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Case Report

Macro-pro-B-type natriuretic peptide (proBNP) and hidden macro-N-terminal proBNP: Case report

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ABSTRACT

B-type natriuretic peptide (BNP) is a cardiac hormone widely used as a biomarker for heart failure. Here, we present the first report of extremely high levels of immunoreactive BNP caused by formation of macro-proBNP.

A 70-year-old woman with left ventricular hypertrophy and normal systolic function presented with extremely high plasma levels of BNP (35,374 pg/ml) and N-terminal proBNP (NT-proBNP; 30,600 pg/ml). Our recently developed proBNP immunoassay showed that nearly 100% of her immunoreactive BNP was proBNP. Polyethylene glycol precipitation tests reported extremely low BNP recovery (1.3%), while protein G addition tests also reported a remarkably low BNP fraction (3.3%). Gel filtration chromatography with normal elution buffer combined with BNP immunoassays showed a BNP peak with a retention time slightly shorter than that of IgG. With acidic elution buffer (pH 3.0), however this peak disappeared and a new BNP peak consistent with glycosylated human proBNP appeared. These results suggest that in this case most BNP immunoreactivity consisted of macro-proBNP, which is an immune complex composed of proBNP and an anti-proBNP autoantibody. Gel filtration chromatography combined with NT-proBNP immunoassays revealed that the NT-proBNP assay cross-reacts with both the proBNP-IgG complex and proBNP. In addition, with acidic buffer, a new large peak appeared with a retention time the same as that of glycosylated NT-proBNP.

These results suggest spuriously high levels of BNP and NT-proBNP are caused by macro-proBNP. Macro-NT-proBNP is not detected by the currently available NT-proBNP assay system.

1. Introduction

B-type natriuretic peptide (BNP) is a cardiac hormone whose plasma levels increase in proportion to the severity of heart failure [1]. Consequently, BNP is widely used as a marker for heart failure [1]. Macro-hormones such as macro-thyroid stimulating hormone, macro-prolactin and others are a rare laboratory interference that can interfere with laboratory hormone measurements leading to spuriously high measured concentrations [2–4]. We present a case of an elderly woman who had extremely high plasma BNP and N-terminal proBNP (NT-proBNP) levels. Our results showed that the extremely high BNP and NT-proBNP immunoreactivity were caused by macro-proBNP, which is an immune complex of proBNP and an anti-proBNP autoantibody.

2. Case report

A 70-year-old woman with a history of type 2 diabetes mellitus, hypertension and angina pectoris was admitted to our hospital for further examination for heart failure. She presented with pretibial pitting edema and had extremely high plasma BNP levels (35,374 pg/ml). Laboratory tests yielded the following results: BNP, 35,117 pg/ml (E test, Tosoh II (BNP), Tosoh, Japan); NT-proBNP, 30,600 pg/ml (Elecsys proBNP2, Roche Diagnostics, U.S.A.), and ANP, 82 pg/ml (E test, Tosoh II (ANP), Tosoh, Japan). Echocardiography showed left ventricular hypertrophy and left atrial dilatation with normal systolic function and abnormal diastolic function. Cardiac catheterization showed normal central hemodynamics (PCWP 4 mmHg, cardiac index 2.6 l/min) and no significant stenosis on coronary arteriography. One month later, the patient had developed sick sinus syndrome, and a permanent pacemaker was implanted. During this period, the patient's plasma BNP

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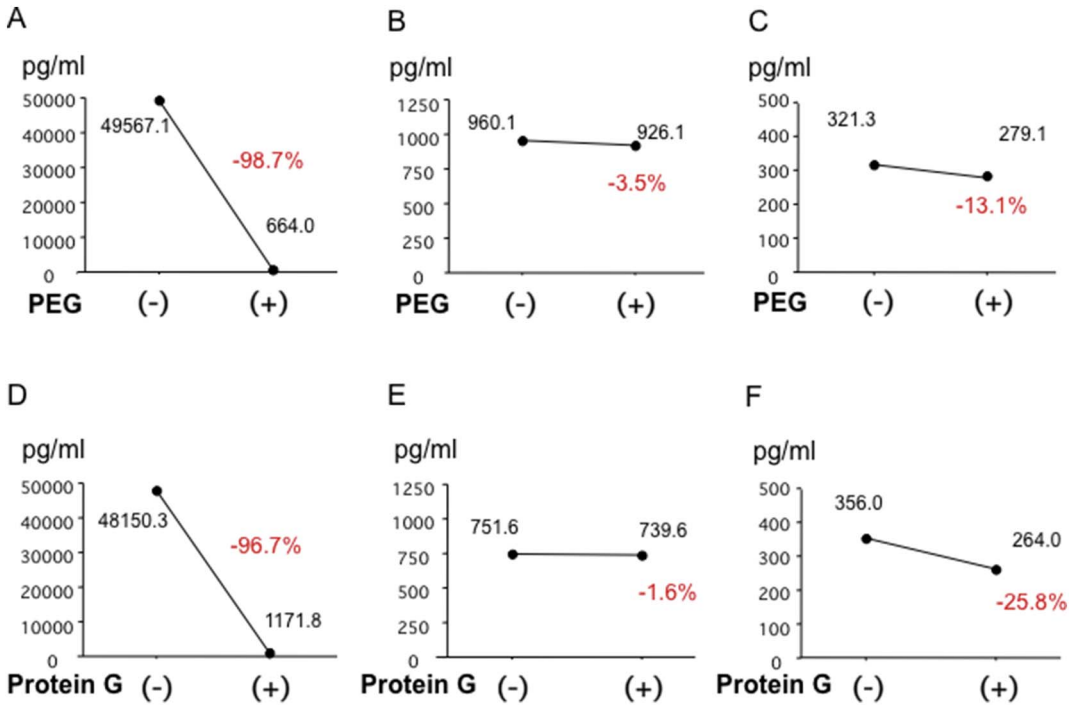

