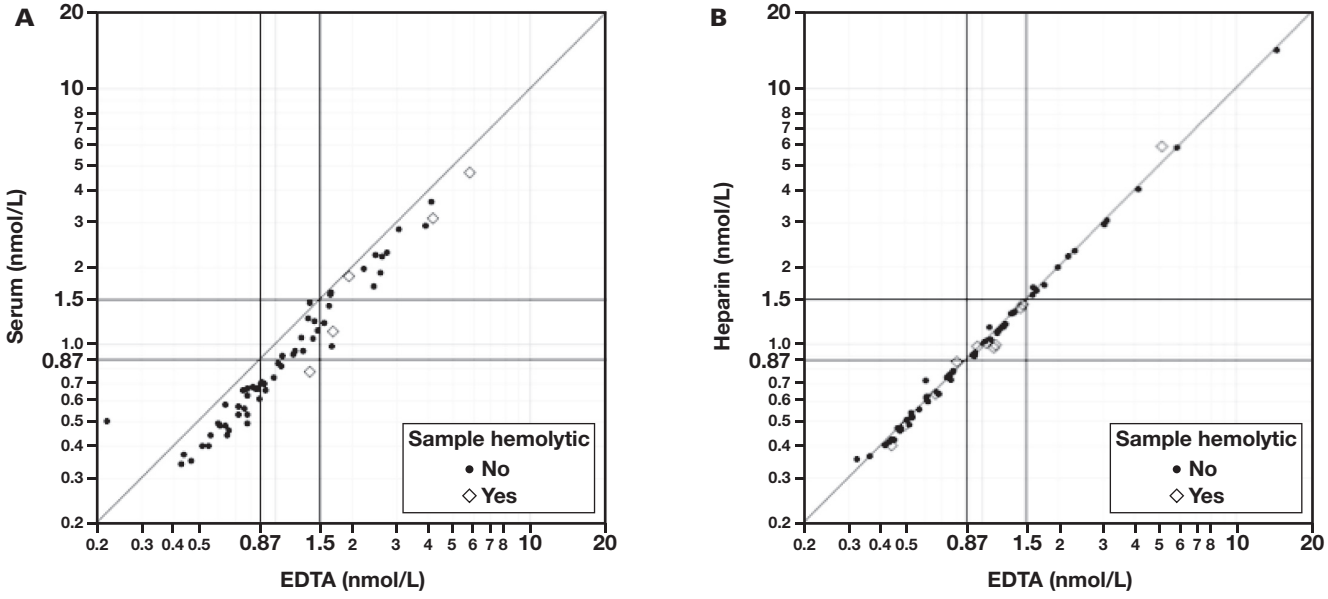
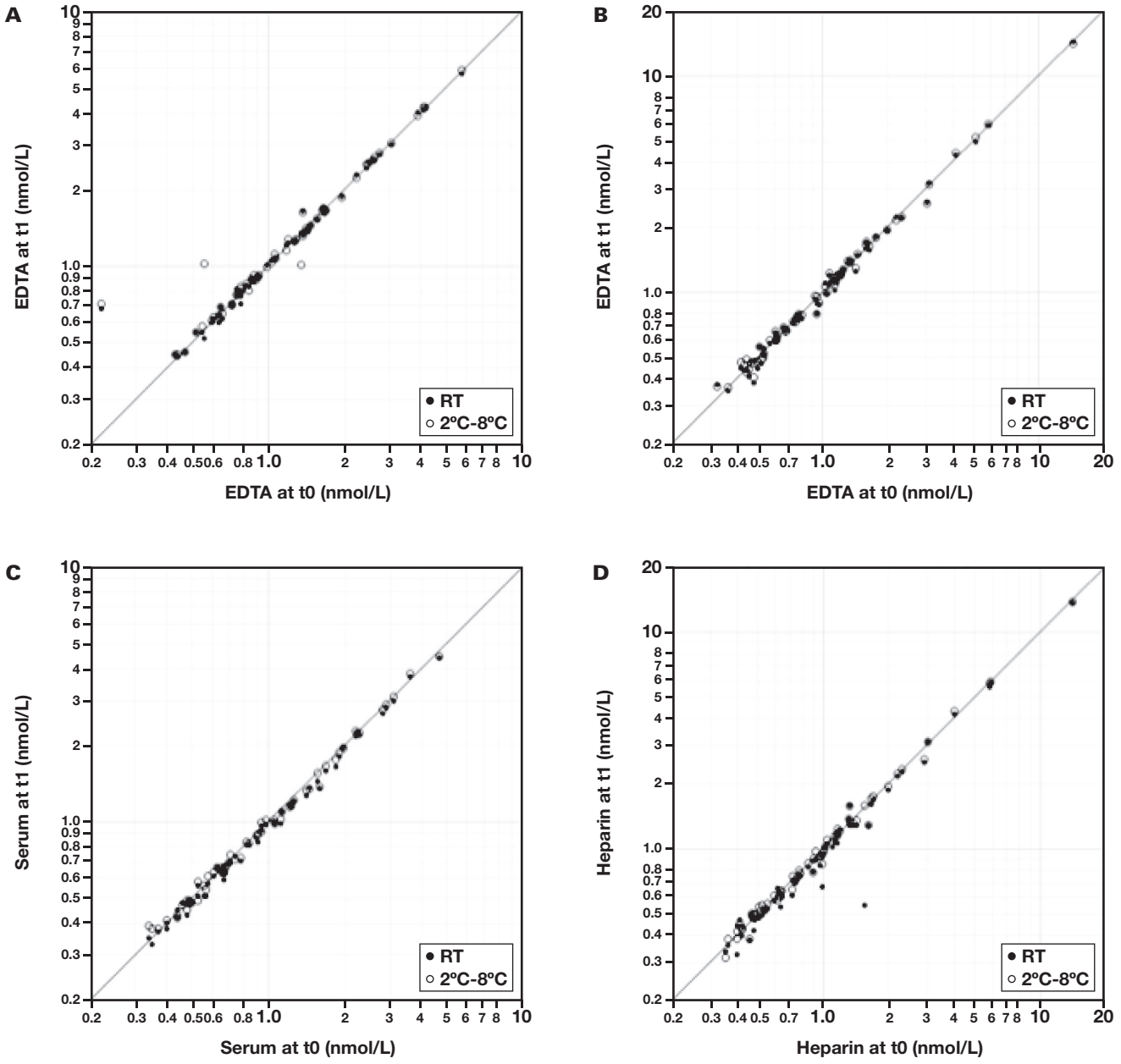


