

Linköping University Postprint

A clinician's experience of using the Cardiac Reader NT-proBNP point-of-care assay in a clinical setting

Alehagen U, and Janzon M

N.B.: When citing this work, cite the original article.

Original publication:

Alehagen U, and Janzon M, A clinician's experience of using the Cardiac Reader NT-proBNP point-of-care assay in a clinical setting, 2008, European Journal of Heart Failure, (10), 3, 260-266.

<http://dx.doi.org/10.1016/j.ejheart.2008.01.005>.

Copyright: Elsevier B.V., <http://www.elsevier.com/>

Postprint available free at:

Linköping University E-Press: <http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-11488>

A clinician's experience of using the Cardiac Reader NT-proBNP point-of-care assay in a clinical setting

Alehagen U, MD, PhD (1), and Janzon M, MD, PhD (1)

Dept. of Cardiology, Linköping University Hospital, Linköping, Sweden (1)

Correspondence to: U Alehagen, Dept of Cardiology, Heart Center, University Hospital of Linköping, SE-581 85 Linköping, Sweden

Telephone: +46-13-22 20 00

E-mail: urban.alehagen@ihs.liu.se

Abstract

The evaluation of natriuretic peptides has become increasingly valuable in a clinical setting, where information is often needed promptly.

Objectives: To compare the usefulness of the recently released Roche Cardiac Reader[®] NT-proBNP assay against the Roche Elecsys[®] NT-proBNP laboratory system in a clinical setting.

Design and Results: Blood samples from 440 patients admitted for acute coronary syndromes, worsening of heart failure, or as polyclinic heart failure patients were evaluated. The relation between the assays was analysed and the diagnostic concordance calculated. A good correlation was found between the assays ($r=0.96$, 95% CI: 0.94-0.97) with a diagnostic concordance of 0.93. A separate analysis was performed in the range where most clinical decisions are made (60-3000 ng/L), with a diagnostic concordance of 88%. The usefulness in a clinical setting where time is important was high.

Conclusion: The Roche Cardiac Reader[®] NT-proBNP assay has been evaluated in a clinical setting. The point-of-care method shows good results, although with a restricted analytical range compared with the reference.

Introduction

The use of cardiac natriuretic peptides has become an increasing part of the clinical routine in handling patients with heart failure. There is extensive information in the literature about the relation between decreased cardiac systolic function and increased plasma concentration of B-type natriuretic peptides (BNP) and the N-terminal fragment of proBNP (NT-proBNP) (1). Similarly, patients with an impaired diastolic function have increased plasma concentration of BNP and NT-proBNP (pseudonormal or restrictive filling pattern) (2). The utility of BNP and NT-proBNP in treating patients with dyspnoea is well documented (3). Moreover, there is very intriguing information concerning the ability to tailor the treatment of heart failure by means of the peptides (4). Lately, interesting data indicate that ischaemia per se could result in increased plasma concentration of BNP and NT-proBNP (5, 6). It has also been shown that a high NT-proBNP plasma concentration is highly predictive of 1-year mortality in patients with non-ST-elevation acute coronary syndrome (7).

Therefore, there is an increasing need to evaluate these natriuretic peptides in the clinical routine. However, the use of point-of-care (POC) systems in the analytical assignment in general is not uncontroversial in light of necessary quality assessments. This is well illustrated in a report which evaluated nine POC devices for monitoring anticoagulation, and in which substantial differences were found between the assays and the laboratory reference method (8).

With regard to natriuretic peptides, only recently has a commercial assay for NT-proBNP that can be used as a POC system become available. Roche Diagnostics have released an assay for analysing NT-proBNP used in the Cardiac Reader[®] system. This POC system has been evaluated in a laboratory environment (9), but not in a clinical setting. As a result, experience from this clinical setting is lacking. Furthermore, it is not obvious that the use of

the POC system would give the same results in a clinical setting compared with the laboratory standard, as pointed out by Kost et al (10). As a result, the usefulness of the reported method in a clinical setting may be ambiguous.

The aim of this study was to evaluate the usefulness of Cardiac Reader POC NT-proBNP assay in a routine clinical setting, and to compare it with the Elecsys NT-proBNP clinical laboratory assay.

Methods

From November 2005 to February 2006, and from June 2007 to August 2007 patients with acute coronary syndromes admitted to the Coronary Care Unit (CCU), or patients admitted to the cardiology ward because of a documented heart failure that had deteriorated, or outpatients at the Department of Cardiology, Heart Center, Linköping University Hospital, Sweden, were consecutively included in the evaluation. The blood samples were drawn with the patients at rest in supine position. They were then analysed on a Cardiac Reader situated in the CCU (using the whole blood sample), and sent to the Laboratory of Clinical Chemistry at Linköping University Hospital as part of the comparison (using plasma samples with lithium heparin as anticoagulant).

Assays

Laboratory system

The laboratory method used to measure NT-proBNP was an electrochemiluminiscence immunoassay utilizing two polyclonal antibodies directed against amino acids 1–21 and 39–50 (Elecsys 2010, Roche Diagnostics, Mannheim, Germany), first described by Karl and co-workers (11). The analytical range was 5–35.000 ng/L (0.6– 4130 pmol/L) according to Roche package insert. The total interassay coefficient of variation (CV) was 4.8% at the level

of 217 ng/L (26 pmol/L) (n=70) and 2.1% at the level of 4261 ng/L (503 pmol/L) obtained at our laboratory. All blood samples were analysed within 1 hour after being drawn from the patient. The NT-proBNP assay used on the Elecsys analytical platform has been extensively evaluated in the literature (12).

