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João Pedro Ferreira, Milton Packer, Naveed Sattar, Javed Butler, Stuart J Pocock, Stefan D Anker, Sandra González Maldonado, Marina Panova-Noeva, Mikhail Sumin, Serge Masson, et al.

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1           **Carbohydrate Antigen-125 Concentrations Across the Ejection Fraction Spectrum in**  
2                                   **Chronic Heart Failure: the EMPEROR Program**

3  
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1 **Abstract**

2 **Background:** Vascular congestion may lead to an increase of carbohydrate antigen 125 (CA-  
3 125). The role of CA-125 as a biomarker of congestion or for prognosis across the full ejection  
4 fraction (EF) spectrum of chronic heart failure (HF) remains unknown.

5 **Methods:** Serum CA-125 was measured in 1111 study participants from the EMPEROR-  
6 Reduced and EMPEROR-Preserved Trials. Congestive signs and symptoms were evaluated  
7 across CA-125 tertiles. Cox regression was used to study the association with outcomes. The  
8 primary outcome was a composite of first HF hospitalization or cardiovascular (CV) death.

9 **Results:** No significant association was present between baseline CA-125 levels and congestive  
10 signs or symptoms. In the overall population, higher CA-125 levels were not associated with an  
11 increased risk of primary outcome (Tertile-3 vs Tertile-1: Hazard Ratio [HR] 1.34; 95%  
12 confidence interval [CI] 0.91-1.96; p-trend=0.11). However, higher CA-125 levels were  
13 associated with an increased risk of primary outcome in patients with HF and reduced EF  
14 (HF<sub>r</sub>EF; Tertile-3 vs Tertile-1: HR 2.25 [95%CI 1.30-3.89]), but not among patients with  
15 preserved EF (HF<sub>p</sub>EF; Tertile-3 vs Tertile-1: HR 0.68 [95%CI 0.38-1.21]); interaction-p-  
16 value=0.02). Patients in the upper CA-125 tertile also showed the steepest eGFR decline over  
17 time (p-trend=0.03). The effect of empagliflozin to reduce the risk of CV death or HF  
18 hospitalization appeared to be attenuated in those with lower baseline CA-125 levels  
19 (interaction-p-trend =0.09).

20 **Conclusion:** Across the range of EF in patients with chronic HF enrolled in the EMPEROR  
21 trials, the majority of whom did not have clinical evidence of congestion, CA-125 concentrations

1 were not significantly associated with congestive signs or symptoms. CA-125 concentrations  
2 may predict HF hospitalization/CV death in patients with HFrEF, but not those with HFpEF.

3 **Trial Registration:** EMPEROR-Reduced (NCT03057977), EMPEROR-Preserved  
4 (NCT03057951)

5

6 **Keywords:** heart failure, CA-125, congestion, diuretic, kidney function, empagliflozin

1 Carbohydrate Antigen 125 (CA-125) is a high molecular weight glycoprotein expressed by  
2 mesothelial cells lining the inner layer of pericardium, peritoneum, and pleura(1). Irritation or  
3 injury to mesothelia (including that induced by mechanical stress, increased hydrostatic  
4 pressures, and inflammatory cytokines) may result in higher CA-125 expression and its release  
5 into the circulation(2-4). The role of CA-125 is not fully understood; it is a repeating epitope  
6 derived from the mucin protein MUC16 and thought to act to protect the mesothelium from  
7 physical stress. It may also have a role in cell adhesion and inflammatory cell recruitment(5, 6).  
8 Owing to its association with malignancy involving the coelomic epithelium, CA-125 is widely  
9 used for diagnostic evaluation and monitoring individuals with ovarian cancer and other  
10 malignancies involving the peritoneum.

11 Although malignancy is the most common context associated with abnormal  
12 concentrations of CA-125, other processes may result in elevation of this biomarker. For  
13 example, studies have implicated volume expansion states with increased CA-125; these include  
14 heart failure (HF), renal failure, and liver cirrhosis(7-10). Mechanistic links between congestion  
15 and CA-125 remain uncertain. In theory, venous plethora might result in mesothelial irritation,  
16 and thus, a clinical role has been proposed for CA-125 as a biomarker of congestion(11).

17 Congestion is an important component of HF symptoms(12-14); defining congestion and  
18 its evaluation remains challenging. As such, there is no gold standard for its routine assessment.  
19 If congestion were to play an important role in the progression of HF, a biomarker associated  
20 with congestion might be expected to add important prognostic information regarding key HF  
21 outcomes. Although natriuretic peptides such as N-terminal pro-B type natriuretic peptide (NT-  
22 proBNP) are modestly associated with fluid overload and symptoms of dyspnea reflective of

1 congestion, concentrations of natriuretic peptides are confounded by other determinants besides  
2 filling pressures/transmural wall stress.

3           Given the proposal for CA-125 to be a surrogate marker of congestion(11) we sought to  
4 1) relate baseline CA-125 concentrations with congestion parameters, 2) evaluate the impact of  
5 empagliflozin on CA-125 concentrations, and 3) investigate the association between CA-125  
6 values at baseline and the effect of empagliflozin on heart failure outcomes and renal function  
7 among study participants across the full left ventricular ejection fraction (LVEF) spectrum in the  
8 Empagliflozin Outcome Trial in Patients with Chronic Heart Failure (EMPEROR) program.

9

## 10 **Methods**

### 11 *Study sample*

12 A total of 9718 individuals were originally enrolled in the EMPEROR-Reduced (n =3730) and  
13 EMPEROR-Preserved (n =5988) trials whose design and results have been published(15, 16).  
14 Briefly, individuals with class II-IV HF and increased circulating natriuretic peptide levels across  
15 a wide range of LVEF were enrolled in the trials. All patients supplied written informed consent,  
16 and the ethics committees of all participating institutions approved the protocol.

17           In addition to their regular HF treatment, eligible patients were randomized double-blind  
18 in a 1:1 fashion to receive either a placebo or the HF drug empagliflozin 10 mg daily. Patients'  
19 key HF outcomes were evaluated during study visits. The combined risk of cardiovascular death  
20 or hospitalization for HF served as the primary outcome for both trials. The combined risk of a  
21 sustained estimated glomerular filtration rate (eGFR) decrease of at least 40%, an eGFR decline

1 to 10–15 ml/min/1.73m<sup>2</sup>, renal replacement therapy, or renal death was the primary renal  
2 outcome.

3 Participation in the trials was not contingent on submitting samples of plasma, serum,  
4 DNA, or urine for biobanking. Bio-samples were only obtained once a separate informed consent  
5 was provided in compliance with local ethical and regulatory regulations. The concepts and  
6 technique of sample collection for biobanking in the EMPEROR trials in addition to the list of  
7 nations that took part in the biobanking for biomarker sub-study have been published  
8 elsewhere(17). **Figure 1** details the study flow for the present analysis; for the purposes of this  
9 analysis, 594 study participants were included from EMPEROR-Reduced (LVEF <40%), while  
10 530 study participants were included from EMPEROR-Preserved (LVEF ≥40%).

