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Analytical and clinical evaluation of a rapid quantitative lateral flow immunoassay for measurement of soluble ST2 in human plasma

Benjamin Dieplinger [a,*], Margot Egger [a], Alfons Gegenhuber [b], Meinhard Haltmayer [a], Thomas Mueller [a]

[a] Department of Laboratory Medicine, Konventhospital Barmherzige Brueder Linz, Linz, Austria
[b] Department of Internal Medicine, Krankenhaus Bad Ischl, Bad Ischl, Austria

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ABSTRACT

Background: Soluble ST2 (sST2) is gaining growing interest as a biomarker in heart failure. So far, the ELISA-format is widely used for commercially available ST2 assays, which hampers their use in clinical routine. Recently, a rapid quantitative lateral flow immunoassay for the measurement of sST2 in human plasma has been developed.
Methods: We evaluated precision and linearity of the ASPECT-PLUS ST2 test, and performed an analytical and clinical assay comparison with the MBL and the PRESAGE ST2 ELISAs. We measured sST2 with these three assays in a clinical cohort of 251 consecutive patients with acute dyspnea as the chief compliant (i.e., 137 patients with dyspnea attributable to heart failure and 114 patients with dyspnea attributable to other reasons).
Results: Within-run and total coefficients of variation of the ASPECT-PLUS ST2 test were < 17% and the assay was linear across its measurement range. We found a constant and proportional bias between the MBL ST2 assay, the PRESAGE ST2 assay and the ASPECT-PLUS ST2 test, respectively. However, at the proposed cut-off of 35 ng/mL, sST2 results obtained with the PRESAGE ST2 assay and the ASPECT-PLUS ST2 test were similar. Testing clinically, the three assays deemed equally useful for the diagnosis of heart failure (AUC, 0.670 for the MBL ST2 assay vs. 0.626 for the PRESAGE ST2 assay vs. 0.630 for the ASPECT-PLUS ST2 test) and for the prediction of 1-year mortality in dyspnoeic patients (AUC, 0.743 for the MBL assay vs. 0.742 for the PRESAGE ST2 assay vs. 0.752 for the ASPECT-PLUS ST2 test).
Conclusion: The ASPECT-PLUS test meets the analytical requirements for point-of-care testing. Test results of the ASPECT-PLUS ST2 and the PRESAGE ST2 methods were comparable at the proposed cut-off, and the diagnostic/prognostic capabilities of the three methods were similar.

1. Introduction

Soluble ST2 (sST2), an interleukin-1 (IL-1) receptor family member, is secreted into the circulation and functions as a "decoy" receptor for interleukin-33 (IL-33), thereby inhibiting IL-33/ST2 signaling [1]. sST2 is currently gaining growing interest as candidate biomarker in heart failure [1–3]. There is increasing evidence that sST2 plasma concentrations provide prognostic information in patients with cardiac disease independently of and additive to other established markers such as cardiac troponins or natriuretic peptides [4–5]. Thus, sST2 and has been included in the 2013 ACCF/AHA guideline for additive risk stratification of patients with acute and chronic heart failure [6].

So far, the enzyme-linked immunosorbent assay (ELISA) format is widely used for commercially available ST2 assays, which hampers their use in clinical routine [5,7]. Recently, the manufacturer of the PRESAGE ST2 ELISA assay (Critical Diagnostics) has developed the ASPECT-PLUS ST2 test, a rapid quantitative lateral flow immunoassay for measurement of sST2 in human plasma.

The aim of this study was to perform (1) an analytical evaluation of ASPECT-PLUS ST2 test; (2) a method comparison with the MBL ST2 assay (Medical & Biological Laboratories International) and the PRESAGE ST2 assay; and (3) a comparison of the clinical utility of these three methods for the diagnosis of heart failure and for the prediction of death in patients with shortness of breath presenting to an emergency department.

2. Materials and methods

2.1. Study protocol

This work was performed at St. John of God Hospital in Linz, Austria. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki, and all study participants gave informed consent. Blood was obtained by conventional venipuncture avoiding venous stasis. Using VACUETTE® polyethylene terephthalate glycol blood collection tubes (Greiner Bio-One), EDTA anticoagulated

* Corresponding author: Department of Laboratory Medicine, Konventhospital Barmherzige Brueder, Seilerstaette 2-4, A-4020 Linz, Austria.
E-mail address: benjamin.dieplinger@bs-lab.at (B. Dieplinger).

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blood samples were collected and plasma aliquots were frozen at −80 °C until further analysis. Further specifications of this analytical and clinical assay evaluation are described in the respective paragraphs of this methods section.