Point-of-care system

The POC system used was the Cardiac Reader[®] (Roche Diagnostics, Mannheim, Germany), utilising one monoclonal and one polyclonal antibody directed against amino acids 27–31 and 39–50. The monoclonal antibody was gold-labelled, and the polyclonal antibody was biotinylated, forming a sandwich structure. This consists of the gold-labelled antibody, the NT-proBNP molecule, and finally the biotinylated antibody. The described complex is attached to a streptavidin molecule, and all this is coupled to the base of the test strip. The colour intensity of the resulting sandwich complex was measured by an optical system in the Cardiac Reader. The total analysis time was 12 minutes. This POC assay uses 150µL of heparinised venous whole blood for the analysis. The stated analytical range was <60–3000 ng/L (< 7.1–353.5 pmol/L). Total CV was 12.8% at the level of 163 ng/L (19.2 pmol/L) and 8.6% at the level of 1166 ng/L (137.4 pmol/L) (n=13) acquired during the study. All blood samples were analysed within 30 minutes after being drawn.

Statistics

Descriptive data are presented as percentage or mean. In the case of continuous variables, analyses have been performed using Student's unpaired two-sided T-test, whereas for the discrete variables, chi-square test was used. In the correlations analysis Cardiac Reader NT-proBNP assay values <60 ng/L were replaced with 60 ng/L, and >3000 ng/L with 3000 ng/L. The same transformation was made for the Elecsys NT-proBNP assay.

In the analyses, regressions of the comparison of methods were performed using Deming, Passing and Bablok regressions (13), whereas the differences in methods are presented as bias plots according to Bland-Altman (14).

Data were analysed using commercially available statistical analysis software packages (Statistica v 7.1, Statsoft Inc, Tulsa, OK, USA; Analyse-it v 1.63, Analyse-it Software Ltd, Leeds, UK).

Results

In the evaluation, 442 patients (males/females: 251/189; mean age: 65) agreed to participate in the study. The majority of the patients had been admitted to the CCU because of acute coronary syndromes (287 out of 442 blood samples). Two blood samples were excluded due to samples mix-up. Therefore 440 blood samples were evaluated in the study. We have decided not to exclude any blood samples due to “outliers”, but to present all samples. The basal characteristic of the population is presented in Tables Ia and Ib.

Table Ia. Basal characteristics of the study population

Variable	Number
Males/females	251/189
Age years, mean (range)	65 (82)
Acute coronary syndrome, (%)	287 (65)
Worsening of heart failure, (%)	153 (35)
Diabetes, (%)	101 (23)
Hypertension, (%)	291 (66)
Lab	
s-creatinine $\mu\text{mol/L}$, mean (SD)	107 (27)
Hb g/L, mean (SD)	135 (18)
Hematocrit mean (SD)	0.41 (0.05)
Triglycerides, mean (SD)	1.7 (1.1)

Table Ib. Basal characteristics of Nt-proBNP plasma concentration in the range 60–3000 pg/mL measured with Cardiac Reader[®] and Elecsys 2010 laboratory system.

	Mean	Median	SD	Lower quartile	Upper quartile
Cardiac Reader (ng/L)	955	603	927	177	1553
Elecsys (ng/L)	1033	650	1022	200	1620

A scatterplot illustrating the relationships between the Cardiac Reader assay and Elecsys NT-proBNP assay is presented in Figure 1. From the scatterplot, a good relation was found between the Cardiac Reader and the Elecsys assays in the range of evaluation ($r=0.96$, 95% CI: 0.94–0.97). There are some discrepancies in the values obtained between the two assays, especially in the upper range. However, the discrepancies are spread equally on both sides of the regression line, and the deviation of the regression line from the identity line is only slight. A statistical analysis of the plasma concentrations of the two assays dividing the analytical range for the Cardiac Reader into two parts (60–1000 ng/L and 1000–3000 ng/L) shows a correlation of 0.91 and 0.87, respectively.

A Bland Altman plot of the regression shows excellent agreement between the methods used (Figure 2). It is possible to identify a couple of outliers, but we have chosen not to exclude them in the calculations.

The Cardiac Reader NT-proBNP assay provides restricted information on plasma concentrations. Values below 60 ng/L are indicated as <60 ng/L, and values higher than 3000 ng/L as >3000 ng/L. An analysis of the diagnostic concordance was performed in the three analytical ranges (<60, 60–3000, and >3000 ng/L) in order to evaluate the impact of this fact. The diagnostic concordance in this study setting was found to be 93%, as presented in Table II.