#### 11 *Biomarker measurement*

12 Biomarkers were measured at baseline (n =1111), week 12 (n =1104), and week 52 (n =708). The  
13 measurements of CA-125 as well as high-sensitive cardiac troponin T (hs-cTnT), insulin-like  
14 growth factor-binding protein 7 (IGFBP-7), growth differentiation factor-15 (GDF-15),  
15 angiopoietin 2 (ANGPT-2), fatty acid-binding protein-3 (FABP-3) and bone morphogenetic  
16 protein 10 (BMP-10) were performed in serum within the Biomarker Research Agreement of  
17 Boehringer Ingelheim, the sponsor of the EMPEROR trials and Roche Diagnostics International  
18 Ltd. These measurements were made using an electrochemiluminescence immunoassay  
19 (Elecsys®, Roche Diagnostics). NT-proBNP was previously measured using a similar  
20 methodology(18). Additionally, plasma samples from the same study participants were included  
21 in a plasma proteomics study using the Olink® Explore 1536 platform, as previously  
22 reported(19).



1 *Assessment of clinical signs and symptoms of congestion*

2           Signs and symptoms of congestion— including NYHA classification, abnormal jugular  
3 vein distension, pulmonary rales, S3 gallop, cardiac murmur, peripheral edema— were assessed  
4 using standard clinical approaches within clinical examination at study visits. In addition,  
5 information about orthopnea and fatigue was derived from Kansas City Cardiomyopathy  
6 Questionnaire.

7 *Statistical analysis*

8 Mean (standard deviation), median (interquartile range), and count (frequency) were used to  
9 describe the distribution of continuous and categorical variables as appropriate. Comparison in  
10 baseline characteristics of the study population across groups defined by tertiles of CA-125 was  
11 performed using ordinal regression. To evaluate the effect of empagliflozin on CA-125  
12 concentrations, comparisons of geometric mean ratio (95% CI) were performed at three months  
13 and 1 year. Cox proportional hazards and negative binomial regression models were used to  
14 analyze the associations between baseline CA-125 and major heart failure outcomes.  
15 Multivariable models included age, sex, geographical region, diabetes, left ventricular ejection  
16 fraction, eGFR, and trial (EMPEROR-Reduced or EMPEROR-Preserved) as covariates, and  
17 were additionally adjusted for baseline hs-cTnT and NT-proBNP, the most important biomarker  
18 predictors of the primary outcome in the EMPEROR studies(20-23). The likelihood ratio test  
19 was used to examine the interaction between baseline CA-125 levels, ejection fraction (HF<sub>r</sub>EF vs  
20 HF<sub>p</sub>EF), and empagliflozin effect on risk of clinical outcomes. With the mentioned baseline  
21 covariates (except for hs-cTnT and NT-proBNP) and baseline CA-125 levels considered, changes  
22 in CA-125 levels over time were examined using mixed effect models for repeated  
23 measurements. These models compared the change in plasma concentrations for each protein

1 from baseline to weeks 12 and 52 between treatment groups (empagliflozin vs. placebo).  
2 Spearman correlations were used to study the correlation between CA-125, the proteins studied  
3 under Elecsys® (IGFBP-7, GDF-15, BMP-10, ANGPT-2, and FABP-3) and the proteins studied  
4 in the Olink® Explore 1536 platform. Statistical analyses were performed using the SAS  
5 software, version 9.4 (SAS Institute, Inc.; Cary, NC). All tests were two-sided and p-values  
6 <0.05 were considered statistically significant.

7

## 8 **Results**

9 This study included 1111 patients with HF and available baseline CA-125 levels. The mean age  
10 (standard deviation) of study participants was 70.1±10.0 years, and 358 individuals (32.2%) were  
11 female. Tertiles of CA-125 were: tertile 1 (T1) ≤11.80 U/mL, tertile 2 (T2) 11.81 U/mL to 20.76  
12 U/mL, and tertile 3 (T3) ≥20.77 U/mL. **Table 1** details the baseline characteristics of study  
13 participants across CA-125 tertiles. Patients with the highest CA-125 concentrations (T3) were  
14 more likely to be male, have lower LVEF, systolic blood pressure, body-mass index and eGFR,  
15 and to have a higher prevalence of coronary artery disease, atrial fibrillation, valvular heart  
16 disease, history of HF hospitalization and higher concentrations of NT-proBNP and hs-cTnT  
17 compared to patients with the lowest CA-125 concentrations (T1). Moreover, patients in T3 of  
18 CA-125 concentrations were more likely to receive diuretics, mineralocorticoid receptor  
19 antagonists, thiazides, ivabradine, glycosides, and anticoagulants. On the other hand, use of  
20 statins, calcium channel blocker, and angiotensin receptor neprilysin inhibitors were less  
21 common in such patients compared to those with lower values (**Supplemental Table 1**).

22 Congestion signs and symptoms at baseline stratified by tertiles of CA-125 are detailed in  
23 **Table 2**. Overall, no statistically significant difference was found across such strata.

1           **Figure 2** shows the cumulative incidence rate of HF hospitalization/cardiovascular death  
2 across tertiles of CA-125 levels. In unadjusted analysis, patients with highest CA-125  
3 concentrations (T3) experienced a higher rate of primary outcome events (a composite of first  
4 HF hospitalization or CV death) compared to patients with lower CA-125 concentrations (T2 and  
5 T1) (p-trend <0.01; **Table 3A**). After adjustment for relevant confounders including other  
6 biomarkers (NT-proBNP and hs-cTnT) and treatment assignment, the association between CA-  
7 125 concentration and HF hospitalization/CV death became non-significant (T3 vs T1 Hazard  
8 ratio [HR] 1.34; 95% confidence interval [CI] 0.91-1.96;p-trend=0.11). This finding was driven  
9 by the fact that CA-125 was not associated with major HF events in those with preserved LVEF  
10 but was associated with major HF events in those with reduced ejection fraction (p-value for  
11 interaction=0.02) (**Tables 3B and 3C**). In those with reduced LVEF, CA-125 retained prognostic  
12 significance even when adjusted for other biomarkers.

13           Considering the components of the primary endpoint, higher concentrations of CA-125  
14 were not associated with CV death; however, patients with the highest concentrations of CA125  
15 (T3) had the highest risk of first HF hospitalization: T3 vs T1 HR 1.56 (95%CI 0.97-2.50), p-  
16 trend =0.06. However, in adjusted analyses the highest CA-125 tertile was not associated with  
17 total HF hospitalizations (T3 vs T1 Risk Ratio [RR] 1.26 (95%CI 0.74-2.16), , p-trend=0.41). A  
18 CA-125 in the highest tertile was associated with a higher (adjusted) risk for first intensification  
19 of diuretic therapy: T3 vs T1 HR 1.44 (95%CI 1.00-2.07), p-value=0.05, driven largely by the  
20 higher rates in HFrEF compared to HFpEF, as shown in **Tables 3B and 3C**.