2.2. sST2 measurements

The ASPECT-PLUS ST2 test is a rapid lateral flow immunoassay for the quantitative measurement of sST2 in human plasma to be used with the ASPECT reader. The ASPECT PLUS ST2 test cassette uses murine mouse monoclonal antibodies against human sST2, goat polyclonal antibodies against murine IgG, and a fluorescent dye. The fluorescent signal is measured with the ASPECT reader and the quantitative sST2 values reported are based on a linear calibration curve unique to each lot and are given as ng/mL.

The measurement of sST2 ASPECT-PLUS ST2 test was performed following the manufacturer's instruction. In brief, after the ASPECT-PLUS ST2 test cassette was warmed to room temperature for 15 min, the foil pouch was removed and 35 μl of plasma sample was pipetted into the sample well; 60 s after loading the plasma sample, 2 drops (i.e., ~110 μL) of test buffer were added into the test buffer well. Finally, the ASPECT-PLUS ST2 test cassette was inserted into the ASPECT reader and the quantitative sST2 results in ng/mL were displayed on the screen approximately 20 min later. sST2 plasma concentrations less than 12.5 ng/mL or greater than 250 ng/ml were reported by the ASPECT Reader as <12.5 ng/ml and >250 ng/mL. Therefore, in the present study all values below 12.5 ng/mL are given as 12.5 ng/mL. For this reason, we were not able to evaluate the limit of detection of the ASPECT-PLUS ST2 test. According to the information in the package insert the limit of detection is 12.5 ng/mL for the ASPECT-PLUS test.

As prescribed previously by our group, the MBL ST2 ELISA was performed on a BEP 2000 instrument (Siemens Diagnostics) with a within-run coefficient of variation (CV) of <7.5% and a total CV of <13% [8]. To our knowledge, there is currently no published data in the scientific literature on the detection limit of the MBL ST2 assay. According to the information in the package insert the limit of detection is 0.032 ng/mL for the MBL ST2 assay.

The PRESAGE ST2 ELISA was also measured on a BEP 2000 instrument with a within-run CV of <2.5% and a total CV of <4.0% [9]. For the PRESAGE ST2 assay there are two previously published studies that determined the limit of detection of the PRESAGE ST2 assay, which have reported a limit of the detection of <2.0 ng/mL for the PRESAGE ST2 assay [9,10]. According to the information in the package insert the limit of detection is 1.3 ng/mL for the PRESAGE ST2 test.

2.3. Precision study

To evaluate the precision of the ASPECT-PLUS ST2 test in our laboratory, we performed a replication study according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guideline EP5-A [11]. Three pooled patient plasma samples were aliquoted into twenty 1.5 mL plastic tubes for each concentration level and frozen at −80 °C. We analyzed these samples in duplicate in one run per day for 20 days on a single ASPECT reader. Within-run and total analytical imprecision (CV) was calculated with the CLSI single-run precision evaluation test [11].

2.4. Evaluation of the linearity

We evaluated the linearity of the ASPECT-PLUS ST2 test according to the CLSI guideline EP6-A [12] using six different analyte concentrations. Fresh plasma samples were used to prepare a high- and a low-concentration pool covering the whole measurement range of the assay. We then conducted a direct dilution series with the low- and high-concentration patient sample pools in the following volume ratios (low-concentration pool + high-concentration pool): pool 1, low only; pool 2, 0.8 low + 0.2 high; pool 3, 0.6 low + 0.4 high; pool 4, 0.4 low + 0.6 high; pool 5, 0.2 low + 0.8 high; and pool 6, high only. Three measurements were done on each concentration, and the default criteria were set at 20% for repeatability and 5 ng/mL for nonlinearity.

2.5. Method comparison

For the method comparison of the MBL ST2, the PRESAGE ST2 and the ASPECT-PLUS ST2 test, we used plasma samples frozen at −80 °C from our previously described cohort of 251 consecutive patients attending an emergency department with acute dyspnea as a chief complaint [13–15]. sST2 plasma concentrations were measured with the MBL ST2 assay from a frozen aliquot as previously described in one batch in 2007 (approximately three years after blood collection) [14]. sST2 plasma concentrations were measured with the PRESAGE ST2 and the ASPECT-PLUS ST2 assay from another frozen aliquot at the same time in one batch in August 2015.