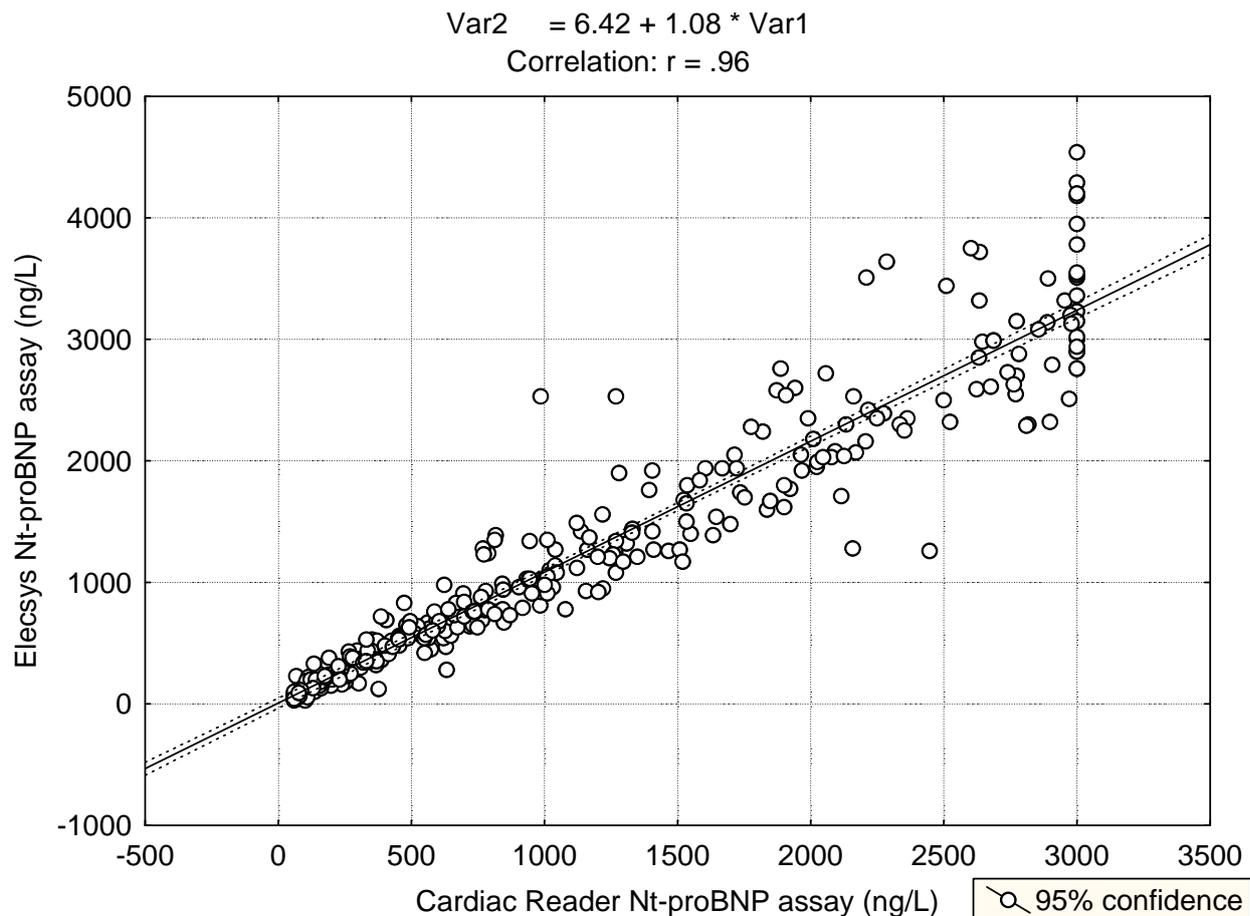


Figure 1. A scatterplot illustrating the correlation between Cardiac Reader Nt-proBNP analysis versus Elecsys Nt-proBNP analysis

Table II. Diagnostic 3x3 comparison of Cardiac reader Nt-proBNP with Elecsys Nt-proBNP

		Elecsys (ng/L)		
		0–59	60–2999	>3000
Cardiac Reader (ng/L)	>3000	n=0 0%	n=5 1.1%	n=60 13.6%
	60–3000	n=6 1.3%	n=322 73.2%	n=13 3.0%
	<60	n=28 6.4%	n=6 1.4%	n=0 0%

Note: Diagnostic concordance: 93.2%

In addition, an analysis of sensitivity and specificity in the three evaluation ranges of the Cardiac Reader (<60, 60–3000, and >3000 ng/L) was performed. In the analytical range <60, the sensitivity was 82% and the specificity was 99%, rendering a positive predictive value of

82% and a negative predictive value of 99%. In the analytical range 60–3000 ng/L, a sensitivity of 97% and a specificity of 83% were found, rendering a positive predictive value of 95% and a negative predictive value of 89%. Finally, in the analytical range >3000ng/L, a sensitivity of 82% and a specificity of 99% were found, rendering a positive predictive value of 97% and a negative predictive value of 92%, when compared with the Elecsys assay used as reference.