Fig. 1. Polyethylene glycol precipitation tests in this case (A) and in other heart failure patients (B and C). Protein G addition tests in this case (D) and in other heart failure patients (E and F).

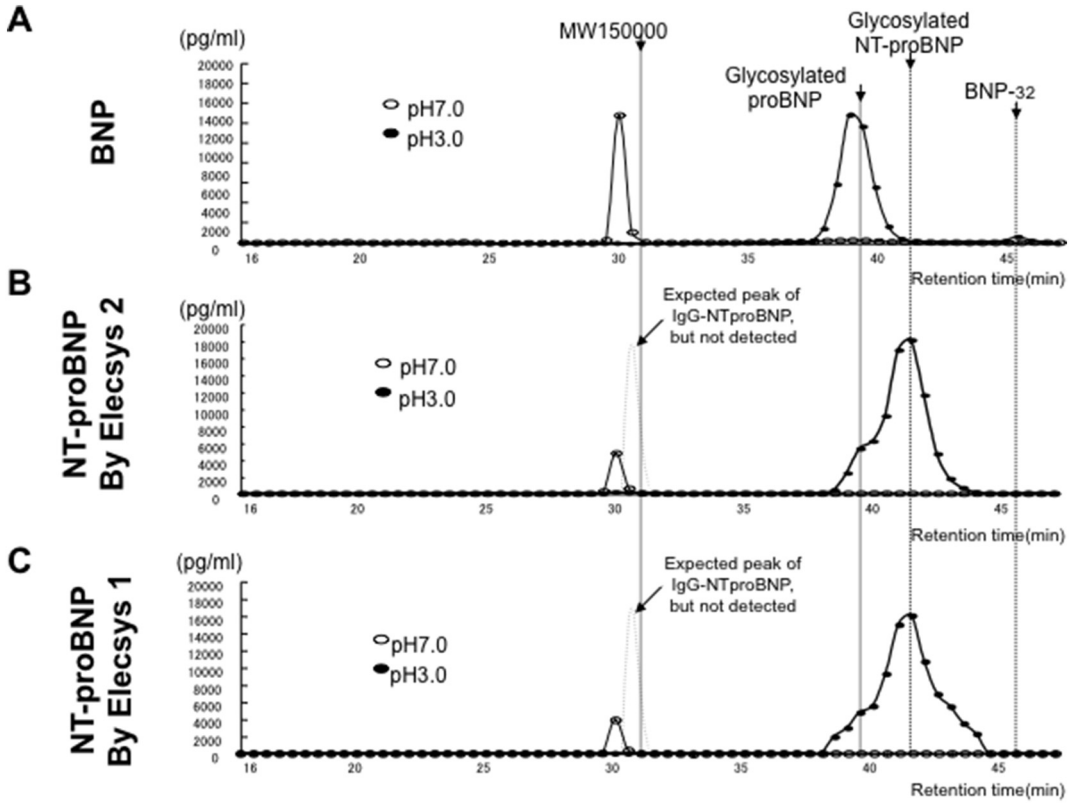

Fig. 2. A. Gel filtration chromatography of plasma samples from this patient eluted using neutral (pH 7.0) (○) and acidic (pH 3.0) (●) eluent combined with BNP immunoassay. Arrows indicate the elution position of IgG (150,000), recombinant glycosylated proBNP, glycosylated NT-proBNP in heart failure patient, and BNP-32. B. Gel filtration chromatography of plasma samples from this patient eluted using neutral (pH 7.0) (○) and acidic (pH 3.0) (●) eluent combined with NT-proBNP immunoassay (proBNP 2). Dotted line indicates an immunoreactivity of expected IgG-NT-proBNP. C. Gel filtration chromatography of plasma samples from this patient eluted using neutral (pH 7.0) (○) and acidic (pH 3.0) (●) eluent combined with another NT-proBNP immunoassay (Elecsys proBNP1). Dotted line indicates an immunoreactivity of expected IgG-NT-proBNP.
Column: TSKgel BioAssist G3SWXL × 2, Eluent(○): 0.1 MPB/0.3 M NaCl/5 mM EDTA/0.1% NaN3 (pH = 7.0), Eluent (●): 0.1 M PB/0.3 M NaCl/5 mM EDTA/0.1% NaN3(pH = 3.0);Flow:0.5 ml/min, Fraction size:0.25 ml/tube.