For the analytical method comparison, we used the approach of evaluating plasma concentrations within the measurement ranges of the three assays only, in order to avoid problems with assay detection limits in case of very low plasma concentrations or necessary sample dilutions in case of plasma concentrations higher than the upper limit of the measurement range. As a consequence, we had a measurement range of 0.1875–12 ng/mL for the MBL ST2 assay (based on a 3-fold dilution of patient samples), a measurement range of 2.8–180 ng/mL for the PRESAGE ST2 assay (based on a 45-fold dilution of patient samples), and a measurement range of 12.5–250 ng/mL for the ASPECT-PLUS ST2 test (based on undiluted patient samples). Of the 251 plasma samples from our short of breath patients, we were able to use 146 plasma samples fulfilling these requirements for our analytical method comparison.

For the clinical method comparison we used all 251 patient samples of the entire cohort to compare the diagnostic and prognostic

Table 1
Data on the analytical assay comparison of the MBL ST2 assay, the PRESAGE ST2 assay and the ASPECT-PLUS ST2 assay.*

sST2 plasma concentrations as measured by the three assays					
Assay	Lowest value	25th percentile value	Median value	75th percentile value	Highest value
MBL ST2	0.189 ng/mL	0.306 ng/mL	0.504 ng/mL	0.796 ng/mL	5.811 ng/mL
PRESAGE ST2	15.4 ng/mL	32.5 ng/mL	45.4 ng/mL	64.8 ng/mL	168.9 ng/mL
ASPECT-PLUS ST2	13.3 ng/mL	31.1 ng/mL	48.0 ng/mL	80.3 ng/mL	250.0 ng/mL

Passing and Bablok regression equitations			
Assays compared	Regression equitation	Intercept (95% CI)	Slope (95% CI)
MBL (variable x) vs. PRESAGE (variable y)	y [ng/mL] = 6.6 ng/mL + 71.1 x [ng/mL]	6.6 ng/mL (2.8 to 11.5)	71.1 (61.0 to 81.9)
MBL (variable x) vs. ASPECT-PLUS (variable y)	y [ng/mL] = −8.0 ng/mL + 112.8 x [ng/mL]	−8.08 ng/mL (−15.3 to −0.3)	112.8 (97.2 to 132.8)
PRESAGE (variable x) vs. ASPECT-PLUS (variable y)	y [ng/mL] = −16.2 ng/mL + 1.5 x [ng/mL]	−16.2 ng/mL (−23.6 to −8.8)	1.5 (1.37 to 1.69)

* Plasma samples of 251 patients with acute dyspnoea were measured with all three ST2 assays and the data is shown for those 146 plasma samples with sST2 plasma concentrations within the measurement range of all three assays.

capabilities of these three sST2 tests. The diagnosis of acute destabilized heart failure as the cause of dyspnea was based on the Framingham score for heart failure plus echocardiographic evidence of systolic or diastolic dysfunction [13]. The outcome measure for the present work was all-cause mortality at 1 year [15].

2.6. Statistical analysis

For the analytical and clinical comparison of the MBL ST2, PRESAGE ST2, and ASPECT-PLUS ST2 tests, Passing and Bablok regression and Spearman's rank correlation analyses were applied. Comparisons of continuous variables between patient groups were performed with the non-parametric Mann–Whitney U test. To evaluate the capabilities of the three sST2 tests for the diagnosis of heart failure and to assess their capabilities for prediction of all-cause mortality at 1-year we used receiver operating characteristics (ROC) analyses. In addition, Kaplan–Meier estimates of the distribution of times from baseline to death were computed according to median sST2 plasma concentrations measured with the three different sST2 tests, and log-rank test was performed to compare the survival between the groups. Our data were analyzed with MedCalc 13.0.0.0 (MedCalc Software) and SPSS 13.0 (SPSS Inc.). Obtained p values were not adjusted for multiple comparisons and are therefore descriptive only.

3. Results

3.1. Assay evaluation — imprecision and linearity

The ASPECT-PLUS ST2 test had a within-run CV of 16.8% and a total CV of 17.0% at a mean concentration of 20.8 ng/mL (pool 1), a within-run CV of 10.1% and a total CV of 10.2% at a mean concentration of 65.6 ng/mL (pool 2), and a within-run CV of 9.6% and a total CV of 10.0% at a mean concentration of 131.8 ng/mL (pool 3).

In the linearity study of the ASPECT-PLUS ST2 test, the low-concentration pool had a mean sST2 concentration of 23.6 ng/mL and the high-concentration pool of 229.6 ng/mL. The standard errors of regression ($S_{y,x}$) and t-tests from regression analyses showed that the first-order model fitted better than the second- and third-order models: first-order model b_1, $S_{y,x} = 9.5$, t-test = 30.8 ($p < 0.001$); second-order model b_2, $S_{y,x} = 9.6$, t-test = 1.0 ($p = 0.336$); third-order model b_3, $S_{y,x} = 9.7$, t-test = 0.82 ($p = 0.224$). In addition, all default criteria were met, so the method was linear within its measurement range.