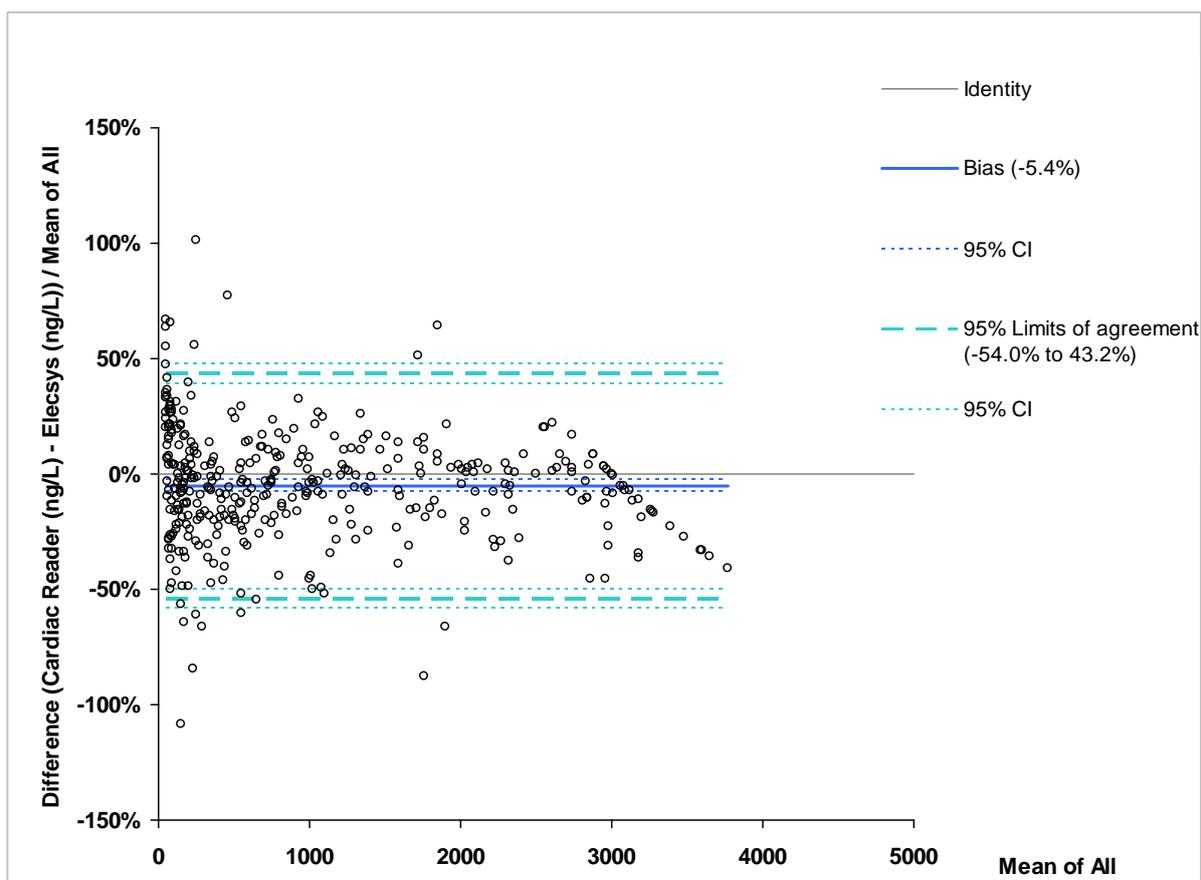


Figure 2. Bland Altman plot of blood samples (n=440) where NT-proBNP values measured by the Cardiac Reader NT-proBNP assay and the Elecsys NT-proBNP assay have been compared.

During the study, quality control was performed on a daily basis, using the internal quality control of the instrument, and on a weekly basis (Roche CARDIAC Control proBNP

Level Low and High; Roche Diagnostics). The analysis showed a CV of 12.8% in the lower range and of 8.6% in the higher range.

In the blood sampling of the patients analysed, Hb (range 66–161 g/L), haematocrit (range 0.20–0.77) and triglycerides (range 0.5–8.1 nmol/L) were also measured. No significant interference could be found using correlation analysis.

Depending on the situation, different cut-off values of NT-proBNP may be needed in the decision process. This has previously been shown by the author using the Elecsys NT-proBNP assay. The upper limit for reference values for healthy elderly patients was found to be <540 ng/L, whereas based on cardiovascular mortality a steep increase in risk was noted if the plasma concentration was >1700ng/L, used here as the decision limit (15). This analytical range is covered by the Cardiac Reader system (60–3000 ng/L), as shown in Table III. In this evaluation the diagnostic concordance was 88%. Another aspect of potentially different cut-off values could be described if the method is used in an environment in which sensitivity is given priority, such as the ED room, compared with a heart failure clinic, where specificity might be prioritised instead.

Table III. Diagnostic 5x5 comparison of reader Nt-proBNP with Elecsys Nt-proBNP

	Elecsys (ng/L)					
	0	0–60	61–300	301–900	901–3000	>3000
Cardiac Reader (ng/L)						
>3000		n=0 0%	n=0 0%	n=0 0%	n=5 1.1%	n=60 13.6%
901–3000		n=0 0%	n=0 0%	n=2 0.5%	n=119 27%	n=13 3.0%
301–900		n=0 0%	n=4 1.0%	n=82 18.6%	n=8 1.8%	n=0 0%
61–300		n=6 1.4%	n=99 22.5%	n=9 2.0%	n=0 0%	n=0 0%
0–60		n=28 6.4%	n=5 1.1%	n=0 0%	n=0 0%	n=0 0%

Note: Diagnostic concordance: 88.1%

As a part of the analysis of a potential high concentration hook effect, all plasma concentrations >4000 ng/L (range 4001–>35000 ng/L) obtained by the Elecsys system were evaluated. In all the samples, the Cardiac Reader displayed >3000 ng/L. Hence no high concentration hook effect could be found.

Moreover, an analysis of potential differences in plasma concentrations between the two assays when analysing males and females in the restricted range that Cardiac Reader NT-proBNP assay could evaluate did not show any significant differences between the two assays (Table IV).