21           **Table 4** details the adjusted eGFR change slope from baseline by tertiles of CA-125 at  
22 baseline. Patients in the highest tertile (T3) had a more rapid decline in eGFR over the course of  
23 the study compared to patients in the lowest (T1), as evidenced by a steeper negative eGFR slope

1 ( $\beta$  coefficient=  $-1.18$ , 95% CI:  $-2.27$  to  $-0.10$ , p-value= $0.03$ ). However, CA-125 concentration  
2 was not associated with sustained eGFR reduction  $\geq 40\%$  or ESRD (T3 vs T1 HR  $1.81$ , 95%CI  
3  $0.78-4.19$ , p-trend= $0.16$ ). Analyses conducted on individuals with HFpEF and HFrEF yielded  
4 similar results (**Supplemental Table 2 and 3**).

5 In the overall study population (across all LVEF spectrum), the effect of empagliflozin to  
6 reduce CV death/HF hospitalization appeared to be attenuated in patients with the lowest CA-  
7 125 tertile (interaction-p-trend= $0.09$ ). No other trends or statistically significant interactions  
8 between baseline CA-125 concentration and effect of empagliflozin on clinical endpoints of the  
9 EMPEROR trials were found (**Table 5**), but for each outcome, the magnitude of the effect of  
10 empagliflozin was smallest in those with the lowest CA-125 tertile (T1). No difference between  
11 LVEF  $<40\%$  and LVEF  $\geq 40\%$  was seen regarding baseline CA-125 interaction and empagliflozin  
12 effect.

13 The top correlations of baseline CA-125 with proteins either measured using Olink  
14 proteomics or other automated immunoassays are shown in **Supplemental Table 4**. At baseline,  
15 most correlations were weak to modest. Strongest correlations of CA-125 were between  
16 biomarkers associated with myocardial structure/function, inflammation, myocardial  
17 development, angiogenesis, and mitochondrial function. As expected, correlation was present  
18 between CA-125 and MUC16.

19 **Table 6** shows the concentration of CA-125 among empagliflozin compared to placebo  
20 groups at baseline and follow-up visits. Treatment with empagliflozin was associated with a  
21 modest reduction of CA-125 level versus placebo to week 12 (adjusted geometric mean ratio:  
22  $0.93$ ; 95% confidence interval [CI],  $0.87-0.99$ , p= $0.03$ ) but not to week 52 (adjusted geometric  
23 mean ratio:  $0.97$ , 95% CI  $0.90-1.06$ ; p= $0.50$ ) (**Figure 3**).

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## **Discussion**

In this analysis across the full EF range in the EMPEROR Trials, there are several key findings regarding CA-125: 1) higher baseline CA-125 levels were not significantly associated with congestive signs or symptoms; 2) in fully adjusted models that included concentrations of NT-proBNP and hs-cTnT, baseline CA-125 levels were not associated with HF hospitalization or CV death; however, a potential difference between HF<sub>r</sub>EF and HF<sub>p</sub>EF was found, with CA-125 being associated with HF hospitalization or CV death only in patients with HF<sub>r</sub>EF; 3) in the whole population, higher CA-125 concentrations were marginally predictive of time to first intensification of diuretic therapy, and were also associated with a more rapid decline in kidney function; 4) the effect of empagliflozin on heart failure outcomes appeared to be attenuated in patients with the lowest CA-125 concentrations (T1); and 5) empagliflozin lowered CA-125 levels by a small degree compared to placebo at week 12 but not at week 52. The evidence from this study suggests that CA-125 may not be an ideal marker for the assessment of congestion and that in the presence of other biomarkers, CA-125 is mainly prognostic for major CV events in HF<sub>r</sub>EF.

Intravascular and interstitial fluid overload or redistribution, known as congestion, is proposed as an important reason for HF decompensation(24). Symptomatic congestion is a complex, multi-factorial phenomenon incorporating changes in intravascular and extravascular hydrostatic pressure, oncotic pressure, vascular and lymphatic capacity. There is no simple gold standard for the non-invasive assessment of congestion among patients with HF. Physical examination findings such rales, jugular venous distension, peripheral edema have limited accuracy for detecting elevated pulmonary capillary wedge pressure(25-27). Concentrations of

1 natriuretic peptides are strongly associated with transmural wall stress and are highly sensitive  
2 for the diagnosis of HF; the increase in fluid retention that underlies congestion may increase  
3 transmural wall stress and natriuretic peptide concentrations(28)(29). However, the association  
4 between natriuretic peptide concentrations and filling pressures is only modest leading to the  
5 search for other biomarkers that might be useful to identify presence of elevated filling pressures,  
6 fluid overload, and the state called “congestion”. Among these is CA-125, which has repeatedly  
7 been suggested as a candidate biomarker for congestion in HF(11)(30)(31-33). Despite these  
8 repeated suggestions, a formal assessment of CA-125 relative to other biomarkers such as NT-  
9 proBNP, as well as an evaluation across LVEF has been lacking.

10 A major strength of this analysis is the ability to study CA-125 across the wide range of  
11 LVEF in the EMPEROR program. In doing so, we found baseline physical findings supporting  
12 presence and severity of congestion were not clearly associated with CA-125 concentrations. A  
13 report from the Dapagliflozin and Prevention of Adverse Outcomes in HF (DAPA-HF) trial of  
14 study participants with LVEF <40% (34) suggested that very elevated concentrations of CA-125  
15 (>35 U/mL, comprising only 12% of the DAPA-HF population) were associated with the  
16 presence of peripheral edema.

17 Previous studies in HF have proposed a role of CA-125 to predict major outcomes  
18 including CV death or HF hospitalization (8, 11, 34-37); some did not consider other biomarkers  
19 such as natriuretic peptides or troponins, and none approached the question agnostic to LVEF. In  
20 fact, most studies focused only on those with reduced LVEF. In the recent DAPA-HF report of  
21 study participants with reduced LVEF, risk for the primary endpoint of worsening HF or CV  
22 death was only present in the highest CA-125 quartile ( $\geq 21.14$  U/mL). In the present study,  
23 across the full LVEF spectrum in EMPEROR, in fully-adjusted models that considered

1 concentrations of NT-proBNP and hs-cTnT, comparably elevated concentrations of CA-125 were  
2 not associated with HF outcomes in the EMPEROR trials. However, an unique strength of this  
3 analysis is the wide range of LVEF in the EMPEROR CA-125 analysis; in this regard, a  
4 statistical interaction between LVEF and prognostic association of CA-125 was found, whereby  
5 higher CA-125 concentrations were associated with HF outcomes in the subset of patients with  
6 HFrEF (EMPEROR-Reduced) but not among those with HFpEF (EMPEROR-Preserved). These  
7 results suggest that in the context of contemporary biomarkers and other clinical variables, CA-  
8 125 is associated with the occurrence of HF events among patients with reduced LVEF but may  
9 not be informative for prognostic assessment among those with mildly reduced or preserved LV  
10 function. The reasons for such differences between HFrEF and HFpEF should be addressed in  
11 future studies.