3.2. Analytical method comparison

The data on 146 patient plasma samples with sST2 plasma concentrations within the measurement range of all three assays is shown in Table 1.

In the 146 dyspnoeic patients, median sST2 plasma concentrations were 0.504 ng/mL (range, 0.189–5.811 ng/mL; 25th–75th percentiles, 0.306–0.796 ng/mL) as measured by the MBL ST2 assay; 45.4 ng/mL (range, 15.4–168.9 ng/mL; 25th–75th percentiles, 32.5–64.8 ng/mL) by the PRESAGE ST2 assay; and 48.0 ng/mL (range, 13.3–250 ng/mL; 25th–75th percentiles, 31.1–80.3 ng/mL) by ASPECT-PLUS ST2 assay.

Non-parametric correlation analyses of the 146 patients revealed a Spearman's coefficient of rank correlation of 0.829 (95% CI, 0.771–0.874; $p < 0.001$) between the MBL ST2 and the PRESAGE ST2, of 0.796 (95% CI, 0.727–0.849; $p < 0.001$) between the MBL ST2 and the ASPECT-PLUS ST2, and of 0.892 (0.854–0.921; $p < 0.001$) between the PRESAGE ST2 and the ASPECT PLUS ST2 assay.

The comparison of sST2 concentrations obtained with the MBL ST2 (variable x) and the PRESAGE ST2 (variable y) assay in the 146 patients is shown in Table 1 and Fig. 1A. The following regression equation was revealed: $y = 6.6$ (95% CI, 2.8 to 11.5) + 71.1 (95% CI, 61.0 to 81.9) x. Thus, Passing and Bablok regression suggests in addition to a rather small but significant constant bias, a high proportional difference between the two methods. The Cusum test showed no significant deviation from linearity ($p = 0.37$).