Table IV. Gender characteristics obtained from the Cardiac Reader NT-proBNP assay and the Elecsys NT-proBNP assay

Variable	Median value, ng/L	IQR
Cardiac Reader males	651	1595
Elecsys males	680	1730
Cardiac Reader females	565	924
Elecsys females	613	1080

Note: IQR: Inter quartile range.

Note: Analyses done after eliminating all patients with a value <60 or >3000 ng/L on the Cardiac Reader

In 120 patients, duplicate samples of NT-proBNP on the Cardiac Reader system were analysed (figure 3). A Bland-Altman plot of the differences between the results is shown in Figure 4. The analysis indicates good precision for the Cardiac Reader POC system. We also wanted to compare the POC system with the usual laboratory system in respect of costs. The cost comparison is shown in Table V. The analysis shows that the POC system is cost-effective. However, we have not added the cost for the nurse inserting the blood sample into the Cardiac Reader, which takes about 2 minutes.

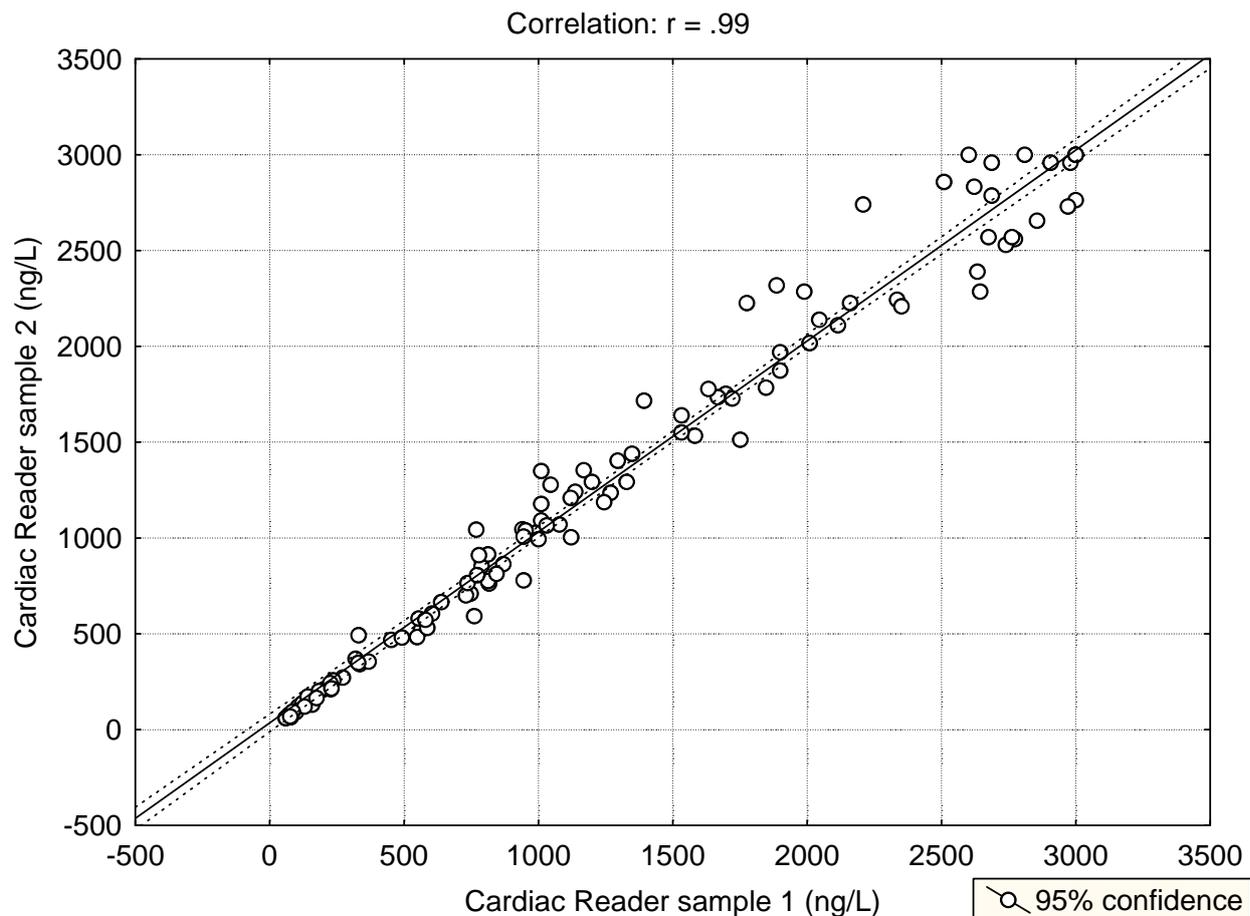


Figure 3. A scatterplot illustrating the correlation between two consecutive blood samples of the same patient ($n=120$ patients) analysed on the same Cardiac Reader system

Table V. Cost analysis of the use of Elecsys NT-proBNP assay versus Cardiac Reader NT-proBNP assay in 440 patients

Elecsys NT-proBNP assay	Cardiac Reader NT-proBNP assay
Cost per analysis: EUR 30.55	Cost per assay: EUR 20.55
Total cost 440 analyses: EUR 13,442	Test control (cost/week): EUR 4.21
	IQC test assay EUR 30.34
	Total cost 440 analyses: EUR 9,093
	Total gain in 440 analyses: EUR 4,349

Discussion

This evaluation demonstrates for the first time that the POC method of testing NT-proBNP is useful in a clinical setting in patients with ACS or HF; that the method is sufficiently robust,

permitting health-care personnel on duty to perform the analyses without unacceptable quality impairments; and that the assay is cost effective compared with the laboratory reference system for analysis of NT-proBNP. However, the use of POC methods raises certain interesting aspects that need to be discussed.