12 In EMPEROR, reductions in major HF outcomes owing to treatment with empagliflozin  
13 appeared to be attenuated in those with a baseline CA-125 in the lowest tertile; this finding was  
14 not present with other outcomes and such a finding was not observed in the DAPA-HF trial,  
15 which studied only patients with a reduced LVEF(34). Overall, these results suggest that CA-  
16 125 cannot be used to help decision making regarding the use of SGLT2 inhibitors in HF  
17 patients. Treatment with empagliflozin was also associated with a small and non-sustained  
18 reduction in CA-125, reminiscent of the lowering of CA-125 by dapagliflozin in reduced LVEF  
19 patients(34) but extended across the whole range of LVEF in this analysis.

20 Deciphering correlations between CA-125 and the large array of biomarkers measured in  
21 EMPEROR might improve the understanding and prognostication of the risk of HF and  
22 represents another unique aspect of this analysis. We found correlations between CA-125 and  
23 several plasma proteins associated with key processes in cardiovascular disease including

1 IGFBP-7(38-41), angiotensin-2(42), bone morphogenetic protein-10(43), and growth  
2 differentiation factor-15 (GDF-15)(44).

3 Our findings should be interpreted in the presence of several limitations. Although CA-  
4 125 was not associated with physical findings consistent with congestion, it is worth keeping in  
5 mind that accuracy of physical examination findings for detecting elevated congestion is limited  
6 and that study participants with higher CA-125 were more likely to receive decongestive  
7 therapies, including diuretics and MRAs at baseline, which might have attenuated associations  
8 between CA-125 and clinical evidence for congestion. Additionally, no “congestion score” was  
9 used in the EMPEROR trials which may decrease the reporting consistency across centers. No  
10 echocardiographic data were available; therefore, we could not correlate CA-125 levels with  
11 echocardiographic measurements (e.g., left atrium volume or inferior vena cava variability). Our  
12 study included chronic HF patients only with more than 70% and 80% of study were men and  
13 white individuals, respectively, limiting the generalizability of our results. Future studies may  
14 investigate the risk of incident HF or death by CA-125 concentration in demographic subgroups  
15 defined by sex, race and ethnicity. Our substudy might have been underpowered to assess the  
16 prognostic value of CA-125, particularly in even smaller subgroups of HFrEF and HFpEF.

17 In conclusion, across the range of EF in patients with chronic HF enrolled in the  
18 EMPEROR trials, the majority of whom did not have clinical evidence of congestion, CA-125  
19 concentrations were not significantly associated with congestive signs or symptoms. These  
20 results call into question the role of CA-125 as a biomarker of clinical congestion in chronic HF  
21 and, in the setting of NT-proBNP testing, suggest that CA-125 may have a modest prognostic  
22 role in HFrEF but not in HFpEF.

23



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33

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38 Publication of Clinical Study Data, scientific and medical researchers can request access to  
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- 1 other criteria are met. Please visit <https://www.mystudywindow.com/msw/datasharing> for further
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1 **Table 1.** Baseline characteristics of study population stratified by tertiles of CA-125.

	Lowest tertile N=370	Middle tertile N=370	Highest tertile N=371	p-value***
<b>Age, years</b>	69.6 ± 9.8	70.1 ± 9.9	70.5 ± 10.4	0.19
<b>Sex, N (%)</b>				0.05
Male	248 (67.0)	231 (62.4)	274 (73.9)	
Female	122 (33.0)	139 (37.6)	97 (26.1)	
<b>Race, N (%)</b>				0.11
White	308 (83.2)	310 (83.8)	316 (85.2)	
Black	9 (2.4)	5 (1.4)	11 (3.0)	
Asian	30 (8.1)	34 (9.2)	32 (8.6)	
Other	22 (5.9)	14 (3.8)	9 (2.4)	
<b>Ethnicity, N (%)</b>				0.37
Not Hispanic / Latino	291 (78.6)	275 (74.3)	281 (75.7)	
Hispanic / Latino	73 (19.7)	82 (22.2)	83 (22.4)	
<b>Region, N (%)</b>				0.44
North America	52 (14.1)	39 (10.5)	65 (17.5)	
Latin America	68 (18.4)	73 (19.7)	76 (20.5)	
Europe	211 (57.0)	220 (59.5)	187 (50.4)	
Asia	27 (7.3)	29 (7.8)	30 (8.1)	
Other	12 (3.2)	9 (2.4)	13 (3.5)	
<b>Smoking status, N (%)</b>				0.33
Never smoked	172 (46.5)	159 (43.0)	157 (42.3)	
Ex-smoker	157 (42.4)	166 (44.9)	178 (48.0)	
Currently smokes	33 (8.9)	36 (9.7)	29 (7.8)	
<b>Drinking alcohol, N (%)</b>				0.82
Does not drink alcohol	222 (60.0)	213 (57.6)	225 (60.6)	
Drinks alcohol	137 (37.0)	148 (40.0)	134 (36.1)	
<b>Time since HF diagnosis, y</b>	4.9 ± 5.4	4.9 ± 5.1	5.2 ± 5.8	0.41
<b>Heart failure duration, y, N (%)</b>				0.51
≤ 1	81 (21.9)	80 (21.6)	85 (22.9)	
>1 to 5	173 (46.8)	161 (43.5)	154 (41.5)	
>5 to 10	60 (16.2)	73 (19.7)	71 (19.1)	
>10	56 (15.1)	56 (15.1)	61 (16.4)	
<b>Diabetes status</b>				
Diabetes	175 (47.3)	181 (48.9)	180 (48.5)	0.74
No diabetes	195 (52.7)	189 (51.1)	191 (51.5)	
<b>LVEF, %</b>	42.6 ± 15.2	40.1 ± 15.1	38.1 ± 14.5	<0.001
<b>LVEF, N (%)</b>				0.001
<50 %	230 (62.2)	254 (68.6)	275 (74.1)	
50 to <60%	67 (18.1)	58 (15.7)	54 (14.6)	
≥ 60%	73 (19.7)	58 (15.7)	42 (11.3)	
<b>SBP, mmHg</b>	130 ± 16	127 ± 15	127 ± 16	0.004
<b>DBP, mmHg</b>	75 ± 11	74 ± 10	75 ± 10	0.63
<b>Heart rate, bpm</b>	69 ± 12	70 ± 11	72 ± 13	0.006
<b>BMI, kg/m<sup>2</sup></b>	29.98 ± 5.95	29.48 ± 5.38	28.56 ± 5.58	0.001
<b>BMI (kg/m<sup>2</sup>), N (%)</b>				0.02
<30	206 (55.7)	201 (54.3)	239 (64.4)	
≥30	164 (44.3)	169 (45.7)	132 (35.6)	
<b>eGFR CKD-EPI, mL/min/1.73 m<sup>2</sup></b>	63.1 ± 19.9	58.9 ± 20.3	59 ± 20.2	0.005
<b>eGFR CKD-EPI (mL/min/1.73 m<sup>2</sup>) classification, N (%)</b>				0.05