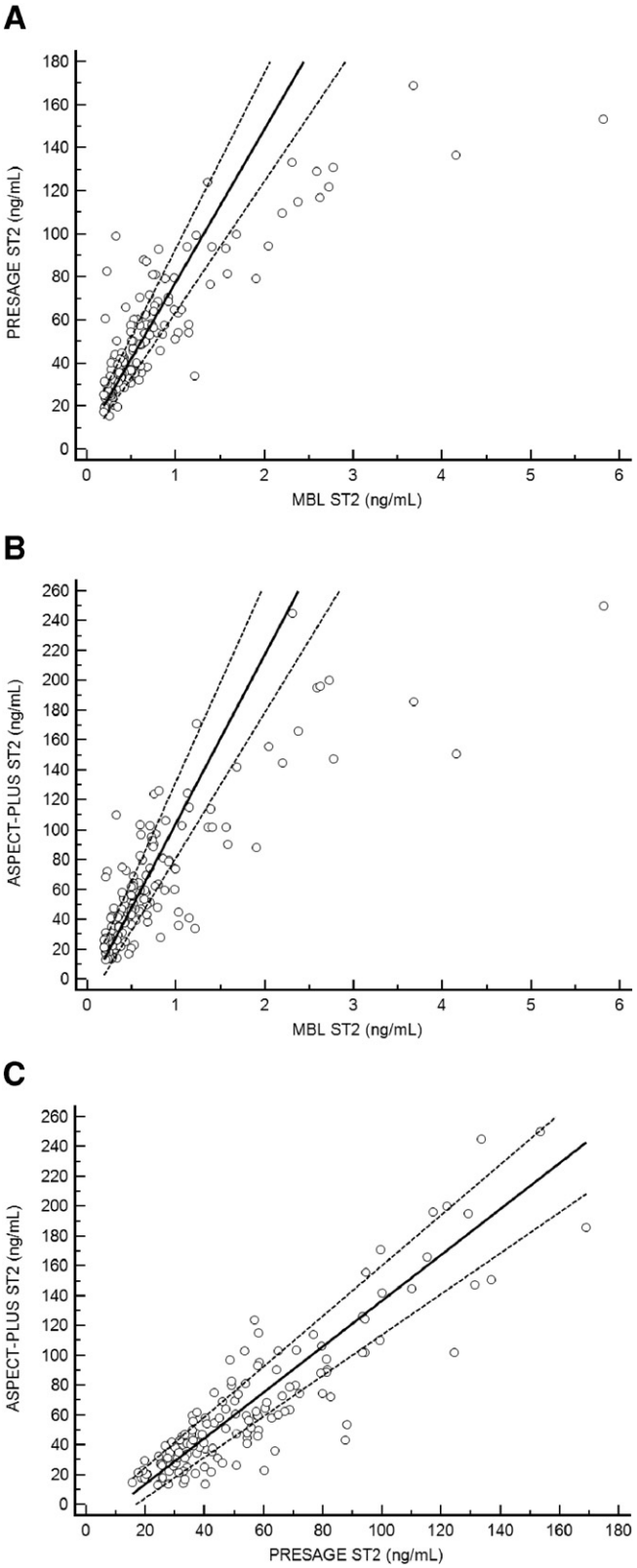

Fig. 1. Scatterplots of sST2 plasma concentrations obtained by the three methods in 146 patients with dyspnea attending an emergency department: (A) MBL ST2 test vs. PRESAGE ST2 test; (B) MBL ST2 test vs. PRESAGE ST2 test; and (C) PRESAGE ST2 test vs. ASPECT-PLUS ST2 test.

Table 2
Clinical comparison of the MBL ST2 assay, the PRESAGE ST2 assay and the ASPECT-PLUS ST2 assay in 251 dyspnoeic patients presenting to the emergency department (n = 251).

Diagnosis: sST2 plasma concentrations according to heart failure classification			
	No heart failure (n = 114)	Heart failure (n = 137)	p value
MBL ST2, ng/mL	0.202 (0.128–0.500)	0.465 (0.255–0.873)	<0.001
PRESAGE ST2, ng/mL	28.5 (19.7–53.5)	40.2 (27.1–68.7)	<0.001
ASPECT-PLUS ST2, ng/mL	21.5 (12.5–58.7)	41.2 (22.5–82.0)	<0.001
Prognosis: sST2 plasma concentrations according to 1-year all-cause mortality			
	Survivors (n = 189)	Decedents (n = 62)	p value
MBL ST2, ng/mL	0.270 (0.147–0.527)	0.755 (0.330–1.794)	<0.001
PRESAGE ST2, ng/mL	30.9 (20.4–50.6)	59.3 (34.1–122.5)	<0.001
ASPECT-PLUS ST2, ng/mL	25.6 (12.5–52.8)	69.3 (33.4–175.0)	<0.001

sST2 plasma concentrations are presented as median (25th–75th percentiles).

Further, the comparison of sST2 concentrations obtained with the MBL ST2 (variable x) and the ASPECT-PLUS ST2 (variable y) assay in the 146 patients is displayed in Table 1 and Fig. 1B. The following regression equation was revealed: y = −8.0 (95% CI, −15.3 to −0.3) + 112.8 (95% CI, 97.2 to 132.8) x. Thus, Passing and Bablok regression suggests in addition to a rather small but significant constant bias, a high proportional difference between the two methods. The Cusum test showed no significant deviation from linearity (p = 0.37).

Finally, the comparison of sST2 concentrations obtained with the PRESAGE ST2 (variable x) and the ASPECT-PLUS ST2 (variable y) assay in the 146 patients is reported in Table 1 and Fig. 1C. The following regression equation was revealed: y = −16.2 (95% CI, −23.6 to −8.8) + 1.5 (95% CI, 1.37 to 1.69) x. Thus, Passing and Bablok regression suggests in addition to a considerable constant bias, a considerable proportional difference between the two methods. The Cusum test showed no significant deviation from linearity (p = 0.76).

3.3. Clinical method comparison — description of the cohort

For the clinical method comparison we used the entire cohort of 251 patients with acute dyspnea presenting to the emergency department (REF HEART, diagnosis, prognosis, ST2). The median ST2 plasma concentrations for the 251 dyspnoeic patients were 0.333 ng/mL (range, 0.036–9.543 ng/mL; 25th–75th percentiles, 0.164–0.729 ng/mL) for the MBL ST2 assay, 35.3 ng/mL (range, 7.6–1874.7 ng/mL; 25th–75th percentiles, 21.7–60.3 ng/mL) for the PRESAGE ST2 assay, and 34.3 ng/mL (range, 12.5–2005.8 ng/mL; 25th–75th percentiles, 13.4–72.6 ng/mL) for the ASPECT-PLUS ST2 assay.

Of these 251 patients, 73 (29%) showed a sST2 plasma concentration below the measurement range of the MBL ST2 assay (i.e., <0.189 ng/mL), none below the measurement range of the PRESAGE ST2 assay (i.e., <2.8 ng/mL), and 57 (23%) below the measurement range of the ASPECT-PLUS ST2 assay (i.e., <12.5 ng/mL).

