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NT-proBNP assays that are based on antibodies which are specific to nonglycosylated regions of NT-proBNP display a similar diagnostic accuracy in distinguishing heart failure patients compared to the Roche NT-proBNP assay

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HyTest

Introduction

An automated NT-proBNP immunoassay manufactured by Roche is widely used for NT-proBNP measurements. This assay employs monoclonal antibodies (mAbs) that are specific to the epitopes 27-31 and 42-46 in the central region of NT-proBNP. One of the mAbs is specific to the partially glycosylated region of NT-proBNP as the epitope 42-46 comprises Ser44, which is modified by glycosidic residues. The presence of O-glycans at this site makes NT-proBNP undetectable by the Roche NT-proBNP assay due to the steric hindrance. In light of this, the assay is able to detect only the NT-proBNP fraction that is nonglycosylated at the 42-46 region and not the "total" NT-proBNP, i.e. both glycosylated and nonglycosylated subfractions. Since O-glycosylation tends to be heterogeneous, its pattern and extent might vary significantly among individuals and this could in turn impact the clinical value of NT-proBNP measurements by glycosylation-sensitive NT-proBNP assays. We have developed two alternative NT-proBNP immunoassays that are not affected by analyte glycosylation and are able to measure the concentration of the "total" NT-proBNP. It's not clear yet if underestimating the NT-proBNP concentration due to the influence of glycosylation of NT-proBNP molecules may have some impact on the clinical significance of this biomarker.

The aim of the present study was: to compare the diagnostic accuracy of measurements of the "total" NT-proBNP (by two prototype immunoassays) with measurements of NT-proBNP subfraction that is nonglycosylated at Ser44 (by the Roche NT-proBNP assay) in distinguishing heart failure (HF) from non-HF patients.

Methods

PLASMA SAMPLES

We used EDTA-plasma samples that were obtained from 51 patients (aged between 60 and 84 years), who had been hospitalized for acutely decompensated heart failure. Patients were NYHA class II–IV. As a control, 53 EDTA-plasma samples from age-matched healthy donors without a history of any chronic illness were also measured.

IMMUNOASSAYS

NT-proBNP levels were measured with three immunoassays: the automated Roche Cobas e 411 analyzer and two HyTest prototype sandwich immunoassays $15C4_{63-71}$-$13G12_{13-20}$ and $29D12_{5-12}$ - $NT34_{25-32}$). The epitope specificities of the mAbs employed in the immunoassays are schematically represented in Figure 1.

STATISTICS

All statistical analysis was performed using XLSTAT software by Microsoft®. The diagnostic accuracy of NT-proBNP assays was analyzed by comparison of the ROC curves as described by Delong and Delong (1988) and Sen (1960).

