Development and diagnostic utility of immunoturbidimetric NT-proBNP assay based on antibodies targeting glycosylation-free regions of NT-proBNP

A-010

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BACKGROUND

Multiple commercial immunoassays are available for the measurement of circulating NT-proBNP levels. Most of the assays use antibodies that bind to the central region of NT-proBNP, which is usually glycosylated. Glycosylation of NT-proBNP restricts the binding of the antibodies and detection of the protein. It has been shown that up to 80% of the circulating NT-proBNP may be glycosylated in the central region (1). Several studies have confirmed that NT-proBNP assays underestimate the concentration of plasma NT-proBNP measuring only a subfraction of the circulating NT-proBNP because of the negative effect of glycosylation on NT-proBNP recognition by the antibodies (2,3). Aim of this study is to develop an NT-proBNP assay which is not affected by glycosylation of the protein and comparison of its diagnostic utility with performance of other commercially available immunoassays.

METHODS

Analytical performance of the assay in optimization phase including linearity, LoQ, precision, antigen access, interference, calibration curve- and onboard reagent stability was evaluated according to Clinical and Laboratory Standards Institute (CSLI) guidelines. Instrument variation study was performed using Mindray BS380 and Beckman Coulter DxC 700 AU instruments.

To determine the total concentration of NT-proBNP, independent of glycosylation, plasma samples were either non-treated or treated with a mixture of glycosidases for 24 h at 37 °C. Following, samples were analysed by a commercially available NT-proBNP immunoassay, based on antibodies targeting a glycosylated region of NT-proBNP and the prototype of the Gentian NT-proBNP assay, based on antibodies targeting regions of NT-proBNP that are free of O-glycans.

RESULTS

Evaluation of the analytical performance of Gentian NT-proBNP assay prototype shows very good performance of the assay. The results from linearity, LoQ, antigen excess, precision, calibration curve stability, on-board stability and interference studies are showed in table 1.

NT-proBNP concentrations were measured in plasma samples either treated or untreated with a mixture of glycosidases. No significant effect of the glycosidase treatment was detected on NT-proBNP values measured by a prototype of Gentian NT-proBNP assay. NT-proBNP concentrations in treated and non-treated samples were similar (slope=0.849) and highly correlated (R^2=0.936), Fig 2A. As a comparison, we used one of the commercially available assays with antibodies targeting the central region of the NT-proBNP. NT-proBNP concentrations showed a good correlation (R^2=0.879), but the assay strongly underestimated concentrations in untreated samples (slope=6.34), Fig 2B. The effect of glycosylation was also shown when results measured with Gentian NT-proBNP assay prototype were compared with results from glycosylated and non-glycosylated samples measured with a commercial assay. Results from samples treated with glycosidases and measured with a commercial assay were significantly closer to results obtained with Gentian assay (R^2=0.8898, Fig 2D) compared to results from non-treated samples (Fig 2C). Instrument variation study showed great correlation between values measured on instruments from Mindray and Beckman Coulter (slope = 0.87 and R^2=0.969) (Fig 3).

RESULTS

Analytical performance	Results from prototype
Linearity	approx. 3 200 ng/L – approx. 20 000 ng/L
Antigen access	>56 000 ng/L
Precision	CV <10%
Calibration curve stability	>8 days
Onboard stability	>30 days
Interference	No interference detected at the tested levels: Bilirubin - 25 mg/dL Haemoglobin - 25 mg/dL Intralipid - 150 mg/dL RF - 1 500 IU/mL HAMA - 2 000 ng/mL Biotin - 50 000 ng/L

Table 1. Summary of results from the evaluation of analytical performance of Gentian NT-proBNP assay prototype