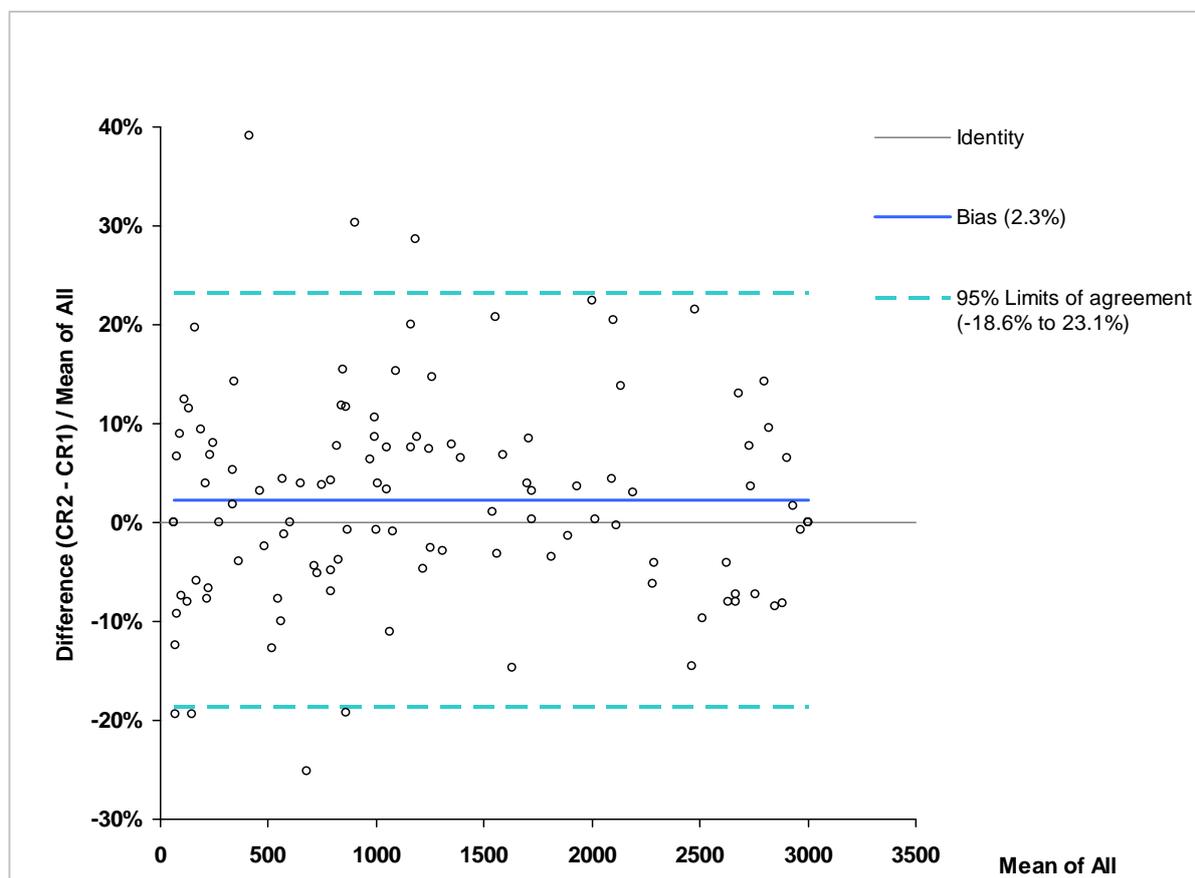


Figure 4. Bland-Altman plot of two consecutive blood samples from the same patient ($n=120$ patients) analysed on the same Cardiac Reader system

In the emergency room, in the CCU and in the outpatient clinic for heart failure patients, where time is important, there is a need for fast, reliable information concerning plasma concentration of natriuretic peptides.

For patients with dyspnoea the positive consequences of having a POC system for BNP in the emergency room are well documented (3). The reports showed that the availability of this POC analysis influenced the correct sorting of patients in a hospital facility where time is

an important dimension of quality. It is, therefore, reasonable to believe that the results can be extrapolated to NT-proBNP when analysed through a POC system.

In the CCU, the evaluation of NT-proBNP in patients with acute coronary syndromes might provide additional prognostic information that could help the clinician to decide on further therapeutic action (16). This has also been shown by other authors e.g. Grabowski et al (17).

In the clinical setting there is a considerable difference between obtaining the measured value after 12 minutes or after a couple of hours, which is the reality for many clinicians even if the sample is marked as an emergency test. The clinical consequence of this might be to make a decision concerning coronary angiography or not in patients for whom the objective facts are not overwhelming. It is also important to have a useful instrument at hand that predicts the level of risk of cardiovascular death (18). The practical consequence of using the POC system in clinical routine at our CCU was that the cardiologists on duty felt that the information given by the POC system within the limited time was an appreciated addition to the cardiac biomarkers used in routine, such as troponin-I, in determining whether a patient should be referred to angiography or not.