≥ 90	37 (10.0)	29 (7.8)	27 (7.3)	
60 to <90	169 (45.7)	140 (37.8)	140 (37.7)	
45 to <60	86 (23.2)	101 (27.3)	100 (27.0)	
30 to <45	66 (17.8)	75 (20.3)	78 (21.0)	
20 to <30	12 (3.2)	23 (6.2)	26 (7.0)	
< 20	0	2 (0.5)	0	
Albumin/Cr ratio*, mg/g	14.0 (6.2, 45.1)	15.9 (6.2, 58.3)	21.2 (8.0, 82.0)	<0.001**
Hemoglobin, mg/dl	13.59 ± 1.39	13.67 ± 1.52	13.58 ± 1.69	0.97
Atrial fibrillation/flutter, N (%)	164 (44.3)	173 (46.8)	205 (55.3)	0.003
Atrial fibrillation, N (%)	155 (41.9)	166 (44.9)	202 (54.4)	0.001
HF hospitalization (within 12 months), N (%)	85 (23.0)	104 (28.1)	112 (30.2)	0.03
Hypertension, N (%)	305 (82.4)	313 (84.6)	306 (82.5)	0.99
Hypercholesterolemia, N (%)	259 (70.0)	260 (70.3)	254 (68.5)	0.65
ACS within 6 months, N (%)	18 (4.9)	19 (5.1)	20 (5.4)	0.75
MI, N (%)	127 (34.3)	137 (37.0)	146 (39.4)	0.16
CAD, N (%)	132 (35.7)	155 (41.9)	168 (45.3)	0.008
CABG or PCI, N (%)	130 (35.1)	145 (39.2)	153 (41.2)	0.09
Valvular heart disease, N (%)	44 (11.9)	60 (16.2)	77 (20.8)	0.001
Invasive EP procedure, N (%)	30 (8.1)	29 (7.8)	34 (9.2)	0.59
Left Bundle Branch Block, N (%)	39 (10.5)	50 (13.5)	40 (10.8)	0.92
Electrical cardioversion in the last 6 m, N (%)	4 (1.1)	10 (2.7)	6 (1.6)	0.61
Electrical defibrillation in the last 6 m, N (%)	3 (0.8)	0	1 (0.3)	0.16
Cardiac Biomarker				
NT-proBNP*, pg/mL	1050 (597, 1881)	1254 (701, 2239)	1866 (1021, 3320)	<0.001**
hs-cTnT*, ng/l	17.0 (12.0, 25.9)	18.6 (13.0, 29.5)	23.1 (15.1, 33.6)	<0.001**

1 Abbreviations: ACS: acute coronary syndrome, BMI: body mass index, CABG: coronary artery bypass graft, CAD:  
2 coronary artery disease, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate, EP  
3 electrophysiology, HF: heart failure, hs-cTnT: high sensitive cardiac troponin T, LVEF: left ventricular ejection  
4 fraction, MI: myocardial infarction, NYHA: New York heart association, NT-proBNP: N terminal pro b type  
5 natriuretic peptides, PCI: percutaneous coronary intervention, SBP: systolic blood pressure.

6 \* Median (IQR) \*\* based on log-transformed values; \*\*\*- p-value from ordinal regression likelihood ratio test

7

1 **Table 2.** Congestion sign and symptoms stratified by tertiles of CA-125 levels at baseline.

	<b>Lowest tertile N=370</b>	<b>Middle tertile N=370</b>	<b>Highest tertile N=371</b>	<b>p-value*</b>
<b>NYHA N (%)</b>				0.14
II	304 (82.2)	298 (80.5)	288 (77.6)	
III	66 (17.8)	71 (19.2)	81 (21.8)	
IV	0	1 (0.3)	2 (0.5)	
<b>KCCQ Orthopnea, N (%)</b>				0.21
Never over past 2 weeks or <1 time/week	306 (82.7)	313 (84.6)	300 (80.9)	
1–2 times/week	36 (9.7)	22 (5.9)	29 (7.8)	
3 times or more but not every day	11 (3.0)	15 (4.1)	20 (5.4)	
Every night	16 (4.3)	19 (5.1)	22 (5.9)	
Missing	1 (0.3)	1 (0.3)	0	
<b>KCCQ Fatigue, N (%)</b>				0.08
Never	105 (28.4)	104 (28.1)	83 (22.4)	
<1 time per week or 1–2 times per week	139 (37.6)	138 (37.3)	130 (35.0)	
3 or more times per week or at least once a day	83 (22.4)	76 (20.5)	107 (28.8)	
Either several times per day or all of time	43 (11.6)	51 (13.8)	51 (13.7)	
Missing	0	1 (0.3)	0	
<b>Peripheral Edema, N (%)</b>				0.07
Absent	278 (75.1)	277 (74.9)	265 (71.4)	
Trace	26 (7.0)	29 (7.8)	46 (12.4)	
Up to ankle	28 (7.6)	29 (7.8)	28 (7.5)	
Above ankle	38 (10.3)	35 (9.5)	32 (8.6)	
Sacral	0	0	0	
Ascites	0	0	0	
<b>Abnormal JVD, N (%)</b>				0.17
No	356 (96.2)	344 (93.0)	348 (93.8)	
Yes	14 (3.8)	26 (7.0)	23 (6.2)	
<b>Pulmonary rales, N (%)</b>				0.07
Absent	331 (89.5)	338 (91.4)	328 (88.4)	
Bases	38 (10.3)	32 (8.6)	38 (10.2)	
One third of lung field or more	1 (0.3)	0	5 (1.3)	
<b>S3 gallop, N (%)</b>				0.99
No	364 (98.4)	360 (97.3)	365 (98.4)	
Yes	6 (1.6)	10 (2.7)	6 (1.6)	
<b>Cardiac Murmur, N (%)</b>				0.36
No	328 (88.6)	331 (89.5)	321 (86.5)	
Yes	42 (11.4)	39 (10.5)	50 (13.5)	

2 Abbreviations: NYHA: New York Heart Association, KCCQ: Kansas City Cardiomyopathy Questionnaire, JVD:  
3 jugular vein distention; \* - p-value from ordinal regression likelihood ratio test

4

**Table 3.** Cox regression results by tertiles of CA-125 levels at baseline in A) all individuals, B) study participants with LVEF <40%, and C) study participants with LVEF ≥ 40%.