Fig. 2A

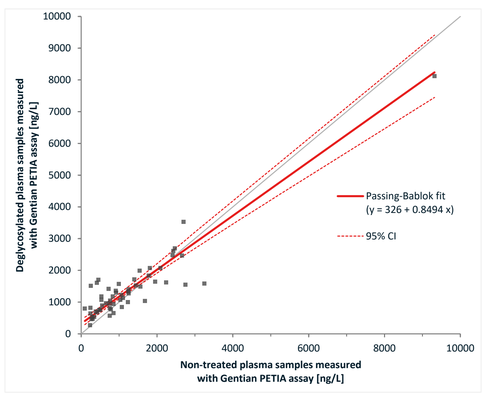

Fig. 2B

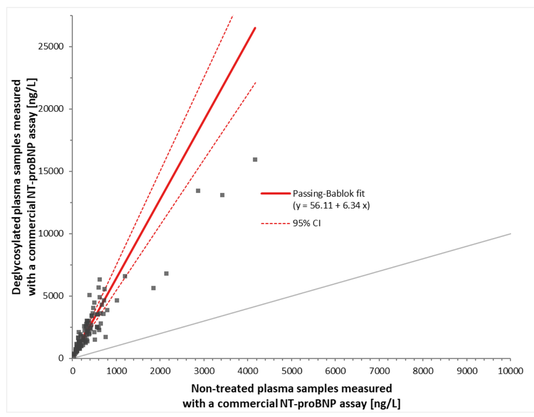

Fig. 2C

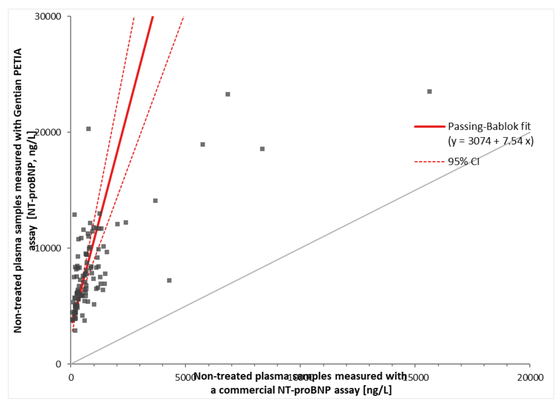

Fig. 2D

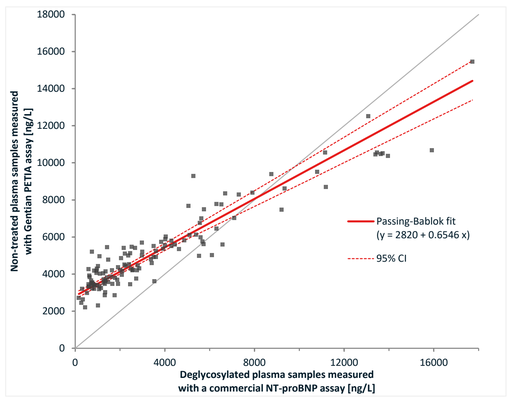

Figure 2. Effect of glycosylation on estimation of NT-proBNP concentration

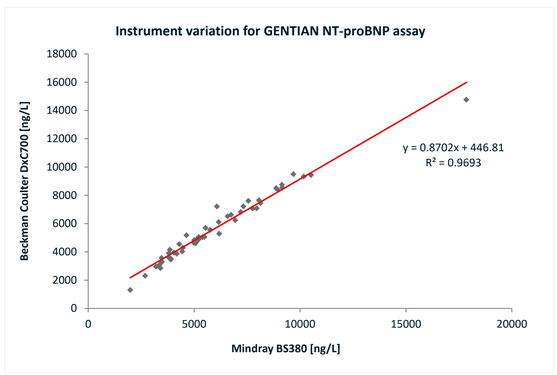

Figure 3. Comparison between results obtained with Gentian NT-proBNP assay on two instruments from different manufacturers

CONCLUSION

Our results confirm the impact of glycosylation on measurement of NT-proBNP and underestimation of NT-proBNP levels by assays based on antibodies targeting glycosylated regions of NT-proBNP. Given the considerable variability in levels and site occupancy of O-glycosylated proteins, it is reasonable to anticipate that immunoassays targeting glycosylation-free regions of NT-proBNP may offer advantages in heart failure (HF) diagnosis and prognosis for specific patient groups and disease states since these assays can detect endogenous NT-proBNP regardless of its glycosylation status. Further investigations, including exploration of the clinical significance are ongoing in order to understand potential benefits and applications of such assays in management of patients with HF.

References: 1. Nishikimi t. et al., (2012). The effect of glycosylation on plasma N-terminal proBNP-76 levels in patients with heart or renal failure. Heart, 98(2), 152–161, 2. Røsjø, H. et al., (2015). Influence of Glycosylation on Diagnostic and Prognostic Accuracy of N-Terminal Pro–B-Type Natriuretic Peptide in Acute Dyspnea: Data from the Akershus Cardiac Examination 2 Study. Clinical Chemistry, 61(8), 1087–1097, 3. Li, L. et al., (2023). Diagnostic utility of total NT-proBNP testing by immunoassay based on antibodies targeting glycosylation-free regions of NT-proBNP. Clinical Chemistry and Laboratory Medicine (CCLM), 61(3), 485–493. https://doi.org/10.1515/cclm-2022-1194

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