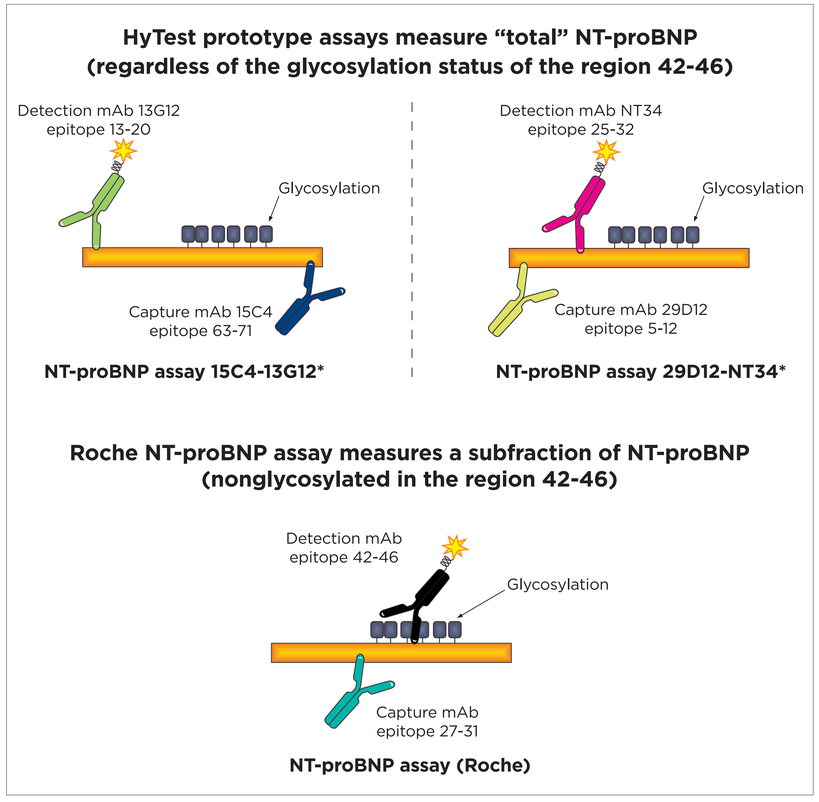

FIGURE 1. Schematic representation of NT-proBNP assays designed by HyTest and Roche.

Results

The diagnostic accuracy of Roche NT-proBNP assay and HyTest prototype NT-proBNP assays in identifying patients with HF was compared using ROC curve analysis. NT-proBNP levels and ROC-AUC values are presented in Table 1. Generated ROC curves are shown in Figure 2.

NT-proBNP concentrations measured with HyTest prototype assays were several-fold higher compared to Roche NT-proBNP assay. The difference can be explained by the influence of glycosylation in the region of NT-proBNP targeted by Roche assay (epitope 42-46) on the binding of antibodies.

Comparing the ROC curves generated for Roche NT-proBNP assays and HyTest prototype NT-proBNP assays we observed that there were no statistically significant differences between them (p-value = 0.369/0.365).

TABLE 1. Results of NT-proBNP measurement in plasma samples.

Assay	NT-proBNP level ng/L		Median ng/L	ROC-AUC	Sensitivity, specificity
Roche Cobas e 411 (proBNP II)	Healthy	0 - 1071	70	0.965	0.86, 0.98
	HF	25 - 11066	2360		
HyTest 15C4-13G12	Healthy	94 - 3645	627	0.946	0.84, 0.98
	HF	419 - 20166	7900		
HyTest 29D12-NT34	Healthy	77 - 1,739	447	0.951	0.86, 0.93
	HF	596 - 27,475	6108		

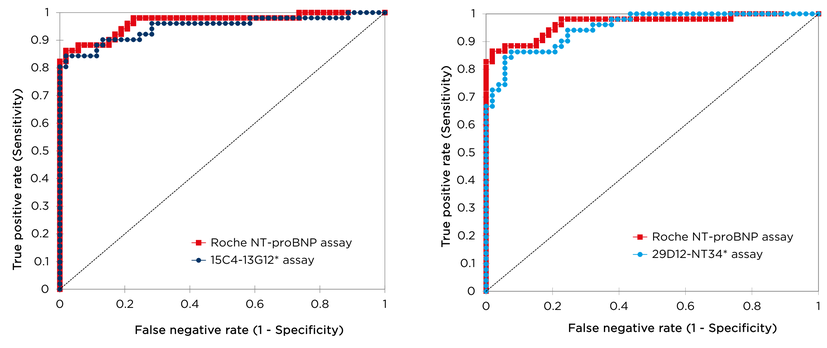

FIGURE 2. ROC curves, showing the ability of automated Roche NT-proBNP immunoassay and HyTest prototype NT-proBNP assays to distinguish patients with HF.

Conclusions

These data show that NT-proBNP immunoassays that are based on antibodies which are specific to nonglycosylated regions of the NT-proBNP molecule (e.g. HyTest's prototype immunoassays) are expected to have at least a similar clinical value for HF diagnosis as the Roche NT-proBNP assay that detects only a subfraction of endogenous NT-proBNP.

Taking into account the known high variability in levels and site occupancy of O-glycosylated proteins, one may expect that immunoassays which measure "total" NT-proBNP levels might be advantageous for HF diagnostics and/or therapy monitoring in certain groups of patients and disease states due to their ability to detect endogenous NT-proBNP independently of its glycosylation status.

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