FIGURE 3. Six-hour stability of MR-proADM in EDTA plasma from the 2 sites. A and C, Samples collected at Campus Bio-Medico University Hospital. B and D, Samples collected at Policlinico Tor Vergata University Hospital.



measurement in duplicate (2 aliquots) started after 45 minutes, after which 1 aliquot of each matrix was stored at either room temperature or at 2°C–8°C for 6 hours, after which MR-proADM was measured again.

Measurements

MR-proADM was measured using the B·R·A·H·M·S MR-proADM KRYPTOR on a KRYPTOR Compact Plus. The assay has a direct measuring range from 0.05 to 10 nmol/L. The allowed sample type is EDTA plasma. The limit of quantification has been estimated in accordance with Clinical and Laboratory Standards Institute guideline EP 17-A to be 0.23 nmol/L. The maximum intra-assay coefficient of variation (CV) was estimated to be 10.8% for low concentrations. The reference range for healthy subjects (based on 144 measurements) was median 0.38 nmol/L and 97.5 percentile at 0.55 nmol/L.

Statistics

The MR-proADM values obtained in the different matrices were compared with Spearman's correlation, Passing and Bablok regression, Bland-Altman plots, and percentage agreement at 2 clinically relevant

TABLE 2. Correlation Between Measurement on Fresh Sample and After 6-Hour Storage

	Storage at RT	Storage at 2°C–8°C
EDTA plasma (at CBM)		
Median bias (IQR)	0% (–2.2 to 2.0)	0.6% (–1.5 to 3.4)
Passing-Bablok		
Intercept (CI)	0.00 (–0.01 to 0.03)	0.01 (–0.00 to 0.04)
Slope (CI)	1.00 (0.98 to 1.01)	1.00 (0.97 to 1.01)
Serum (at CBM)		
Median bias (IQR)	–4.0% (-–6.7 to –1.0)	–2.1% (–4.7 to 2.1)
Passing-Bablok		
Intercept	0.02 (–0.00 to 0.03)	0.02 (–0.01 to 0.03)
Slope	0.94 (0.93 to 0.97)	0.96 (0.94 to 0.99)
EDTA plasma (at PTV)		
Median bias (IQR)	–0.4% (–3.9 to 2.7)	0.4% (–3.7 to 3.2)
Passing-Bablok		
Intercept (CI)	0.00 (–0.00 to 0.00)	–0.01 (–0.03 to 0.02)
Slope (CI)	1.00 (0.97 to 1.03)	1.01 (0.97 to 1.03)
Heparin plasma (at PTV)		
Median bias (IQR)	–3.5% (–6.0 to 0.2)	0% (–3.7 to 3.3)
Passing-Bablok		
Intercept (CI)	–0.00 (–0.03 to 9.02)	0.01 (–0.02 to 0.02)
Slope (CI)	0.97 (0.93 to 1.00)	0.99 (0.97 to 1.02)

CBM, Campus Bio-Medico University Hospital; CI, confidence interval; IQR, interquartile range; PTV, Policlinico Tor Vergata University Hospital; RT, room temperature.

cut-offs, namely 0.87 nmol/L[11,17–20] and 1.5 nmol/L.[11,17,21,22] Mean bias with interquartile range was calculated.

The statistical analysis was carried out with Analyse-It and JMP from SAS.

Results

EDTA Plasma vs Serum

Serum and EDTA plasma was collected from 61 patients (40 female/21 male; mean age, 69 years). Seven patients received anticoagulation, and in 5 patients at least 1 sample was visibly hemolytic.