A)

	Lowest tertile N=370	Middle tertile N=370	Highest tertile N=371	Continuous <sup>§</sup> N=1111
<b>CV death/HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	7.04 (5.13, 9.24)	8.37 (6.19, 10.87)	14.76 (11.71, 18.17)	
Hazard ratio* (95% CI)	reference	1.01 (0.67-1.53)	1.34 (0.91-1.96)	1.07 (0.98, 1.17)
p-value	--	0.95	0.14	0.14
p for trend	--	0.11	--	--
<b>First HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	4.22 (2.78, 5.96)	6.49 (4.59, 8.71)	11.26 (8.61, 14.25)	
Hazard ratio* (95% CI)	reference	1.30 (0.79,2.14)	1.56 (0.97,2.50)	1.08 (0.98, 1.20)
p-value	--	0.30	0.07	0.13
p for trend	--	0.06	--	--
<b>CV death</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	4.06 (2.67, 5.72)	3.06 (1.84, 4.58)	5.92 (4.12, 8.03)	
Hazard ratio* (95% CI)	reference	0.64 (0.35,1.16)	1.05 (0.62,1.79)	1.05 (0.90, 1.22)
p-value	--	0.14	0.85	0.52
p for trend	--	0.77	--	--
<b>Total HF hospitalizations**</b>				
Unadjusted event rate (events per 100 patient years at risk)	7.09	10.01	17.42	
Adjusted event rate ratio / event rate ratio (95% CI)	reference	1.27 (0.74, 2.18)	1.26 (0.74, 2.16)	1.09 (0.95-1.24)
p-value	--	0.381	0.40	0.21
p for trend	--	0.41	--	--
<b>Sustained eGFR reduction ≥40% or ESRD</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	1.66 (0.76, 2.90)	1.95 (0.94, 3.34)	4.17 (2.55, 6.18)	
Hazard ratio* (95% CI)	reference	1.12 (0.44,2.80)	1.81 (0.78,4.19)	1.15 (0.95, 1.39)
p-value	--	0.82	0.16	0.14
p for trend	--	0.14	--	--
<b>First event of intensification of diuretic therapy</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	8.43 (6.27, 10.89)	10.08 (7.64, 12.87)	15.03 (11.90, 18.52)	
Hazard ratio* (95% CI)	reference	1.08 (0.74,1.59)	1.44 (1.00,2.07)	1.09 (0.99, 1.19)
p-value	--	0.69	0.05	0.07
p for trend	--	0.05	--	--

**B)**

	<b>Lowest tertile N=193</b>	<b>Middle tertile N=193</b>	<b>Highest tertile N=194</b>	<b>Continuous<sup>§</sup> N=580</b>
<b>CV death/HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	8.19 (5.00-12.15)	13.47 (9.09-18.70)	23.78 (17.70-30.73)	
Hazard ratio* (95% CI)	Reference	1.40 (0.78-2.51)	2.25 (1.30-3.89)	1.16 (1.03, 1.31)
p-value	--	0.26	0.004	0.01
p for trend	--	0.003	--	--
<b>First HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	5.32 (2.83-8.58)	10.78 (6.90-15.49)	19.58 (14.11-25.96)	
Hazard ratio* (95% CI)	Reference	1.70 (0.85-3.40)	2.70 (1.40-5.19)	1.17 (1.02, 1.33)
p-value	--	0.13	0.003	0.02
p for trend	--	0.002	--	--
<b>CV death</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	4.71 (2.43-7.27)	4.12 (1.98-7.04)	7.63 (4.60-11.43)	
Hazard ratio* (95% CI)	Reference	0.69 (0.28-1.69)	1.17 (0.53-2.54)	1.07 (0.88, 1.30)
p-value	--	0.42	0.70	0.50
p for trend	--	0.58	--	--
<b>Total HF hospitalizations**</b>				
Unadjusted event rate (events per 100 patient-years at risk)	11.42	15.72	28.92	
Adjusted event rate ratio (95% CI)	Reference	1.18 (0.59-2.38)	1.68 (0.85-3.30)	1.11 (0.96, 1.28)
p-value	--	0.64	0.13	0.16
p for trend	--	0.13	--	--
<b>Sustained eGFR reduction ≥40% or ESRD</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	1.99 (0.54-4.35)	2.11 (0.58-4.63)	4.53 (2.07-7.93)	
Hazard ratio* (95% CI)	Reference	NA	NA	1.03 (0.81, 1.31)
p-value	--	NA	NA	0.80
p for trend	--	NA	--	--
<b>First event of intensification of diuretic therapy</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	10.33 (6.62-14.86)	13.57 (9.16-18.84)	21.63 (15.83-28.31)	
Hazard ratio* (95% CI)	Referent	1.08 (0.62-1.88)	1.82 (1.08-3.06)	1.11 (0.99, 1.25)
p-value	--	0.79	0.02	0.08
p for trend	--	0.02	--	--

C)

	Lowest tertile N=177	Middle tertile N=177	Highest tertile N=177	Continuous <sup>§</sup> N=521
<b>CV death/HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	7.14 (4.67-10.14)	5.01 (3.02-7.50)	8.19 (5.44-11.49)	
Hazard ratio* (95% CI)	Referent	0.58 (0.32-1.06)	0.68 (0.38-1.21)	0.90 (0.72, 1.13)
p-value	--	0.08	0.19	0.38
p for trend	--	0.20	--	
<b>First HF hospitalization</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	4.12 (2.31-6.45)	3.43 (1.82-5.53)	5.56 (3.35-8.32)	
Hazard ratio* (95% CI)	Referent	0.69 (0.33-1.47)	0.70 (0.34-1.47)	0.93 (0.70, 1.23)
p-value	--	0.34	0.35	0.59
p for trend	--	0.37	--	
<b>CV death</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	3.96 (2.22-6.20)	2.28 (1.04-3.99)	4.46 (2.55-6.90)	
Hazard ratio* (95% CI)	Referent	0.50 (0.21-1.15)	0.86 (0.41-1.82)	0.98 (0.73, 1.32)
p-value	--	0.10	0.69	0.89
p for trend	--	0.72	--	
<b>Total HF hospitalizations**</b>				
Unadjusted event rate (events per 100 patient-years at risk)	5.02	5.84	8.69	
Adjusted event rate ratio (95% CI)	Referent	1.19 (0.50-2.86)	0.99 (0.40-2.45)	1.08 (0.78-1.50)
p-value	--	0.69	0.98	0.65
p for trend	--	0.99	--	
<b>Sustained eGFR reduction ≥40% or ESRD</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	1.91 (0.70-3.71)	1.49 (0.48-3.05)	3.72 (1.86-6.22)	
Hazard ratio* (95% CI)	Referent	0.82 (0.23-2.84)	1.59 (0.53-4.75)	1.31 (0.91, 1.87)
p-value	--	0.75	0.41	0.14
p for trend	--	0.35	--	
<b>First event of intensification of diuretic therapy</b>				
Incidence rate, patients with events per 100 patient years at risk (95% CI)	8.50 (5.69-11.86)	7.18 (4.69-10.19)	9.79 (6.70-13.46)	
Hazard ratio* (95% CI)	Referent	0.80 (0.46-1.36)	0.96 (0.57-1.63)	1.08 (0.88, 1.32)
p-value	--	0.41	0.88	0.48
p for trend	--	0.88	--	

CV denotes: cardiovascular; HF denotes: heart failure; CI denotes: confidence intervals; \* Hazard ratio based a Cox model adjusted for age, sex, baseline eGFR (CKD-EPI), baseline LVEF, log (NT-proBNP) at baseline, log (HS Troponin T) [ng/L], region, diabetes at baseline, treatment arm (empagliflozin vs placebo), study (EMPEROR-REDUCED vs EMPEROR-PRESERVED). § Hazard ratio for a 2-fold change (doubling) in CA-125. \*\* Estimates from a negative binomial model

**Table 4.** Adjusted eGFR change slope from baseline by tertiles of CA-125 at baseline. Model adjusted as noted, including by treatment assignment.