In the entire study cohort of 251 dyspnoeic patients, 137 patients were classified as having dyspnoea attributable to heart failure, and 114 patients were classified as having dyspnoea attributable to other reasons. Furthermore, during the 1-year follow-up 62 patients (25%) died and 189 (75%) survived. Median sST2 plasma concentrations measured with the MBL ST2, the PRESAGE ST2 and the ASPECT-PLUS ST2 assay according to patients with heart failure and without heart failure as well as according to survivors and decedents are displayed in Table 2. sST2 plasma concentrations measured with all three assays were significantly higher in patients with heart failure compared to patients with dyspnea due to other reasons and also increased in decedents when compared to survivors (Table 2).

3.4. Clinical method comparison — diagnostic capabilities

In distinguishing between patients with dyspnoea caused by heart failure (n = 137) and patients with dyspnea attributable to other causes (n = 114), the areas under the curve for the three sST2 assays were similar: 0.670 (95% CI, 0.608–0.728) for the MBL ST2; 0.626 (95% CI, 0.563–0.686) for the PRESAGE ST2; and 0.630 (95% CI, 0.567–0.690) for the ASPECT-PLUS ST2 assay. Corresponding ROC plots for the diagnosis of heart failure are displayed in Fig. 2.

3.5. Clinical method comparison — prognostic capabilities

The prognostic capabilities of the three different sST2 assays for all-cause mortality at 1 year in the 251 study participants are visualized by the ROC curves of Fig. 3. The areas under the curve for the prediction of death were: 0.743 (95% CI, 0.675–0.788) for the MBL ST2; 0.742 (95% CI, 0.683–0.795) for the PRESAGE ST2; and 0.752 (95% CI, 0.694–0.0.804) for the ASPECT-PLUS ST2 assay.

Fig. 4 shows the Kaplan–Meier curves of the 251 study participants, who were stratified into two groups each according to median sST2 plasma concentrations measured with the MBL ST2 assay (i.e. ≥0.333 ng/mL, Fig. 4a), the PRESAGE ST2 assay (i.e. ≥35 ng/mL, Fig. 4B), and the ASPECT-PLUS ST2 test (i.e. ≥34 ng/mL, Fig. 4C), respectively. All-cause mortality at 1-year was significantly higher in patients with increased plasma concentrations (logrank test, p < 0.001 for each sST2 test): hazard ratio of 3.73 (95% CI, 2.26–6.15) for the MBL ST2 assay; hazard ratio of 3.40 (95% CI 2.06–5.60) for the PRESAGE ST2 assay; and hazard ration of 3.37 (95% CI 2.04–5.55) for the ASPECT-PLUS ST2 test.

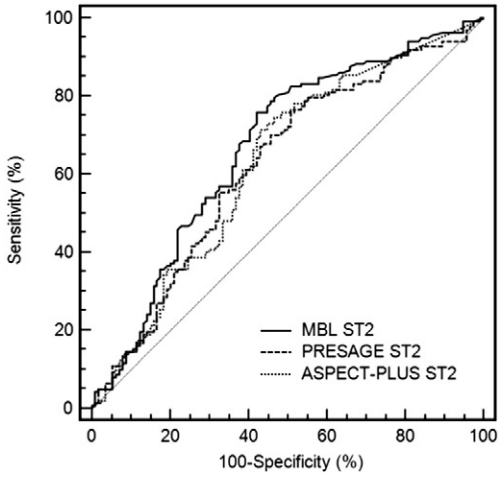

Fig. 2. Receiver operating characteristic curves for the biochemical diagnosis of heart failure by sST2 plasma concentrations measured with the MBL ST2 test, the PRESAGE ST2 test, and the ASPECT-PLUS ST2 test in 251 patients with shortness of breath presenting to the emergency department (137 patients with dyspnea attributable to heart failure vs. 114 patients with dyspnoea from other causes).

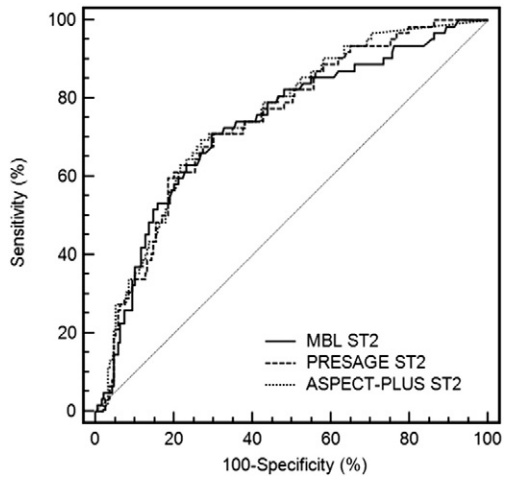

Fig. 3. Receiver operating characteristic plots demonstrating the ability of sST2 plasma concentrations measured with the MBL ST2, the PRESAGE ST2, and the ASPECT-PLUS ST2 test to predict 1 year all-cause mortality in 251 patients with shortness of breath presenting to the emergency department (62 decedents vs. 189 survivors).

