Influence of the matrix type over the concentration of GDF-15

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ABSTRACT

Growth differentiation factor 15 (GDF-15) has been suggested as a prognostic biomarker for bleeding and mortality in atrial fibrillation (AF). To date, serum and EDTA matrices are standardized for the GDF-15 assay but it is unclear if it can be measured also in citrate. In this study, we aim to investigate if the Elecsys GDF-15 assay (Roche Diagnostics, Mannheim, Germany) can be determined accurately in citrate samples in a cohort of 10 patients with AF and 10 healthy controls. From January 2018 to March 2018, we included healthy controls and patients with AF under vitamin K antagonists in a tertiary hospital. Blood samples were drawn in both groups. We included 10 controls (50% males, mean age 36.4±8.9 years) and 10 patients with AF (80% males, mean age 76.5±16.6 years). The mean GDF-15 levels were increased in patients with AF in comparison to healthy controls, as expected by the presence of a heart-related condition and the higher age of this population. In healthy controls, GDF-15 levels showed an optimal correlation between EDTA-serum (r=0.975; p<0.001), EDTA-citrate (r=0.972; p<0.001), and serum-citrate (r=0.997; p<0.001) samples. This was also observed in patients with AF: EDTA-serum (r=0.975; p<0.001), serum-citrate (r=0.835; p=0.003), and EDTA-citrate (r=0.768; p=0.009). Our results demonstrate that citrate samples may be used for the determination of GDF-15 in AF given the positive and good correlation with EDTA and serum matrices. Further studies should validate these observations.

INTRODUCTION

Growth differentiation factor 15 (GDF-15) is a cytokine from the transforming growth factor β superfamily. GDF-15 is expressed in low concentrations in most organs under normal conditions but it is also expressed and secreted by macrophages and cardiomyocytes in response to oxidative stress and inflammation. Thus, cardiovascular disease is a major driver of GDF-15 production and increasing evidence indicates that GDF-15 predicts adverse outcomes of cardiovascular disease, independent of traditional risk factors.[1 2] In particular, GDF-15 has been suggested as a new prognostic biomarker for bleeding and mortality in atrial fibrillation (AF).[3 4]

To date, serum and EDTA matrices are standardized for the GDF-15 assay.[5] However, it remains unclear if GDF-15 can be measured also in citrate samples. In this study, we aim to investigate if GDF-15 can be determined accurately in citrate samples in a cohort of 10 patients with AF and 10 healthy controls.

METHODS

This is a pilot study performed between January 2018 and March 2018. During this period, we included healthy controls with no known disease and 10 patients with AF hemodynamically stable under oral anticoagulation therapy with vitamin K antagonists in a tertiary hospital from the Hospital Clínico Universitario Virgen de la Arrixaca.

In all healthy subjects and patients with AF, a blood sample was drawn at fasting condition, at rest, atraumatically and without stasis by antecubital puncture using a 21-gauge needle. Samples were placed into different vacutainer collecting tubes containing serum and anticoagulated tubes EDTA and trisodium citrate. Samples were centrifuged at 2200 *g* and 4°C for 10 minutes, and the supernatants were stored in aliquots at −80°C until further use. GDF-15 levels were assessed by electrochemiluminescence in an automated analyzer (Cobas 8000, Roche Diagnostics, Mannheim, Germany) in a single center.

The study was conducted according to the ethical principles of the Declaration of Helsinki and its later amendments and Good Clinical Practice guidelines.

Statistical analyses

Categorical variables were presented as absolute frequencies and percentages. Continuous variables were presented as mean±SD after tested for normality by the Kolmogorov-Smirnov (K-S) test. Comparisons of GDF-15 levels within the 3 matrices were performed using the Student's t-test, whereas correlations between GDF-15 levels in the different matrices were tested by Pearson's correlation coefficient.

Table 1 Comparison of growth differentiation factor 15 levels between patients with atrial fibrillation and healthy controls measured in 3 different matrices

Matrix	Patients with atrial fibrillation	Healthy controls	P value
EDTA (pg/mL), mean±SD	2022.3±1211.2	890.5±555.2	0.015
Serum (pg/mL), mean±SD	1780.5±1011.7	828.6±486.4	0.015
Citrate (pg/mL), mean±SD	1414.1±970.2	736.1±418.6	0.048

RESULTS AND DISCUSSION

We included 10 healthy controls (50% males, mean age 36.4±8.9 years) and 10 patients with AF (80% males, mean age 76.5±16.6 years). There were significant differences in GDF-15 levels between patients with AF and healthy controls. Thus, in the 3 matrices, the mean GDF-15 levels were increased in patients with AF, as expected by the presence of a heart-related condition and the higher age of this population (table 1). Nevertheless, in both groups, the highest levels of GDF-15 were detected in EDTA matrices, whereas the lowest levels were detected in citrate (figure 1A).