	<b><math>\beta</math></b>	<b>95 % CI</b>	<b>p-value</b>
Lowest tertile slope* [/year]	-1.169	(-1.916 -0.421)	0.002
Middle tertile slope* [/year]	-1.588	(-2.352 -0.824)	<0.001
Highest tertile slope* [/year]	-2.352	(-3.140 -1.565)	<0.001
Middle vs Low	-0.419	(-1.490 0.651)	0.44
High vs Low	-1.184	(-2.273 -0.095)	0.03

Model includes baseline eGFR (CKD-EPI), age, baseline LVEF, log (NT-proBNP) [pg/mL] at baseline, log (HS Troponin T) [ng/L] at baseline as linear covariate(s) and region, diabetes at baseline, sex, treatment, study, baseline eGFR (CKD-EPI) by Time interaction, Time by tertiles of CA-125 at baseline interaction, tertiles of CA-125 at baseline as fixed effect(s). Intercept and slope allowed to vary randomly between patients.

\* Slope measured at baseline eGFR (CKD-EPI) = 60.34.

**Table 5:** Effect of empagliflozin on endpoints in the EMPEROR Trials as a function of baseline CA-125.

	<b>Placebo incidence rate (Events per 100 patient years; 95% CI)</b>	<b>Empagliflozin 10 mg incidence rate (Events per 100 patient years; 95% CI)</b>	<b>Hazard ratio (95% CI)</b>	<b>Interaction trend p-value</b>
<b>CV death/HF hospitalization</b>				
Tertile 1	6.74 (4.22-9.83)	7.56 (4.84-10.86)	1.10 (0.62-1.97)	0.09
Tertile 2	10.28 (7.03-14.14)	6.37 (3.78-9.64)	0.62 (0.35-1.11)	
Tertile 3	18.21 (13.23-23.97)	12.11 (8.53-16.31)	0.57 (0.37-0.89)	
<b>First HF hospitalization</b>				
Tertile 1	3.98 (2.12-6.42)	4.72 (2.64-7.40)	1.21 (0.57-2.54)	0.14
Tertile 2	7.39 (4.68-10.70)	5.31 (2.97-8.32)	0.69 (0.36-1.32)	
Tertile 3	13.24 (9.06-18.21)	9.82 (6.63-13.63)	0.59 (0.36-0.98)	
<b>CV death</b>				
Tertile 1	3.82 (2.04-6.16)	4.22 (2.31-6.70)	1.06 (0.50-2.26)	0.22
Tertile 2	3.89 (2.07-6.28)	2.37 (0.95-4.41)	0.67 (0.27-1.69)	
Tertile 3	7.84 (4.85-11.53)	4.54 (2.54-7.11)	0.57 (0.29-1.12)	
<b>Total HF hospitalizations</b>				
Tertile 1	7.36	7.28	1.09 (0.49-2.39)	0.43
Tertile 2	11.11	8.49	0.84 (0.40-1.75)	
Tertile 3	20.16	15.46	0.71 (0.37-1.37)	
<b>First intensification of diuretic</b>				
Tertile 1	9.46 (6.34-13.21)	7.59 (4.81-10.99)	0.77 (0.45-1.34)	0.41
Tertile 2	12.14 (8.50-16.41)	7.59 (4.70-11.16)	0.59 (0.34-1.01)	
Tertile 3	19.00 (13.80-25.01)	12.40 (8.73-16.71)	0.57 (0.36-0.88)	

CV denotes: cardiovascular; HF denotes: heart failure; CI denotes: confidence intervals; hazard ratio based on a Cox regression model with terms for age, baseline eGFR (CKD-EPI), baseline LVEF, region, diabetes at baseline, sex, study, treatment assignment, tertiles of CA-125 at baseline and treatment by tertiles of CA 125 at baseline interaction. Too few events were present to analyze sustained eGFR reduction  $\geq 40\%$  or ESRD in this analysis. Caption: No statistical interaction was present between empagliflozin and CA-125 concentration was detected for each outcome.