Similar results for all three assays were obtained in evaluating the prognosis of the 137 patients with heart failure (n = 137) as well (data not shown).

4. Discussion

Our analytical evaluation of the novel ASPECT-PLUS ST2 test, revealed that this rapid lateral flow immunoassay for the quantitative measurement of sST2 in human plasma meets the quality specifications for laboratory medicine considering that this is a point-of-care assay. The precision study of the ASPECT-PLUS ST2 assay showed that the within-run and total imprecision CVs were <17%, which were adequate, especially for a point-of-care format assay. Furthermore, we reported that the assay is linear within its whole measurement range. However, the limit of detection of the ASPECT-PLUS ST2 assay is 12.5 ng/mL and, therefore, it has a disadvantage with respect to its low-end sensitivity compared to the PRESAGE ST2 assay which is considered a "high-sensitivity" assay [9]. Consequently, the ASPECT-PLUS ST2 test is probably not as suitable for risk stratification of healthy and/or population based cohorts as it has been shown for the PRESAGE ST2 assay [16].

In the present study, we did find a considerable constant and proportional bias between the PRESAGE ST2 and the ASPECT-PLUS ST2 assay. However, at the proposed cut-off value of 35 ng/mL the sST2 results obtained with both methods were very similar. Reasons for the differences are most probably different assay formats as well as different antibodies and reagents used for the PRESAGE ST2 and the ASPECT-PLUS ST2 assays. Furthermore, we confirmed the results of our previous assay comparison studies on the MBL ST2 and the PRESAGE ST assay [7,17]. We found about 100-fold higher median sST2 plasma concentrations using the PRESAGE ST2 assay compared with the MBL ST2 assay. In addition, we report similar differences in median sST2 plasma concentrations obtained with the MBL ST2 assay and the ASPECT-PLUS ST2 assay.

In this context, the median plasma concentrations of the entire cohort of 251 short of breath patients were 0.333 ng/ml for the MBL ST2 assay, 35 ng/mL for the PRESAGE ST2 assay, and 34 ng/mL for the ASPECT-PLUS ST2 assay. The median we found for the PRESAGE ST2 assay in our cohort fits perfectly to the proposed cut-off value of 35 ng/ml for risk stratification of patients with heart failure.

Another important result of our study is that we found no relevant differences in the diagnostic and prognostic capabilities of the three sST2 assays in our clinical cohort of dyspnoeic patients.

As shown in previous reports on this study cohort of dyspnoeic patients, the diagnostic value of sST2 measured with the MBL ST2 assay for acute heart failure was only very modest [14]. With the present study we now further extend this finding to the PRESAGE ST2 and the ASPECT-PLUS ST2 assay with similar areas under the curve for the