An important aspect in the clinical routine concerning the measurement of natriuretic peptides is that, in the same peptide, the same epitopes and the same reference values be evaluated both in the POC system and in the clinical laboratory routine at the hospital. Otherwise there will be confusion in the interpretation of values gained from the two systems as the patient is moved from the emergency room or CCU to the ordinary ward. For this reason, the different cut-off values for different assays have to be incorporated in the interpretation of results (19). Roche Diagnostics has released a POC method that analyses NT-proBNP, uses the same epitopes in the analysis, and has the same reference values as the NT-proBNP assay used in the multi-analysing system, Elecsys 2010, which is used in many

hospital laboratories. The results obtained from the POC system can therefore be directly compared with the results obtained by the Elecsys multi-analysing platform. Therefore we wanted to evaluate the usefulness of the Cardiac Reader NT-proBNP assay in comparison with the Elecsys platform in a real clinical setting in which the Cardiac Reader was handled by the nurses on duty at the CCU, and not by analytical technicians, as is often the case in technical evaluations of laboratory techniques (9). The nurses at the CCU who performed the analyses on the Cardiac Reader found it easy to learn and to handle without any obvious impairment of the results obtained.

In the evaluation, the Cardiac Reader correlated well with the method used as a reference, the Elecsys assay, in measuring NT-proBNP. The precision is higher in the reference system, but as illustrated in the Bland-Altman analysis, the divergences are acceptable for use in clinical routine. The regression analyses show good correlation between the methods used.

No gender difference could be found using the Cardiac Reader NT-proBNP assay. However, reports in the literature indicate an increased plasma concentration of NT-proBNP in healthy females compared with healthy males (20). One possible explanation for the lack of gender differences in our study population might be that we have been analysing a diseased population in which the influence of disease overwhelms the gender-specific differences.

The results show that the POC system provides information in the range where most blood samples are obtained. In the clinical routine, it is certainly a disadvantage not to be given reliable information about the entire plasma concentration range as provided by the laboratory system (5-34000 ng/L). However, for the clinician that is involved in everyday clinical routine, the patients with greatly increased NT-proBNP concentration (>3000 ng/L) usually do not present themselves as diagnostic problems. In this range, the clinician would certainly feel more comfortable knowing the exact figure of the plasma concentration, even if

the information given by the Cardiac Reader >3000 ng/L indicates a greatly increased risk of cardiovascular mortality (21).

Restricted information about patient samples with a plasma concentration < 60 ng/L will probably not influence the decision by the clinician regardless of the fact that the exact numerical figures are not given. The information that the concentration is low is often sufficient. However, patients with a moderately increased peptide concentration are those that can present as diagnostic and therapeutic problems. In patients with acute coronary syndrome, an increased peptide concentration might strengthen the decision to perform angiography because the patients are at risk. The Cardiac Reader will provide information in this important plasma concentration range (60–3000 ng/L). In a recent publication, Januzzi et al. showed certain decision limits for patients with acute heart failure derived from a large pooled multicenter study (22). The Cardiac Reader POC system was able to present all but one of the cut-off values used by Januzzi.

In discussing the advantages and disadvantages of a POC, one argument is the wider CV values that are documented. For the Cardiac Reader NT-proBNP assay, the CVs were 12.8 and 8.6%, respectively, in the lower and higher control levels tested. These coefficients of variation are wider than the laboratory system, but not are unacceptable for the analyses performed. Kupchak et al reported the influence of various widths of CV values on the analysis of BNP when analysing the AUC in a ROC analysis. They used a dataset from a clinical setting, but with theoretically different coefficients of variation. The surprising result was that the width of CV values had little affect on the ROC analysis measuring the AUC for the method (23). Therefore, when comparing different methods using a ROC analysis, a wider CV might be acceptable. Thus we found that the Cardiac Reader NT-proBNP POC system was useful for the clinician in certain areas in which limited time is a reality.

The interesting, and most important, issue is whether the POC method analyses the variable that the manufacturer claims it does. This aspect is discussed by Hawkrige et al. in a setting of patients with high levels of BNP32 as indicated by the POC assay. When analysed by high precision mass spectrometry, they did not reveal any BNP32 at all (24). In the case of the present POC assay, no such studies have been conducted to our knowledge, but the same antibodies and the same epitopes are used in the laboratory reference routine, which makes them easier to compare.

Cost aspects are important as the use of cardiac natriuretic peptides in clinical routine will probably expand. Ongoing studies are evaluating the effect on mortality and hospitalization for heart failure patients when treatment is tailored based on the result of cardiac natriuretic peptide measurements. If the tailoring regime shows positive effects, the need for fast information on the plasma concentration of cardiac natriuretic peptides will increase the use of POC systems in the management of heart failure patients in outpatient clinics. Our basal cost analysis of the two systems supports the use of NT-proBNP POC system.

Limitations

The present report should be regarded as a clinician's experience of using the POC system in a clinical setting. Hence, more extensive information is lacking on the imprecision of the system evaluated. Zugck et al, in a recently published multicenter study, made an excellent evaluation of this new POC system that showed good analytical precision of the system and a lack of interference from some of the major substances that may influence the peptide concentration obtained (9).

Conclusion

In conclusion, a comparison with the Elecsys NT-proBNP assay shows that the Cardiac Reader NT-proBNP assay may be used in a clinical setting with excellent results. The usefulness of the POC method is high in this setting. Our results suggest that the limitations of wider CV values, and the limited analytical range of the Cardiac Reader NT-proBNP, does not interfere with the clinical assessment made, and might instead contribute important information because of the short processing time.