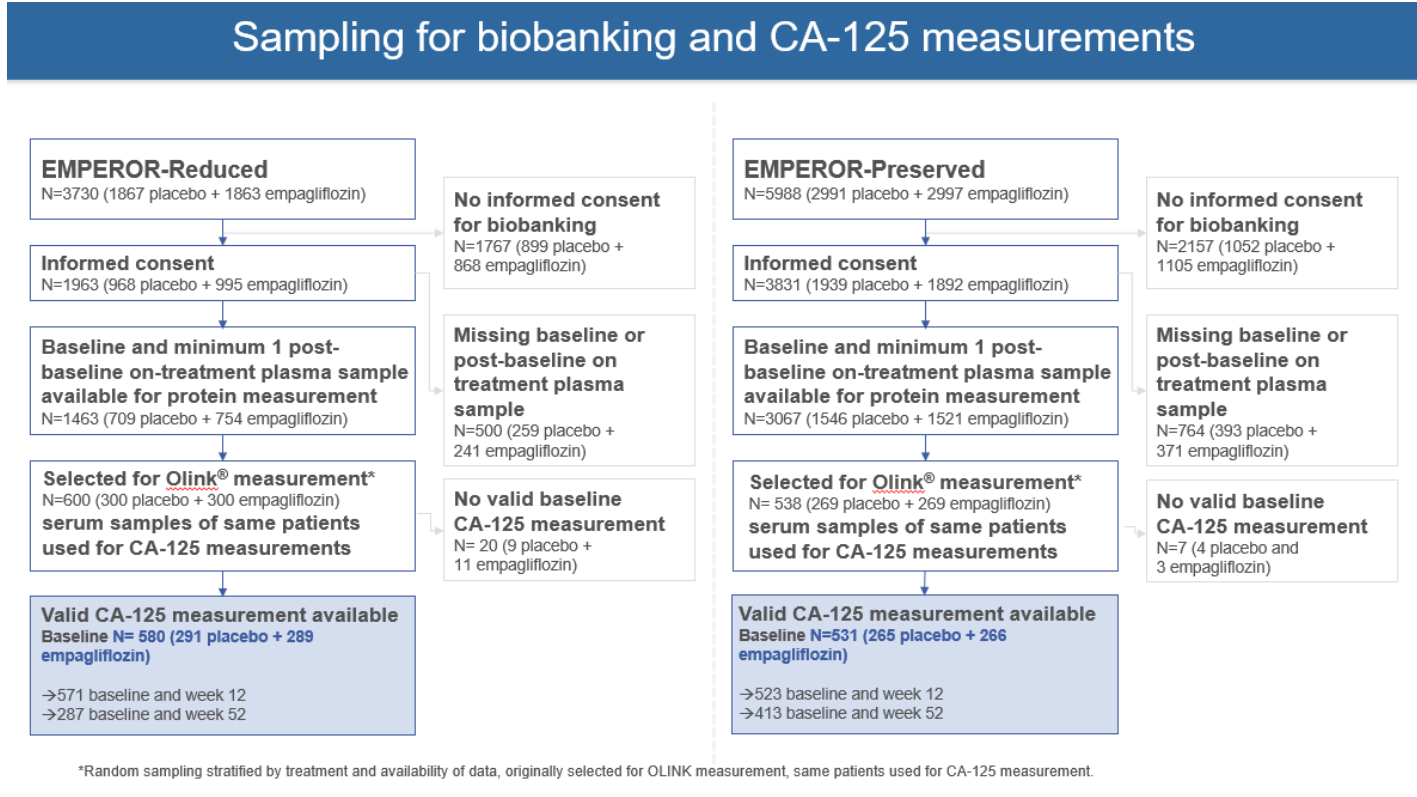


**Table 6.** CA-125 concentration (U/mL) among placebo and empagliflozin across study visits.

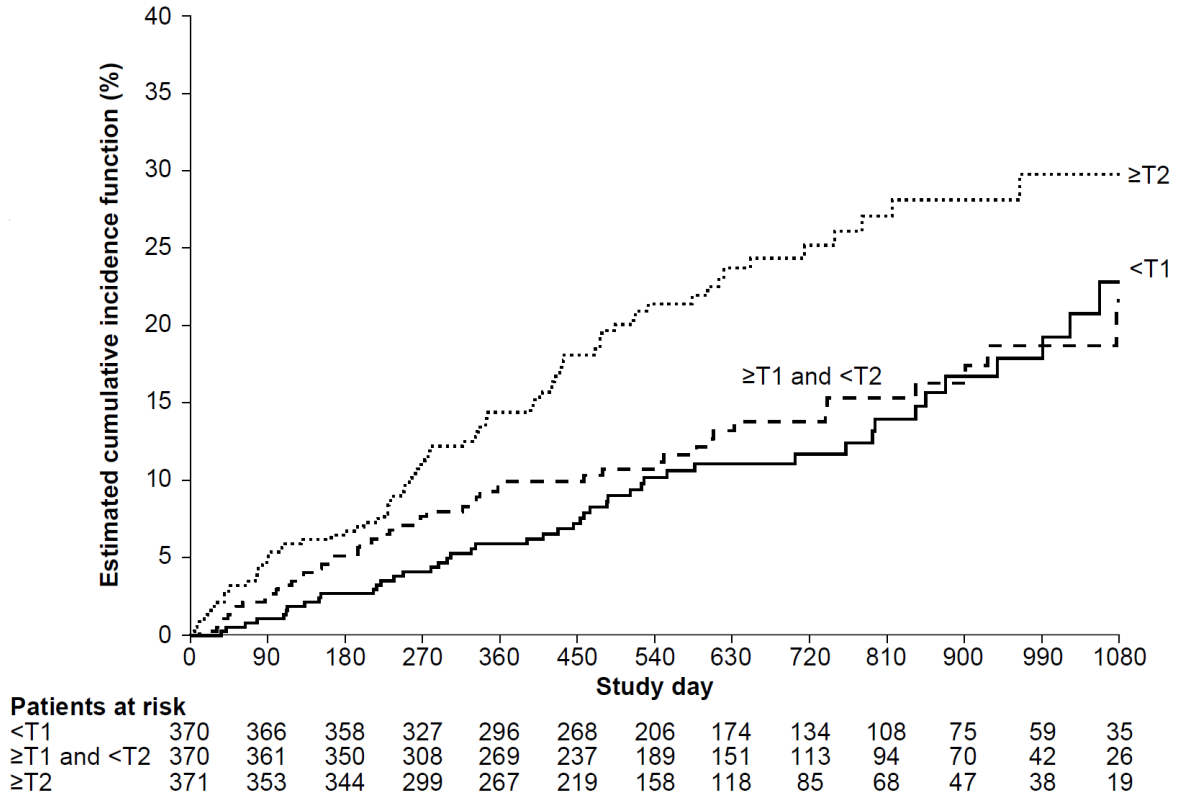
<b>Statistic</b>	<b>Placebo</b>	<b>Empagliflozin 10mg</b>
<b>Number of patients</b>	<b>552</b>	<b>551</b>
Baseline geometric mean (gCV [%])	17.724 (123.664)	20.710 (173.387)
<b>Week 12</b>		
Values at visit		
Number of patients	549	545
Geometric mean (gCV [%])	17.786 (115.159)	18.879 (163.618)
Adjusted* geometric mean	18.555	17.264
95% confidence interval	(17.745, 19.402)	(16.509, 18.054)
Relative change from baseline		
Geometric mean ratio (gCV [%])	0.997 (65.026)	0.907 (58.327)
Adjusted* geometric mean ratio	0.987	0.918
95% confidence interval	(0.944, 1.032)	(0.878, 0.960)
Comparison vs Placebo (ratio of relative change)		
Adjusted* geometric mean ratio	reference	0.930
95% confidence interval	---	(0.873, 0.991)
p-value	---	0.03
<b>Week 52</b>		
Values at visit		
Number of patients	351	349
Geometric mean (gCV [%])	17.009 (110.463)	18.017 (126.716)
Adjusted* geometric mean	18.700	18.189
95% confidence interval	(17.660, 19.801)	(17.177, 19.262)
Relative change from baseline		
Geometric mean ratio (gCV [%])	0.991 (67.468)	0.944 (70.835)
Adjusted* geometric mean ratio	0.995	0.967
95% confidence interval	(0.939, 1.053)	(0.914, 1.024)
Comparison vs Placebo (ratio of relative change)		
Adjusted* geometric mean ratio	reference	0.973
95% confidence interval	---	(0.897, 1.055)
p-value	---	0.50

\* Model includes age, baseline eGFR (CKD-EPI), baseline LVEF as linear covariate(s) and study, region, baseline diabetes status, sex, treatment by Visit interaction, log(baseline Carbohydrate antigen 125) by Visit interaction as fixed effect(s).

**Figure 1. Study Flowchart**



**Figure 2.** Cumulative rate of heart failure hospitalization/cardiovascular death stratified by CA-125 tertiles. Patients in the highest CA-125 tertile had shorter time to first heart failure hospitalization/cardiovascular death than other tertiles (log-rank test p-value <0.01).



**Figure 3.** Relative changes (empagliflozin vs placebo) in the circulating levels of CA-125 at week 12 and week 52. Reduction in CA-125 from empagliflozin treatment was present at 12 weeks, but no longer evident by 52 weeks.