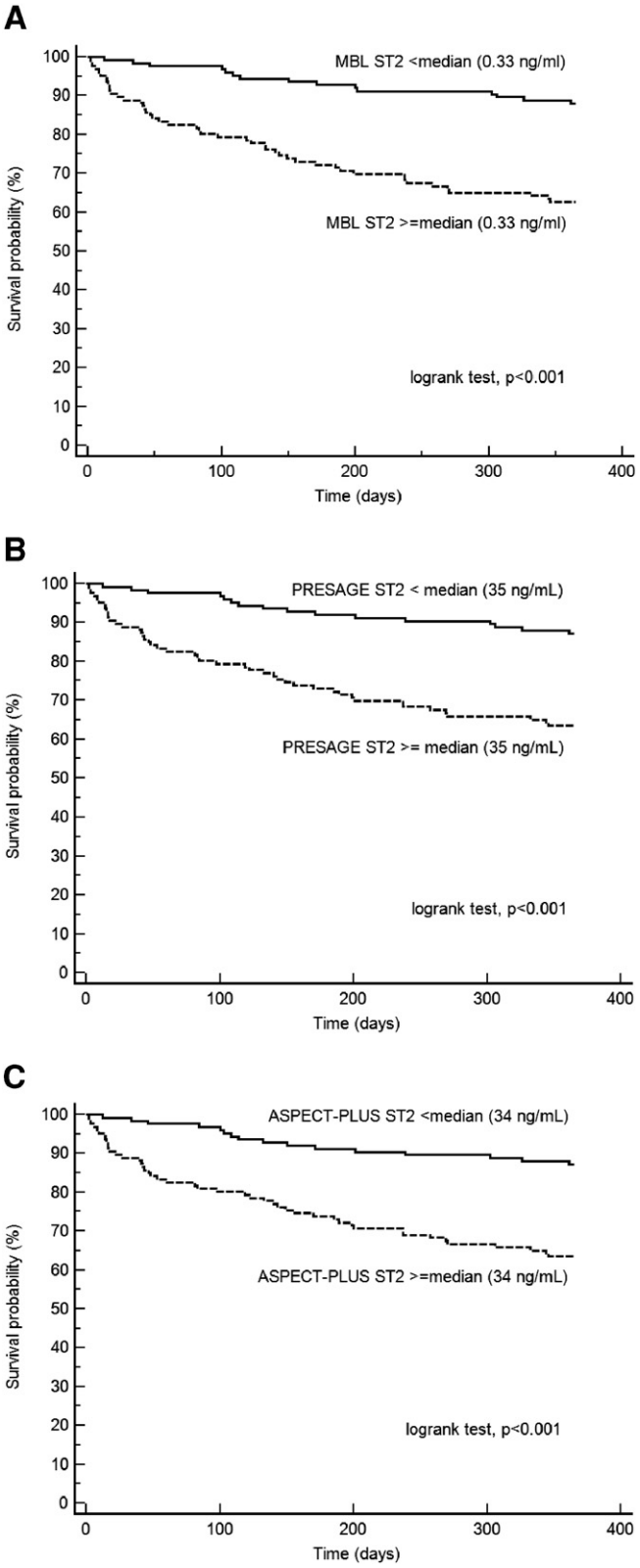

Fig. 4. Kaplan–Meier plots showing survival in 251 patients with acute dyspnea stratified according to median sST2 plasma concentrations measured with the (A) MBL ST2 test; the (B) Presage ST2 test; and (C) the ASPECT-PLUS ST2 test.

diagnosis of heart failure in ROC curve analyses. As previously published and now confirmed, the analyte sST2 lacks disease specificity [5,7,14] and is, therefore, not a valuable marker for the diagnosis of heart failure.

In contrast, we and others have previously shown that sST2 can serve as a prognostic marker in patients with heart failure and in patients with dyspnea [8,15,18–20]. Here, we now show for the first time a comparison of the prognostic capability of three different sST2 assays in patients with acute dyspnea. Interestingly, there was no clinical relevant difference in the prognostic performance of one of the three assays. We saw similar areas under the curve in ROC curve analysis for the prediction of 1-year all-cause mortality for the MBL, the PRESAGE and the ASPECT-PLUS ST2 tests. Furthermore, when stratified according to median sST2 plasma concentrations we found similar hazard ratios, with a ~3.5 fold increased risk for patients with ST2 plasma concentrations above the median.

So far, the PRESAGE ST2 assay is the only method that has been cleared by the U.S. Food and Drug Administration (FDA) and has received Conformitè Europèenne (CE) mark. The MBL ST2 assay is a research use only assay. Recently, the ASPECT-PLUS ST2 test and the ASPECT reader have received CE mark. However, FDA clearance is still due for the ASPECT-PLUS ST2 test.

Another important point for implementation of sST2 testing in clinical routine is laboratory costs. When taking into account the reagent price only for sST2 measurement with the three different assays (i.e., excluding costs for staff and equipment), the costs for a single sST2 measurement are roughly 6 € for the MBL ST2 test, 10 € for the PRESAGE ST2 test, and 20 € for ASPECT-PLUS ST2 test. However, one has to keep in mind the different assay formats (ELISA-format for the MBL ST2 and the PRESAGE ST2 test and point of care testing-format of the ASPECT-PLUS ST2 test) with different hands-on and turn-around times.

In conclusion, the ASPECT-PLUS assay meets the analytical requirements for point-of-care testing. An advantage of the ASPECT-PLUS ST2 test is the point-of-care format with low hands on time and the fast turn-around time, which makes timely and single sample measurements sST2 feasible. Even though, there was a considerable constant and proportional bias between the ASPECT-PLUS ST2 and the PRESAGE ST2 assay, we obtained similar results at the proposed cut-off of 35 ng/mL with both methods. Importantly, we found no relevant difference with respect to the diagnostic and prognostic capabilities of the ASPECT-PLUS ST2 and the PRESAGE ST2 in acute dyspnoeic patients attending an emergency department.

Conflict of interest statement

Competing interests: none declared.

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