According to the K-S test, GDF-15 followed normal distribution in EDTA (K-S statistic=0.173; p=0.121), serum (K-S statistic=0.168; p=0.142), and citrate (K-S statistic=0.189; p=0.064). Correlation of GDF-15 levels within the 3 specimens in the whole sample was good between EDTA-serum (r=0.842), serum-citrate (r=0.888) and EDTA-citrate (r=0.842), all significant with p value <0.001 (figure 1B).

When we analyzed the groups as separate, there were no significant differences in GDF-15 levels between EDTA and serum matrices (p=0.184) in healthy controls. However, GDF-15 levels were significantly higher in EDTA and serum matrices when compared with citrate (p=0.023 and p=0.040, respectively) in this group. Correlation tests showed optimal correlations between EDTA-serum (r=0.975; p<0.001), EDTA-citrate (r=0.972; p<0.001), and serum-citrate (r=0.997; p<0.001).

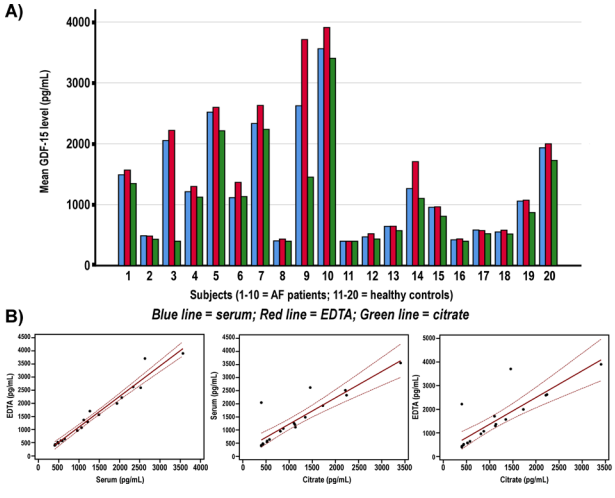

Figure 1 (A) Mean growth differentiation factor 15 (GDF-15) level for each subject according to different samples. (B) Correlation graphs for EDTA-serum, serum-citrate and EDTA-citrate. AF, atrial fibrillation.

On the other hand, there were significant differences in GDF-15 levels between EDTA and serum (p=0.040) despite that both matrices are standardized for the GDF-15 assay, and EDTA and citrate (p=0.035) in patients with AF, whereas no differences were observed between serum and citrate (p=0.073). As in healthy controls, the correlation of GDF-15 levels was optimal between EDTA-serum (r=0.975; p<0.001) and serum-citrate (r=0.835; p=0.003), and good between EDTA-citrate (r=0.768; p=0.009) in patients with AF.

Finally, we investigate the percentage of reduction of GDF-15 levels from the matrix showing the highest level (ie, EDTA) to the matrix showing the lowest level (ie, citrate) in patients with AF. Thus, GDF-15 showed a reduction of 13.58% from EDTA to serum matrices, a reduction of 43.01% from EDTA to citrate matrices, and a reduction of 25.91% from serum to citrate matrices.

During the last years, GDF-15 has gained attention in AF since it has shown to be associated with adverse clinical outcomes,[6–10] but there is no standardized cut-off point yet for this condition. In the present study, we show that GDF-15 levels are increased in patients with AF compared with controls, even in citrate samples. Nevertheless, this is a novel biomarker that requires further investigation, and future studies should validate such observation.

We also observed differences in the GDF-15 levels when measured in EDTA or citrate, but not when measured in serum or citrate. Interestingly, we found significant differences in GDF-15 levels between EDTA and serum samples, despite that both matrices are standardized for the GDF-15 assay. However, it is also important to note that the correlation was good within EDTA, serum and citrate matrices, which certify that this biomarker can be determined in either EDTA, serum, or citrate matrices.

However, it needs to be considered that the concentration might be different depending on the matrix used to collect the blood sample. Thus, it is still required to clarify some important issues about GDF-15 (temporal variability in the determination, type of sample, cut-off point, and so on) before standardizing its use for risk stratification.

Limitations

There are limitations in this study that we must acknowledge. First, this is only a pilot study with small sample size. However, GDF-15 is expensive, so we need to investigate in an initial small cohort if GDF-15 could be determined in citrate samples. Once we have demonstrated this for the first time, we will investigate GDF-15 in higher sample size cohorts. On the other hand, only patients with AF were included, so our results should be validated also in patients with other conditions. Finally, we acknowledge that the

populations studied here are heterogeneous and that the presence of a heart-related condition and age could be influencing these higher GDF-15 levels in patients with AF. Nevertheless, we also included healthy controls despite that we expected to find lower GDF-15 levels in order to investigate if even in this population, this biomarker could be determined accurately in citrate.

CONCLUSION

Our results demonstrate that citrate samples may be used for the determination of GDF-15 in AF given the positive and optimal correlation with EDTA and serum matrices, even when some differences can be detected measuring the biomarker in the different samples. However, further studies are required to validate these results.

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