The main results are shown in **TABLE 1**. Although the Spearman correlation between serum and EDTA plasma was good (0.97), the mean bias (–2.3%) and the Passing and Bablok slope (0.83) indicate a systematic deviation that can be seen in the Bland-Altman and scatter plots (**FIGURES 1** and **2**, respectively). This is also reflected in the poor agreement at clinically relevant cut-offs (0.87 and 1.5 nmol/L). Although hemolytic samples can give imprecise results, there was no indication that this influenced the results (data not shown). Also, the use of anticoagulants did not influence the result (data not shown)

EDTA Plasma vs Heparin Plasma

EDTA plasma and heparin plasma was collected from 63 patients (33 female/30 male; mean age, 62 years). Seven patients received anticoagulant therapy, and in 11 patients at least 1 sample was visibly hemolytic. The main results are shown in **TABLE 1**. Spearman correlation between heparin plasma and EDTA plasma was excellent (1.00), and the low mean bias (2.6%) and the Passing and Bablok slope (0.98) also indicate an excellent correlation. The Bland-Altman and scatter plots (**FIGURES 1** and **2**, respectively) show no signs of concentration-dependent deviation. This is also reflected in the excellent agreement at clinically relevant cut-offs (0.87 and 1.5 nmol/L). Although hemolytic samples can give imprecise results, there was no indication that this influenced the results. Also, the use of anticoagulants did not influence the result (data not shown).

Stability at Room Temperature and at 2°C–8°C

All samples were tested for 6-hour stability after centrifugation at room temperature and at 2°C–8°C. Comparison was done for heparin plasma, serum, and EDTA plasma (separate for the 2 centers) (**FIGURES 3**). When compared to their original measurement at t0, all matrices showed good correlation reflected in the Passing-Bablok slope and the low median bias (**TABLE 2**). No apparent difference in stability was seen between storage at room temperature and at 2°C–8°C.

Discussion

This study shows for the first time the concentration of MR-proADM in serum and plasma (EDTA and heparin) in matched samples collected from routine clinical patients. The clear deviation of MR-proADM concentration in serum compared to EDTA plasma unequivocally shows that serum should not be used as the sample matrix. Heparin plasma, however, showed only minimal bias compared to EDTA plasma. The excellent correlation and the agreement at clinically relevant cut-offs show that the use of heparin plasma can be used without reservation in clinical routine and emergency samples.

We were not able to reproduce the findings in serum by Lorubbio et al,[13] who found a similar 95th percentile for studies using serum samples from heathy blood donors and studies in EDTA plasma. However, cohort-specific age and body mass index distribution might have masked the differences, as there was a significant correlation of MR-proADM concentration with both.[13,15,16] In addition to healthy blood donors,[16] we have now confirmed the breakdown of MR-proADM in the serum of in-hospital patients. The lower bias in patients compared to healthy blood donors (21% vs >30%) probably relates to the higher concentration in patients; that is, all values were well above the functional assay sensitivity.

Despite the difference in MR-proADM in serum compared to EDTA plasma, after clotting and sample preparation, the concentration was stable in all 3 matrices for at least 6 hours independent of storage temperature, meaning that the breakdown of MR-proADM in serum took place during the coagulation phase. Although in serum, proteases and cofactors are activated during coagulation, in plasma, they are mainly inhibited by the use of anticoagulants, such as EDTA (metallo- and Ca2+-dependent proteases) and heparin (thrombin, factor Xa).[23] Indeed, the total proteolytic activity was shown previously to be the highest in serum and citrate plasma followed by heparin plasma and EDTA plasma,[24] with each matrix having its own specific mix of active peptidases recognizing specific amino acid motifs. Therefore, some peptides might be broken down in serum and EDTA or heparin plasma, whereas others

are only broken down in one of them, depending on their amino acid composition.

Conclusions

This study shows that, in addition to the available EDTA plasma, heparin plasma can be used to measure MR-proADM levels. Values are completely comparable; the clinical cut-offs established in EDTA plasma[10,11] can also be used on values obtained from heparin plasma. This may potentially decrease the number of blood samples taken from a patient for diagnostic testing and increase the implementation of this biomarker.

Another collateral but no less important aspect is the possibility of using heparin samples for emergency room analysis. In fact, the use of lithium or sodium heparin samples is reserved for the assay of biomarkers for emergency tests (such as myocardial biomarkers). In this regard, the use of the same sample for different biomarkers could reduce the amount of blood drawn from the patient, and additional tests, like MR-proADM, could be very helpful to facilitate the clinical diagnostic and/or prognostic pathway. The use of this biomarker in different settings has been evaluated and it could be advantageous in clinical practice. In the emergency setting, its value has been demonstrated in sepsis diagnosis and prognosis as well as in bacterial or viral infection.[10,25–28] Very recently, in COVID-19 patients, MR-proADM was also evaluated as a helpful marker of endothelial damage, disease severity, and mortality.[29–31]

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