Acknowledgements

The study was supported by grants from The Linköping University Research Foundation CIRC, and was supported by a research grant from Roche Diagnostics Sweden. We would like to thank Mrs Britt-Marie Ödlund, Roche Diagnostics Sweden, for advice.

References

1. Rothenburger M, Wichter T, Schmid C, et al. Aminoterminal pro type B natriuretic peptide as a predictive and prognostic marker in patients with chronic heart failure. *J Heart Lung Transplant* 2004; 23(10):1189-1197.
2. Lubien E, DeMaria A, Krishnaswamy P, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. *Circulation* 2002; 105(5):595-601.
3. Mueller C, Scholer A, Laule-Kilian K, et al. Use of B-type natriuretic peptide in the evaluation and management of acute dyspnea. *N Engl J Med* 2004; 350(7):647-654.
4. Troughton RW, Frampton CM, Yandle TG, et al. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 2000; 355(9210):1126-1130.
5. Goetze JP, Gore A, Moller CH, et al. Acute myocardial hypoxia increases BNP gene expression. *Faseb J* 2004; 18(15):1928-1930.
6. Foote RS, Pearlman JD, Siegel AH, Yeo KT. Detection of exercise-induced ischemia by changes in B-type natriuretic peptides. *J Am Coll Cardiol* 2004; 44(10):1980-1987.
7. James SK, Lindahl B, Timmer JR, et al. Usefulness of biomarkers for predicting long-term mortality in patients with diabetes mellitus and non-ST-elevation acute coronary syndromes (a GUSTO IV substudy). *Am J Cardiol* 2006; 97(2):167-172.
8. Gosselin R, Owings JT, White RH, et al. A comparison of point-of-care instruments designed for monitoring oral anticoagulation with standard laboratory methods. *Thromb Haemost* 2000; 83(5):698-703.

9. Zugck C, Nelles M, Katus HA, et al. Multicentre evaluation of a new point-of-care test for the determination of NT-proBNP in whole blood. *Clin Chem Lab Med* 2006; 44(10):1269-1277.
10. Kost GJ, Ehrmeyer SS, Chernow B, et al. The laboratory-clinical interface: point-of-care testing. *Chest* 1999; 115(4):1140-1154.
11. Karl J, Borgya A, Gallusser A, et al. Development of a novel, N-terminal-proBNP (NT-proBNP) assay with a low detection limit. *Scand J Clin Lab Invest Suppl* 1999; 230:177-181.
12. Sokoll LJ, Baum H, Collinson PO, et al. Multicenter analytical performance evaluation of the Elecsys proBNP assay. *Clin Chem Lab Med* 2004; 42(8):965-972.
13. Passing H, Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *J Clin Chem Clin Biochem* 1983; 21(11):709-720.
14. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1(8476):307-310.
15. Alehagen U, Goetze JP, Dahlstrom U. Reference intervals and decision limits for B-type natriuretic peptide (BNP) and its precursor (Nt-proBNP) in the elderly. *Clin Chim Acta* 2007; 382(1-2):8-14.
16. James SK, Lindahl B, Siegbahn A, et al. N-terminal pro-brain natriuretic peptide and other risk markers for the separate prediction of mortality and subsequent myocardial infarction in patients with unstable coronary artery disease: a Global Utilization of Strategies To Open occluded arteries (GUSTO)-IV substudy. *Circulation* 2003; 108(3):275-281.
17. Grabowski M, Filipiak KJ, Malek LA, et al. Admission B-type natriuretic peptide assessment improves early risk stratification by Killip classes and TIMI risk score in

patients with acute ST elevation myocardial infarction treated with primary angioplasty. *Int J Cardiol* 2006.

18. Bibbins-Domingo K, Gupta R, Na B, et al. N-terminal fragment of the prohormone brain-type natriuretic peptide (NT-proBNP), cardiovascular events, and mortality in patients with stable coronary heart disease. *Jama* 2007; 297(2):169-176.
19. Tang WH, Philip K, Hazen SL, et al. Comparative sensitivities between different plasma B-type natriuretic peptide assays in patients with minimally symptomatic heart failure. *Clinical cornerstone* 2005; 7 Suppl 1:S18-24.
20. Raymond I, Groenning BA, Hildebrandt PR, et al. The influence of age, sex and other variables on the plasma level of N-terminal pro brain natriuretic peptide in a large sample of the general population. *Heart* 2003; 89(7):745-751.
21. Jernberg T, James S, Lindahl B, et al. NT-proBNP in unstable coronary artery disease--experiences from the FAST, GUSTO IV and FRISC II trials. *Eur J Heart Fail* 2004; 6(3):319-325.
22. Januzzi JL, van Kimmenade R, Lainchbury J, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. *Eur Heart J* 2006; 27(3):330-337.
23. Kupchak P, Wu AH, Ghani F, et al. Influence of imprecision on ROC curve analysis for cardiac markers. *Clin Chem* 2006; 52(4):752-753.
24. Hawkrigde AM, Heublein DM, Bergen HR, 3rd, et al. Quantitative mass spectral evidence for the absence of circulating brain natriuretic peptide (BNP-32) in severe human heart failure. *Proc Natl Acad Sci U S A* 2005; 102(48):17442-17447.